Preparation and Covalent Surface Modifications of Silica Coated Spherical and Rod-Shaped Gold Nanoparticles

by

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Abstract

The surface chemistry of silica-coated gold nanoparticles (Au NPs) was investigated by modifying these surfaces using alcohol-based reagents. Alcohol condensation reactions were facilitated by dielectric heating using a microwave reactor. The attachment of two alcohol-based reagents, a carboxylic acid functionalized alcohol and a chelating agent with a terminal alcohol functional group, was achieved on the surfaces of spherical gold nanoparticles and gold nanorods, respectively. A fluorescent probe was coupled to the carboxylic acid functionalized silica-coated Au NPs as a confirmation of the surface functionalization, and as a demonstration of an application for these nanoparticles. The silica-coated gold nanorods modified with a chelating species were evaluated for their ability to capture metal ions present in an aqueous solution. A wide range of surface chemistry for silica-based nanoparticles can be achieved using the methods presented herein to meet the requirements of their future applications.

Keywords: Gold nanoparticles; silica nanoparticles; surface modification; alcohols; microwave.
To my mother and father. Thank you for always being there for me in your own ways. Thank you for always encouraging me to chase my own dreams and never be afraid of failures.
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I would like to thank my Senior Supervisor, Professor Byron D. Gates, for his guidance and training throughout my academic research career. I deeply appreciate the opportunity to work with him since the time when I was an undergraduate student. I am also grateful for his support and encouragements on pursuing a future academic path that is meaningful for me.

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<th>Description</th>
</tr>
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<tbody>
<tr>
<td>12-HDA</td>
<td>12-hydroxydodecanoic acid</td>
</tr>
<tr>
<td>AEM</td>
<td>Analytical electron microscopy</td>
</tr>
<tr>
<td>Au NPs</td>
<td>Gold nanoparticles</td>
</tr>
<tr>
<td>Au NRs</td>
<td>Gold nanorods</td>
</tr>
<tr>
<td>BF</td>
<td>Bright field</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge coupled device</td>
</tr>
<tr>
<td>CMC</td>
<td>Critical micelle concentration</td>
</tr>
<tr>
<td>CTAB</td>
<td>Cetyltrimethylammonium bromide</td>
</tr>
<tr>
<td>DF</td>
<td>Dark field</td>
</tr>
<tr>
<td>DI water</td>
<td>Deionized water</td>
</tr>
<tr>
<td>DIC</td>
<td>diisopropyl carbodiimide</td>
</tr>
<tr>
<td>EDC</td>
<td>(N)-(3-dimethylaminopropyl)-(N)’-ethylcarbodiimide</td>
</tr>
<tr>
<td>EDS</td>
<td>Energy dispersive X-ray spectroscopy</td>
</tr>
<tr>
<td>HAADF</td>
<td>High-angle annular dark field</td>
</tr>
<tr>
<td>HBTU</td>
<td>(N, N, N', N')tetramethyl-(O)-(1(H)-benzotriazol-1-yl)uronium hexafluorophosphate</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>LSPR</td>
<td>Localized surface plasmon resonance</td>
</tr>
<tr>
<td>NHS</td>
<td>(N)-hydroxysuccinimide</td>
</tr>
<tr>
<td>NIR</td>
<td>Near infrared</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly(ethylene glycol)</td>
</tr>
<tr>
<td>PMD</td>
<td>Photonic mode density</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
</tr>
<tr>
<td>SA</td>
<td>Salicylic acid</td>
</tr>
<tr>
<td>SERS</td>
<td>Surface enhanced Raman scattering</td>
</tr>
<tr>
<td>SPDP</td>
<td>(N)-succinimidyl 3-[2-pyridyldithio]-propionate</td>
</tr>
<tr>
<td>STEM</td>
<td>Scanning transmission electron microscopy</td>
</tr>
<tr>
<td>Sulfo-SMCC</td>
<td>sulfo-succinimidyl-(4-(N)-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC)</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TEOS</td>
<td>Tetraethyl orthosilicate</td>
</tr>
<tr>
<td>WCA</td>
<td>Water contact angle</td>
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## Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>Colloidal Dispersion</td>
<td>Colloids of one substance with dimensions between 1 nm and 1 μm suspended in another substance or continuous phase homogeneously.</td>
</tr>
<tr>
<td>Colloidal Stability</td>
<td>A measure of a colloidal dispersion’s properties. For example, a stable colloidal dispersion refers to a suspension of colloids that is resistant to aggregation, coagulation, flocculation, and phase separation.</td>
</tr>
<tr>
<td>Condensation Reaction</td>
<td>A chemical reaction in which two molecules combine, usually in the presence of a catalyst, and eliminate water or another simple molecule as a byproduct.</td>
</tr>
<tr>
<td>Dielectric Constant</td>
<td>A measure of a material’s property on their ability to prevent the transmission of both electronic and electrostatic charges in an applied electric field. A slower propagation of charges makes a material more easily polarizable electrically.</td>
</tr>
<tr>
<td>Dielectric Heating</td>
<td>A result of molecular dipole rotation within a dielectric material induced by electronic heating generated by a high-frequency alternative electric field, including microwave electromagnetic radiation.</td>
</tr>
<tr>
<td>Dynamic Light Scattering</td>
<td>A sample of nanoparticles held in the solution-phase is exposed to a laser beam, which leads to the scattering of light at all angles. The intensity of the scattered light is dependent on the size and motion of the nanoparticles. Backscatter detection is used in this technique: a detector positioned at either 90° or 173° with respect to the light path measures the scattered light intensity. The results from these measurements are the nanoparticle size distribution of the sample derived from the Mie theory.</td>
</tr>
<tr>
<td>Electron Diffraction</td>
<td>Ordered arrays of atoms in a crystal lattice scatter a beam of focused incident electrons in a periodic manner. Qualitative and quantitative information including crystallinity, crystal phases and orientation, d-spacing, and texture of the sample can be collected and analyzed from electron diffraction measurements.</td>
</tr>
<tr>
<td><strong>Energy Dispersive X-ray Spectroscopy</strong></td>
<td>An analytical technique coupled to the STEM imaging techniques (see below). X-rays are generated from the specimen following the impact from high energy incident electrons. An X-ray detector located above the sample stage collects X-rays emitted in the range of 0.3 to 0.03 steradian from the sample. An EDS spectrum of energy against counts consists of two components: (1) characteristic X-ray peaks that arise from all elements present in the excited area of the specimen, with the exceptions of H and He, which do not generate X-rays; and (2) a continuum of bremsstrahlung X-rays that occurs at all energies (up to the incident beam energy).</td>
</tr>
<tr>
<td><strong>Extinction Spectroscopy</strong></td>
<td>An analytical technique which measures the absorption and scattering activities of the incident light following its interaction with a solution of plasmonic nanoparticles. The most intense extinction (absorption and scattering) both occur at the wavelength corresponding to the surface plasmon resonance of the nanoparticles. A spectrum of the intensity of extinction is obtained as a function of wavelength from each measurement.</td>
</tr>
<tr>
<td><strong>Fluorescence Spectroscopy</strong></td>
<td>An analytical technique that measures the fluorescence activity of fluorophores contained in the sample solution following excitation using an external light source. Upon absorption of light, the electrons in the fluorophores are promoted from their ground electronic state to excited states. After a finite time spent in the exited states, these electrons return to the ground state via radiative and nonradiative decays. Fluorescence is emitted during the radiative decay process and is measured by the spectrometer. A spectrum of wavelength against fluorescence intensity is obtained from each measurement.</td>
</tr>
<tr>
<td><strong>Full-Width at Half-Maximum</strong></td>
<td>The distance between the points on a Gaussian or Lorentzian curve at which the function of the curve reaches half its maximum value.</td>
</tr>
<tr>
<td><strong>Hexagonal Close Packing</strong></td>
<td>An arrangement of atoms of alternating layers overlaid on one another. Each atom is surrounded by 12 other atoms, organized into a close-packed configuration. The atoms of the second layer are situated at the interstitial sites of the first layer, and the atoms in the next layer has the same x and y coordinates as those in the first layer.</td>
</tr>
<tr>
<td><strong>High Angle Annular Dark Field Imaging</strong></td>
<td>An imaging mode associated with STEM imaging. A high angle annular detector is positioned below the sample stage and collects transmitted electrons that are scattered at high angles (&gt; 50 milliradian). The energies of electrons ejected at these angles are atomic number dependent and can be used to identify the elements present in the sample.</td>
</tr>
</tbody>
</table>
Microwave
A form of electromagnetic radiation. The range of microwave wavelengths is 1 mm to 1 m, and with frequencies from 300 GHz to 300 MHz, respectively.

Monochromatic Excitation
An incident beam of electrons of a single wavelength. These electrons have a very narrow distribution of energies.

Ostwald Ripening
The phenomena associated with dissolution of smaller particles in the solution-phase followed by further growth of larger particles driven by thermodynamics of the system. The surfaces of the smaller particles are more energetically unstable than the surfaces of larger particles. The surface molecules of smaller particles are more likely to dissolve into solution. The saturated solution with these molecules subsequently drive their deposition onto larger particles.

Scanning Transmission Electron Microscopy
A focused beam of electrons is scanned across the specimen in a similar manner to a scanning electron microscope. Instead of detecting backscattered electrons with a detector located above the sample stage, scanning transmission electron microscopy (STEM) collects electrons that are transmitted (forward scattered) at relatively high angles (> 10 milliradian) using an annular detector located below the sample stage. In addition to contributions from the material density and thickness of the sample, the contrast of a STEM image can also be directly related to the atomic number of the elements contained in the sample.

Self-Assembled Monolayers
Single-molecule thick assemblies that form spontaneously via intermolecular interactions at an interface.

Single Crystalline
A solid with an ordered three-dimensional arrangement of atoms that is consistent throughout the whole volume of the solid and is free from crystallographic defects.

Surface Enhanced Raman Spectroscopy
A surface-sensitive technique that provides enhanced Raman scattering activities of analytes through adsorbing the analytes onto the metal surfaces. Examples of such substrates include plasmonic nanoparticles, surfaces prepared through electrochemical roughening and metallic coatings on nano-patterned surfaces. A spectrum of Raman shift against Raman intensity is acquired for each measurement. The spectral pattern can be used as a fingerprint to identify the presence of specific molecular species of interest.
Surface Plasmon Resonance

The frequency of the collective oscillations of the free electrons at the surfaces of metallic nanoparticles (e.g., gold, silver, and copper) upon their interaction with an appropriate wavelength of incident light. These free electrons exhibit an intense absorption of light at the surface plasmon resonance frequency. The spectral position of the surface plasmon resonance is highly dependent on the size, shape, structure and morphology of the nanoparticle, as well as their dielectric environment.

Transmission Electron Microscopy

A transmission electron microscope (TEM) illuminates samples with a thickness <100 nm that are held on a thin grid (~10 to 35 μm thick) using a beam of collimated, high energy electrons. These electrons travel towards the sample stage under an applied accelerating potential (typically 80 to 300 kV). The transmitted beam of electrons is focused by objective lenses to form images on a charge coupled device (CCD). The contrast of such images arises from variations in the density and thickness within the sample. The resolution of transmission electron microscopes can reach the atomic scale; lattices of crystalline materials can be resolved because of the short de Broglie wavelength of the incident electrons.

Transmission Electron Microscopy – Bright Field Imaging

A TEM image formed from the detection of transmitted electrons after they pass through the sample. Meanwhile, the diffracted electrons are blocked by an objective aperture. These images have a bright background with dark features.

Transmission Electron Microscopy – Dark Field Imaging

A TEM image formed from the detection of diffracted electrons following their interactions with the sample. An objective aperture placed at a specific angle with respect to the sample stage, which is responsible for allowing electrons diffracted over a narrow range of angles to reach the detector and to form an image.
Chapter 1.

Introduction

Silica-coated gold nanoparticles (Au NPs) of different shapes ($\varnothing \leq 100$ nm) are an important class of nanoscale materials. The silica-coated surfaces are chemically modifiable by a large selection of functional molecules. Another important aspect of these materials is their tunable optical properties due to the inherent properties of the underlying Au NPs. This chapter begins with an introduction to the surface modifications currently available for Au NPs and the motivation to pursue a silica encapsulation for these nanoparticles, followed by an overview of the current methods for modifying the surfaces of silica-based coatings on nanoparticles. The second part of this chapter includes an introduction to the syntheses and growth mechanisms of Au NPs that were prepared for the investigations of this thesis. This chapter is concluded by a summary of the objectives of the thesis work presented in Chapters 3 and 4.

1.1. Introduction to the Surface Modifications of Gold Nanoparticles

The preparation of gold nanoparticles of various shapes requires the use of surfactants for stabilization of the nanoparticles and dispersity of the nanoparticles in a solution-phase solvent. As-synthesized Au NPs and gold nanorods (Au NRs) with well-controlled size and shape, and that are highly monodispersed can be prepared by a limited number of surfactant systems. The most common surfactants used in the synthesis of Au NPs and Au NRs are sodium citrate, cetyltrimethylammonium bromide (CTAB), and polyvinylpyrrolidone (PVP). These surfactants are important for the synthesis of gold nanoparticles because they enable highly reproducible protocols for highly monodispersed as-synthesized nanoparticles. Sodium citrate, CTAB, and PVP cannot, however, provide the desired surface functionalities for many applications. As a result, it is often essential to modify the surfaces of gold nanoparticles for the specific requirements of an application. Regardless of the shape of the nanoparticles, the most common surface modifications of Au NPs are achieved through ligand exchange reactions with
functionalized thiolated compounds, particularly alkane thiols. Thiol-containing molecules are known to favorably adsorb onto gold (111) surfaces by forming a relatively strong thiolate-Au bond (40-50 kcal mol$^{-1}$). Alkane thiols can form self-assembled monolayers (SAMs) on the surfaces of Au NPs. Self-assembled monolayers are highly ordered assemblies of organic molecules formed by their spontaneous adsorption from a solution or gas phase onto the surfaces of solids. The original surfactant species on the as-synthesized Au NPs can be replaced by thiolated molecules with relative ease. Since the first report of a ligand exchange reaction using alkane thiols on the surfaces of Au NPs, alkane thiols with terminal functional moieties including alkyl halides, amines, azides, carboxylic acids, maleimides, phenols, alcohols, carbohydrates, amphiphilic polymers, amino acids, nucleic acids, peptides and proteins have been used to successfully modify the surfaces of gold nanoparticles (Figure 1). One challenge from using gold-thiol chemistry is the large number of intermediate steps that are required to achieve a final surface functionality due to the hydrophobic nature of many thiol compounds. Another challenge is the stability of the gold-thiol bond, which is prone to oxidation under ambient conditions.

![Figure 1.1](image)

**Figure 1.1** Examples of surface modifications to gold nanoparticles (Au NPs). X can be maleimides, polymers, nucleic acids, peptides and proteins. The types of functionalized silicon oxides that can be used to modify the Au NP surfaces are not limited to the examples shown.

Another common approach to enable changes to the surface chemistry of Au NPs is to encapsulate them with a shell of silica. The silica shell offers a new platform where silanol (Figure 1) and alcohol chemistry can be utilized as alternatives to thiol chemistry, which can sometimes be costly and present a high risk of contaminating other surfaces in the laboratory. The following discussions provide an overview of the formation of a silica shell on the surfaces of Au NPs and the subsequent approaches to their further surface
modification. In addition, a summary is also included of the types of current applications of silica-coated Au NPs.

1.1.1. Silica-Coated Gold Nanoparticles and Their Surface Modifications

A thin shell of silica on the surfaces of Au NPs can enhance their thermal stability while preserving their optical properties. A silica coating also renders the gold nanoparticles biocompatible, as CTAB is known to be a toxic cationic surfactant to cultured cells and animals. An important aspect of encapsulating gold nanoparticles in a silica shell is to diversify the means to which their surfaces can be functionalized using silanes or other chemistries. This thesis extends the surface modification of these silica coatings to alcohol condensation reactions. Further chemical modifications to the silica surfaces can be enabled through the association of functionalized silane-based or alcohol-based reagents, click chemistry, surface-initiated group transfer polymerizations, and carbodiimide crosslinker chemistry. It is worth noting that unlike thiol chemistry, both silane- and alcohol-based chemistries involve covalent modifications of surfaces.

Coating gold nanoparticles with both a spherical and rod-like shape generally involves the Stöber process with the aid of templates provided by surfactants. For example, CTAB can form a template of ordered micelles on the surfaces of gold nanoparticles and can screen the electrostatic repulsion between silica oligomers. The low dissociation constant, or a low tendency to separate reversibly into ionic forms, of CTAB enables their self-assembly at concentrations around the critical micelle concentration (CMC) of CTAB in a water-methanol cosolvent system. Methanol and ethanol are two common solvents in which the silica precursors, such as tetraethyl orthosilicate (TEOS), are suspended. A CTAB concentration slightly above the CMC is the most common for the silica coating process, because templating can be achieved by the formation of a mesoporous CTAB bilayer. Variations in the structures of the CTAB layer surrounding the nanoparticle surfaces can occur at concentrations below the CMC of CTAB, resulting in nonuniform silica coatings. At concentrations well above the CMC, on the other hand, a large amount of excess free CTAB molecules will be present in the reaction mixture. The result is the formation of empty CTAB micelles (without Au cores) and possibly solid silica spheres.
The formation of silica shells on Au NPs proceeds through a hydrolysis and condensation reaction of the silicon alkoxides (e.g., TEOS), commonly referred as the sol-gel chemistry of silica.\textsuperscript{19,20} Prior to the addition of TEOS, the Au NPs first required the addition of CTAB to modify their surfaces. Following the incubation with a CTAB solution, the surfaces of Au NPs are primarily coated with a bilayer of CTAB. The sol-gel reactions of silicon alkoxides, when carried out at neutral pH, are relatively slow, they often require the addition of a Brønsted-Lowry acid or base catalyst. The type of catalyst can determine the structure of the resulting gel because of differences in the relative rates of hydrolysis and condensation facilitated by the catalyst. During the hydrolysis reaction catalyzed by an acid (Figure 1.2) or a base (Figure 1.3), an alk oxy group is replace by a hydroxyl group via a pentacoordinated transition state.\textsuperscript{20} In addition, the Si to H\textsubscript{2}O ratio and specific reaction conditions including catalyst concentration and reaction duration can change the number of alkoxy groups that are hydrolyzed in a reaction. The stability of the transition state for both types of catalyzed reactions rely on the relative electronic properties of the hydroxyl groups relative to the alkoxy groups. Overall, the rate of hydrolysis reactions depends on the stability of the transition state. Here, successive hydrolysis reactions become progressively slower when catalyzed by an acid, and faster when using a base.\textsuperscript{20}

\textbf{Figure 1.2} Hydrolysis of silicon alkoxides catalyzed by an acid.

\textbf{Figure 1.3} Hydrolysis of silicon alkoxides catalyzed by a base.

In addition to silicon alkoxide hydrolysis, both an acid and a base can catalyze the subsequent condensation reactions to form O-Si-O, siloxane bonds (Figures 1.4 and 1.5, respectively).\textsuperscript{20} Condensation reactions can initiate when at least one silanol group is present on a silicon atom. Under basic reaction conditions, the process of hydrolysis of all
three available sites on each silicon atom can be completed before condensation takes place, allowing for the formation of \((\text{OH})_3\text{Si}-\text{O}-\text{Si(OH)}_3\), which possess six silanol groups for subsequent condensation reactions. Hence, under basic conditions, the rate of siloxane bond formation increases as the reaction progresses. The resulting agglomerates are highly branched and can form colloidal, gel-like particles via crosslinking. Under acidic conditions, on the other hand, condensation begins before the hydrolysis process is complete. These condensation reactions are likely to take place only on terminal silanol groups, resulting in the formation of linear molecular chains that assemble into a network. In this thesis, the base catalyzed approach was used to prepare silica coatings on both spherical and rod-shaped Au NPs.

Figure 1.4  Condensation of silicon alkoxides catalyzed by an acid.

Figure 1.5  Condensation of silicon alkoxides catalyzed by a base.

Further control of the surface chemistry of the silica shells can be achieved by both post-synthesis surface modifications and co-condensation reactions.\(^{21}\) Many functional groups can be attached to silica surfaces using silane chemistry. These functional groups include primary and secondary amine groups, as well as alkyl halides, azide, carboxyl, and hydroxyl groups.\(^{21}\) Amine-functionalized silica shells can be covalently attached to many biomolecules including nucleic acids, proteins, and peptides, as well as fluorescent dyes, small organic molecules, and other nanoparticles. These bonds with amine-functionalized surfaces can be achieved by coupling with carboxylic acids, alkyl halides, and \(N\)-hydroxysuccinimide esters that are present on the biomolecules.\(^1\) The coupling reactions typically require coupling agents to activate the functional groups during the coupling process. Examples of coupling agents include \(N\)-(3-dimethylaminopropyl)-N-
ethylcarbodiimide (EDC), diisopropyl carbodiimide (DIC), N-hydroxysuccinimide (NHS) esters, [N,N,N',N'-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate] (HBTU), N-succinimidyl 3-[2-pyridyldithio]-propionate (SPDP), and sulfo-succinimidyl-(4-N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC). Primary amino groups can also be coupled to aldehydes and ketone moieties to form Schiff bases; with isothiocyanates to form thiourea derivatives; and with metal complexes for chelation processes in the presence of a catalyst (e.g., Wilkinson’s catalyst). Thiol-functionalized silica shells can be prepared using thiolated silanes such as 3-mercaptopropyltriethoxysilane. Further coupling reactions to antibodies, fluorescent molecules, peptides, and proteins first require thiol functionalization on these molecules followed by subsequent reduction reactions with the thiol groups on the surfaces of the silica shells to form redox-active disulfide bonds. Maleimide-functionalized biomolecules can also link to the thiol-functionalized silica surfaces via the formation of thioether bonds. Previous reports have also shown the surface modification of azide-functionalized silica nanoparticles using alkyne substituted species through click chemistry.1

The diversity of available silane-based surface modifications for silica-based surfaces greatly enable silica nanoparticles and gold nanoparticles coated with a silica shell to be used for applications across many fields. The challenges of using silane-based surface modifications include the extensive procedures required for these reactions, the susceptibility of silane molecules to side reactions and their sensitivity to environmental conditions (e.g., moisture), as well as their robustness upon interacting with silica-based surfaces.23 Alternative approaches to modifying silica-based surfaces can offer opportunities to simplify the surface modification procedures, as well as to improve the robustness of the surface-bound species.

1.1.2. Applications of Silica-Coated Gold Nanoparticles

A silica coating on gold nanoparticles retains the large surface area to volume ratio of the nanoparticles, offers biocompatibility, and gives access to a large selection of available silane-based surface modifications that are discussed in the previous section. With these qualities, silica-coated gold nanoparticles have been incorporated into many biomedical applications. These applications span across fields that include biosensing,24,25 cell imaging,26,27 drug delivery,28,29 photothermal therapy,8,30 and photoacoustic imaging.31,32
1.2. Introduction to Gold Nanoparticles

Noble metal nanoparticles interact with light in unique ways that are distinct from properties of their respective bulk materials. The physical and chemical properties of matter are primarily determined by the type of motion its electrons can accomplish. The electronic motion is determined by the type of material and by the space that is accessible to its electrons (i.e., the degree of confinement). As the size of matter decreases to below the electron mean free path (e.g., ~40-50 nm in bulk gold) or the distance an excited electron travels between collision events with the lattice, its electrons are confined to the nanometer scale. Electronic motion becomes restricted due to this confinement, and new/enhanced properties of the material can appear. In addition, the emergence of new properties also depends on the overall shape of the material. An example of such properties is the nanoparticles’ radiative properties, such as absorption and scattering.

The oscillating electromagnetic field of an incident light can induce a collective coherent oscillation of the free electrons within the noble metal nanoparticles. The size of these nanoparticles ranges from 1 nm to 100 nm. The energy of the incident light of interest is within the range of visible to near infrared (NIR) regions of the electromagnetic spectrum. The induced oscillation of the electrons at the surfaces of the nanoparticles results in a charge separation with respect to their atomic lattice. This charge separation creates a dipole that oscillates in the direction of the electric field of the incident light. The amplitude of the electron oscillation reaches a maximum at a specific frequency, which is referred to as the localized surface plasmon resonance (LSPR). In addition, the effects of the LSPR can result in an enhancement of the electric field component of the incident light. As a result of this effect the radiative properties of noble metal nanoparticles, including light absorption, fluorescence, Rayleigh scattering, and Raman scattering, can be enhanced by a few orders of magnitude. The strength of the propagating surface electromagnetic fields around the nanoparticles decays exponentially as a function of distance from the surfaces of the nanoparticles and becomes negligible at a distance comparable to the size of the nanoparticles (or less). The properties (e.g., Raman scattering, photoisomerization rate, and radiative and nonradiative relaxation rates) of chemical and biological species can be, therefore, influenced and/or altered when brought within the distance of the enhanced fields at or near the surfaces of the
Noble metal nanoparticles’ ability to modify the properties of other materials in close proximity to their surfaces has created a focus of their study across many fields as they are sought to be incorporated into diverse applications.\textsuperscript{1,34}

1.2.1. Properties of Gold Nanoparticles of Different Shapes

Gold structures of the sub-100 nm size regime possess unique properties that are distinct from bulk gold materials. Since Michael Faraday’s discovery of “fine particles” from treating aqueous solutions of gold (III) chloride with phosphorus dissolved in carbon disulfide in 1857,\textsuperscript{36} nanometer-sized structures of gold have been prepared and studied by many scientists including Nobel Laureates in Chemistry, Richard Zsigmondy and Theodor Svedberg.\textsuperscript{37} The chemistry and physics of gold nanoparticles of various shapes and sizes have since become focuses of subdisciplines including the fields of colloids and surface chemistry.

The most unique characteristic of colloidal Au NPs, or Au NPs uniformly suspended in a solvent, is their interaction with light. Gustav Mie first established the relationship of the intense red color of Faraday’s samples under white light illumination by solving Maxwell’s equations, which explained the apparent red color of these solutions that result from absorption and scattering of light by the gold nanoparticles.\textsuperscript{38} In general, as the size of a noble metal is reduced to tens of nanometers, a beam of incident light of an appropriate energy can trigger the collective oscillation of the free electrons of the material at the surfaces of these materials at the same frequency as the incident light. This phenomenon of triggered electron oscillations is described as the coupling between electronic properties of the nanoparticles and the incident electromagnetic radiation, also termed the LSPR of the nanoparticles.

Any nanoscale material with an adequately high electron density can possess the LSPR property. These materials include noble metals, especially gold and silver, and doped semiconductors.\textsuperscript{34,37,39,40} The LSPR bands of gold, silver, and copper nanoparticles are the most interesting of all plasmonic materials for our studies, because their LSPR bands are located in the visible and NIR regions of the electromagnetic spectrum.\textsuperscript{41,42} In the 700 nm to 1100 nm range of the electromagnetic spectrum, low absorption of light from water and blood, as well as low scattering from soft tissues have been identified,\textsuperscript{43} making them especially attractive for applications in the biological and biomedical fields.
In addition to the inherent photostability and chemical inertness of Au NPs, their optical properties can be easily manipulated in a number of ways. These include controlling particle size, shape, structure and morphology, as well as the dielectric environment around the nanoparticles dictated by surfactants and solvent. These parameters have been extensively investigated in the research community and a high degree of control is most commonly achieved during the growth phase of the nanoparticles via solution-phase methods.

The shapes of gold nanoparticles that have received the most attention are spheres, rods, nanocages, and nanoshells. Gold nanospheres are simple to prepare with a high level of uniformity and reproducibility. Protocols have been developed to prepare them in a wide range of sizes with their LSPR bands occurring between 500 nm and 600 nm. Gold nanorods, on the other hand, possess two LSPR bands. One originates from the electronic motion along the long (longitudinal) axis of the nanorods and the other from that of the short (transverse) axis. Although they are more challenging to prepare compared to spherical Au NPs, the tunability of their dimensions or aspect ratio (length to width ratio) offers the ability to tune the LSPR during their synthesis across the electromagnetic spectrum. By carefully modifying the concentrations of key reagents and the size of the seeds, Au NRs can be synthesized with a longitudinal LSPR positioned across the visible and NIR regions of the optical spectrum (e.g., from ~600 nm to > 1200 nm). Gold nanocages are cubic structures with a hollow interior prepared from a galvanic replacement reaction using silver nanocubes as a sacrificial template. The LSPR of gold nanocages are also highly tunable, from ~450 nm to ~900 nm. Gold nanoshells are prepared via chemical reduction reactions on the surfaces of silica or polymer particles (e.g., polystyrene colloids). Their optical properties are determined by the ratio of the diameter of the particle to the thickness of the gold shell. Their LSPR is located in the visible region of the spectrum, but is generally less prominent than that for the other types of Au NPs discussed above.

The studies in this thesis involve the preparation and subsequent use of spherical and rod-shaped Au NPs. Two of the widely used preparation methods for these shapes of Au NPs are the citrate reduction method for spherical Au NPs, and the seed-mediated surfactant-directed growth approach for Au NRs. The following discussion introduces these two synthetic methods.
1.2.2. The Preparation of Spherical Gold Nanoparticles Using the Citrate Reduction Method

Spherical gold nanoparticles are commonly prepared using the citrate reduction approach. This method is based on the modifications made by Turkevich et al. in 1951 from Hauser and Lynn’s work reported in 1940. Since the Turkevich report, this synthetic scheme has been extensively studied and further optimized. As a result, the mechanism of the process, as well as the role of the components of the system have been substantially discussed in the literature. The size of the spherical Au NPs prepared using this method can be tuned from ~10 nm to ~200 nm. A typical synthesis of spherical gold colloids involves the reduction of gold (III) chloride trihydrate using sodium citrate tribasic dihydrate in an aqueous solvent. The role of sodium citrate tribasic dihydrate, or trisodium citrate in short, is not, however, limited to serving only as a reducing agent.

Trisodium citrate serves three roles in the preparation of spherical Au NPs: (i) reducing agent for the conversion of Au(III) to Au(0); (ii) surface protecting groups (or ligands); and (iii) a pH mediator. The process of this synthesis can be described in three stages. First, the formation of nuclei by fast nucleation, which is accompanied by a slow reduction of Au(III) to Au(0) via coalescence or Ostwald ripening growth mechanisms. Second, the nuclei grow through a “growth by diffusion” model in which the size of the nuclei increases with a decreasing polydispersity and a constant number of particles. Third, the reaction proceeds with further growth into nanoparticles. The reduction of Au(III) can be viewed as a step-wise process as well, such as outlined by Equations 1.1 to 1.4 below. Briefly, tetrachloroauric acid dissociates into AuCl$_4^-$ ions in aqueous environments, followed by substitutions of the Cl$^-$ by OH$^-$ at pH 6. The resulting metallocomplex is shown in Equation 1.2. This complex is capable of coordinating with the carboxylate group of the citrate (Equation 1.3), which then undergoes decarboxylation, shown in Equation 1.4.

\[
\text{HAuCl}_4 + 2\text{H}_2\text{O} \rightleftharpoons [\text{AuCl}_2(\text{OH})_2]^+ + 3\text{H}^+ + 2\text{Cl}^- \quad (1.1)
\]

\[
[\text{AuCl}_2(\text{OH})_2]^+ + [\text{C}_6\text{H}_5\text{O}_7]^{3-} \rightarrow [\text{AuCl(OH)}_2\text{C}_6\text{H}_5\text{O}_7]^{3-} + \text{Cl}^- \quad (1.2)
\]

\[
[\text{AuCl(OH)}_2\text{C}_6\text{H}_5\text{O}_7]^{3-} \rightarrow [\text{Au(OH)}_2]^+ + [\text{C}_5\text{H}_4\text{O}_5]^+ + \text{CO}_2 + \text{Cl}^- \quad (1.3)
\]
\[
\text{[Au(OH)\textsubscript{2}]^{-}+[C\textsubscript{5}H\textsubscript{4}O\textsubscript{5}]^{-}\rightarrow\text{Au}^{0}+2\text{OH}^{-}+2\text{CO}_{2}+[C\textsubscript{3}H\textsubscript{4}O]^{-}} \quad (1.4)
\]

As a surfactant, citrate anions are known to coordinate to the surfaces of gold nanoparticles through their carboxylate groups via inner-sphere complexation. Here, the anions directly interact with the gold surfaces without intervening water molecules.\textsuperscript{77} Specifically, dihydrogen citrate anions (H\textsubscript{2}Citrate\textsuperscript{-}) adsorb onto the gold surfaces by a \(\eta^2\)-COO\textsuperscript{-} bridging coordination mode from the central carboxylate group (Figure 1.6).\textsuperscript{78} Here, the \(\eta^2\) coordination mode refers to the two oxygen atoms in the central carboxylate group both being coordinated to a gold atom on the surfaces (Figure 1.7a). Each adsorbed citrate also interacts with adjacent citrate anions by hydrogen bonding between their terminal carboxylic acid groups. An adsorbed citrate anion can form cyclic COOH dimers with dangling anions and acyclic COOH dimers with adjacent adsorbed anions (Figure 1.7a).\textsuperscript{78} Dangling anions refer to those that are not in direct contact with the gold surfaces. Disordered citrate anions can also be observed at the gold surfaces. One proposed configuration of such disordered citrate anions includes a \(\eta^2\)-COO\textsuperscript{-} coordinated central carboxylate group, a terminal \(\eta^1\)-COO\textsuperscript{-} group on side, and a non-interacting terminal COOH group on the either side (Figure 1.7b).\textsuperscript{78} As the hydrogen-bonded H\textsubscript{2}Citrate\textsuperscript{-} anions gather at the surfaces, they are hypothesized to form one-dimensional chains, which collectively become SAMs through van der Waals interactions between neighbouring CH\textsubscript{2} moieties. The thickness of the citrate layer is estimated to be 8 to 10 Å, including the length of the Au-COO\textsuperscript{-} bond. The three most common facets on the gold nanoparticle surfaces that bind to citrate anions are the (111), (110), and (100) facets, with Au(111) being the most favored facet that has the highest surface coverage by citrate anions.\textsuperscript{78}

*Figure 1.6*  The chemical structure of sodium citrate tribasic dihydrate.
Figure 1.7 A proposed mechanism of a unit of citrate trimer coordinated at the surfaces of a Au NP. Configurations of ordered (a) and disordered (b) citrate anions are shown. The red bonds indicate locations of hydrogen bonding with an adjacent citrate anion.

1.2.3. The Seed-Mediated Synthesis of Gold Nanorods in a Binary Surfactant System

The synthesis of rod-shaped gold nanoparticles, or Au NRs, represent the first successful example of preparing plasmonic nanostructures with an anisotropic symmetry by solution-phase chemistry techniques. Seeded growth of Au NRs in the solution-phase was pioneered by Murphy et al. nearly two decades ago. These NRs have since become one of the most studied colloidal plasmonic nanomaterials because of its remarkable longitudinal LSPR properties that can be tuned across the visible and NIR regions of the electromagnetic spectrum. The seed-mediated synthesis method involves the use of a “soft template”. In this case a cationic surfactant, cetyltrimethylammonium bromide (CTAB), plays a crucial role of directing the formation of rod-shaped structures.

An important property of some surfactant molecules is that at concentrations higher than their critical micelle concentration in an aqueous environment, they form micelles through the self-assembly process. Some cationic surfactants are known to induce rod-like or wormlike micelles at high concentrations. Alkyltrimethylammonium halides and alkylpyridinium halides are two examples of such surfactants. A 16-carbon alkyltrimethylammonium halide with a bromide counter ion (i.e., CTAB) is the most
commonly used surfactant for the synthesis of gold and silver rod-shaped nanoparticles. Ion density profiles constructed from force field-based molecular dynamics simulations showed that on the Au(111) surface the bromide ions (Br\(^-\)) form two layers at the gold surfaces, with a higher number of anions in the layer closer to the gold. The Br\(^-\) species also can also directly adsorb onto the gold surfaces and compete with neighbouring water molecules adsorbed within the first layer.\(^8\) In comparison, chloride ions (Cl\(^-\)) prefer to remain in solution and the surface ion density of Cl\(^-\) (~0.26 ions nm\(^{-2}\)) is relatively negligible with respect to that for Br\(^-\) (~2.06 ions nm\(^{-2}\)). On the other hand, iodide ions (I\(^-\)) interact with the gold lattice in a nearly covalent fashion, leading to weakened Au-Au interactions and increased mobility of the outermost atoms on the surfaces of the nanoparticles.\(^8\) The result of this effect during the formation of the gold nanorods is a significantly reduced amount of rod-shaped structures, and an increased yield of triangular prisms, decahedrons, and truncated tetrahedrons.\(^8\) Many efforts have been made to refine the original recipe of the synthesis of Au NRs to achieve a high degree of monodispersity with negligible shape impurities. One of the approaches was based on an improved control of the micellization behavior of CTAB using derivatives of salicylic acid.\(^5\) Salicylic acid (SA) contains a hydrophobic benzene ring that enables it to associate with the hydrophobic alkyl chains of CTAB. The CTAB molecules themselves form a bilayer over the surfaces of the gold nanorods with the alkyl chains in the center and the polar headgroups either attached to the gold surfaces or interacting with the aqueous solution.\(^5,8\) Proton (\(^1\)H) nuclear magnetic resonance (NMR) studies of Au NRs synthesized with a surfactant mixture of CTAB and 5-bromosalicylic acid which demonstrated that: (1) the 5-bromosalicylic acid was imbedded in the hydrocarbon region of the CTAB assemblies; and (2) the polar substituents of 5-bromosalicylic acid, such as the carboxyl and hydroxyl groups, projected away from the hydrophobic alkyl chain region of the CTAB and into the aqueous environment. This suggested that the aromatic species can be found at the interfaces of the CTAB bilayers.\(^5\)

The intercalation of these aromatic species results in changes to the micellar packing parameter of the CTAB micelles:

\[
p = \frac{v}{A \times l} \quad (1.5)
\]
where $v$ is the effective volume of the alkyl chain, $A$ is the effective area of the polar headgroup, and $l$ is the length of the alkyl chain. The presence of the phenyl moiety from the salicylic acid in the bilayer can increase $v$, but the carboxyl groups help to reduce the electrostatic repulsion among the quaternary ammonium headgroups and hence reduce $A$. The overall effect is a more dense packing of the CTAB bilayer, as well as an easier micellar transition from spherical to rod-shaped soft templates.

![Figure 1.8](image.png)

**Figure 1.8** A schematic of the synthesis of gold nanorods (Au NRs). This illustration is not drawn to scale.

A typical synthesis of the Au NRs prepared in this thesis is outlined in Figure 1.8. This procedure was adapted from the report discussed above with a reduced total growth solution volume from ~500 mL/synthesis to ~50 mL/synthesis. Prior to the preparation of the growth solution, gold seeds were first prepared in a separate reaction vessel. These seeds were formed by reducing a gold precursor (HAuCl$_4$) using sodium borohydride in the presence of the CTAB surfactant. The majority of the seed particles would have a diameter of less than 2 nm. Next, a series of precursors were added sequentially into the growth solution containing CTAB and SA, while keeping the reaction flask submerged in a water bath at 30 °C (Figure 1.8). This temperature control is necessary for preventing crystallization of the CTAB.

The growth of the Au NRs begins simultaneously in all directions, but with different rates of growth. The longitudinal growth takes places in the [001] direction and has been proposed to take place in five stages. The first proposed stage is characterized by the isotropic growth of the seed particles with facets {100}, {110} and {111} until reaching a diameter of approximately 6 nm during the first two minutes. As the area of the facets becomes comparable with the diameter of a CTAB micelle (~2.9 nm), the micelles in solution begin adsorbing onto the gold surfaces, preferring to adhere to the {100} facets.
over the \{111\} facets. This preference is due to energy differences between the facets upon adsorption of micelles. The \{100\} facets become increasingly coated by CTAB micelles in comparison to the \{111\} facets, which become more exposed for further attachment of gold atoms. This increased population of CTAB on the \{111\} facets is considered to be the second stage, during which anisotropic growth is initiated and rod-like shapes are formed (e.g., at 2 to 5 min). These rods are bound by alternating \{100\} and \{110\} planes along their longitudinal sides.

In the next proposed stage, rapid rod growth continues in a non-uniform fashion (e.g., 5 to 20 min): longitudinal growth gradually slows and becomes outcompeted by growth of the diameter of the nanorods. This increased growth in diameter results from adatom (an atom located on the surfaces of a crystalline material) addition at the boundaries between \{111\} and the alternating \{100\} and \{110\} facets: the migration of the adatoms on the \{111\} facets to the \{100\} or \{110\} facets. The result of this growth stage is the formation of dumbbell shaped nanoparticles.

Stage four is characterized by reconstruction of the side facets along the length of the nanorods at 20 to 45 min into the reaction. The growth rate of the nanorod further decreases due to consumption of the reducing agent, ascorbic acid. Meanwhile, CTAB micelles continue to adsorb onto the nanorods and rearrange to form more uniform bilayers. The increased CTAB coating also further prevents gold atoms (Au\(^0\)) from accessing the \{100\} and \{110\} facets. At the ends of the nanorods, the newly deposited gold atoms also rearrange from the \{111\} facets to the \{100\} facets or \{110\} facets due to the lower surface energy of the latter two facets. The result of this arrangement is truncated square prism shaped structures at the end of this growth stage.

In the final growth stage, the relatively thermodynamically unfavorable \{110\} facets of the nanorods slowly evolve into the more thermodynamically stable facets via an intermediate stage that is consisted of \{120\} facets (Figure 1.9 a and b) through continuous adatom migration (e.g., at 45 min and up to 2 weeks). The final form of the nanorods is made up of \{250\} facets surrounding all the longitudinal sides with an octagonal cross-section (Figure 1.9 a and c). The reason that the final form adopts the \{250\} facets, and not the most thermodynamically stable \{100\} facets, is that the more open and accessible structure of the \{250\} facets can allow greater local structural flexibility, and the more rounded facets help to decrease the stress of the single-crystalline rod structures.
ends of the nanorods in their final form contains alternating \{110\} and \{111\} facets, with a preference towards the more thermodynamically stable \{111\} facets.

Figure 1.9 The facets of a Au NR during its surface reconstruction at the end of its growth process. (a) An atomic model of the evolution from a \{110\} surface to a \{250\} surface through a \{120\} intermediate stage. The same evolution is depicted from a view of the cross-section of a Au NR (b and c).

The fully grown Au NRs possess a positive surface potential.\cite{88,89} The CTAB bilayer is difficult to be completely displaced from the surfaces of these Au NRs.\cite{90,91} Ligand exchange using thiolated compounds,\cite{1,92} and encapsulation using silica\cite{18,93} or polymers\cite{94,95} are two approaches to modify the surface chemistry of the CTAB coated Au NRs. The challenges associated with working with gold-thiol chemistry were discussed in section 1.1. In this thesis, we pursued the direction of encapsulating Au NPs using a silica shell to overcome the challenges associated with gold-thiol chemistry. The surface modification method demonstrated here also aims to provide an alternative approach to the silane-based surface modifications on silica-based surfaces, with a relatively shorter reaction time, a reduced reaction sensitivity to environmental factors including ambient moisture in the laboratory, and at a reduced cost of reagents.
1.3. Objectives of the Thesis

This thesis aims to demonstrate an alternative method to the surface modification of silica-coated gold nanoparticles with spherical and rod-like shapes through a microwave-assisted alcohol condensation reaction. The syntheses, surface modifications, characterization, and applications of both types of nanoparticles are the components of the thesis. The aim of these studies is to build a foundation for the covalent surface modification of silica-based core-shell nanoparticles with alcohol containing reagents. Such covalent surface modifications of planar silica surfaces using functional alcohols was previously demonstrated with the use of microwave radiation for this process. This approach was shown to be highly effective for the formation of monolayers derived from alcohol-based reagents on planar silica surfaces.

The work in Chapters 3 and 4 shows that microwave heating is also effective in modifying the surfaces of nanoparticles through facilitating the alcohol condensation reaction. Chapter 3 also includes a comparison to the use of a heated oil bath to carry out these surface modifications, which exhibited a much slower progress for the same reactions. To further verify the modified surface chemistry and the utility of these nanoparticles, a fluorescent dye was coupled to the functionalized surfaces of the silica-coated gold nanoparticles in Chapter 3. The work discussed in Chapter 4 involves the microwave-assisted surface modification of silica-coated rod-shaped gold nanoparticles using an alcohol-based chelating agent. A subsequent metal chelation test demonstrated that metal ions can be effectively captured at the surfaces of these nanoparticles. Overall, this thesis intends to demonstrate successful surface modifications using alcohol condensation reactions on silica-based nanoparticles with the aid of microwave radiation or radiative heating achieved using an oil bath. Alcohol condensation reaction can potentially be applied to the attachment of any alcohol-based species onto silica-based nanoparticles, diversifying the types of functional species that are available for surface modifications of nanoparticles. This work also seeks to provide an alternative method for preparing a diverse range of functional nanoparticles at an efficient rate and a reasonable cost of reagents.
Chapter 2.

Characterization of Functionalized Silica-Coated Gold Nanoparticles and Nanorods

In this thesis, the preparation of the gold nanoparticles, as well as their subsequent surface modifications were monitored using a selection of tools. The nanoparticles’ optical properties, surface chemistry, elemental compositions, and colloidal stabilities were evaluated through these analyses. The details of these characterization techniques and the justification for their use for the intended studies will be discussed in this Chapter.

2.1. Extinction Spectroscopy Measurements

Extinction spectroscopy is one of the most convenient tools to characterize plasmonic nanoparticles. The studies presented in this thesis rely on this technique to assess the successful synthesis of Au NPs and their subsequent surface modifications. The surface plasmon resonance of the Au NPs is highly sensitive to changes in their size, shape, and surface chemistry, as well as the dielectric properties of the surrounding environment. Their resonance peak(s) respond to these changes by shifting spectral positions to higher or lower energies, as well as changing the shape [e.g., full-width at half-maximum (FWHM)] and intensity of the peak(s). As all the experiments were performed in the solution-phase, extinction spectroscopy measurements offered a convenient method to initially characterize the products. These measurements were performed on both the as-synthesized and surface modified gold nanoparticles while suspended in solution. Most other characterization techniques required extensive sample preparations such as exchange of solvent, isolation of the nanoparticles, and/or drying them on a substrate.

Extinction spectroscopy measures the total extinction of light for a transmitted incident beam of light. The extinction process is the result of energy loss following an interaction between an electromagnetic wave and matter. For Au NPs, energy loss can take the form of absorption and scattering. For spherical Au NPs of 20 nm in diameter, their total extinction is nearly all due to contributions from absorption processes. As the particle size increases to 40 nm, the scattering component begins to be more prominent.
and at 80 nm, the two components contribute nearly equally to the total extinction.\textsuperscript{96} In an extinction measurement of plasmonic nanoparticles, light absorption and scattering are greatly enhanced at the resonance frequency of their localized surface plasmon, resulting in an extinction maximum or a LSPR peak. This effect is particularly strong for gold and silver nanoparticles when compared to other metals. The LSPR peak intensity and spectral position depend on several factors that dictate the electron charge density on the surfaces of the nanoparticles. These factors include the type of metal, nanoparticle size, shape, morphology, composition, and the dielectric constant of the surrounding solvent. For spherical particles less than 20 nm in diameter, the LSPR enhanced extinction processes can be quantitatively explained by the Mie theory,\textsuperscript{38,96,97}

\[
C_{\text{ext}} = \frac{24\pi^2 R^3 \varepsilon_m^{3/2}}{\lambda} \frac{\varepsilon_i}{(\varepsilon_r+2\varepsilon_m)^2+\varepsilon_i^2} \tag{2.1}
\]

where \( C_{\text{ext}} \) denotes the extinction cross-section, which is determined by \( \varepsilon_m \), the dielectric constant of the surrounding medium; \( \varepsilon_m \) is related to the refractive index of the medium through \( \varepsilon_m = n_m^2 \). The radius of the nanoparticles \( R \), the wavelength of the incident light \( \lambda \), and the complex dielectric constant of the metal \( \varepsilon \) are the factors that make up the extinction cross-section, and \( \varepsilon = \varepsilon_r(\omega) + i\varepsilon_i(\omega) \), where \( \varepsilon_r(\omega) \) is the real part and \( \varepsilon_i(\omega) \) is the imaginary part of the dielectric function of the metal.\textsuperscript{96,97} The spectral position of the LSPR band is determined by the real part of the dielectric constant of the metal, and the maximum extinction of the LSPR band occurs at approximately \( \varepsilon_r \approx -2\varepsilon_m \).

For noble metal nanoparticles, their LSPR bands arise within the ultraviolet (UV), visible, and NIR regions of the electromagnetic spectrum. Hence, the LSPR properties of these nanoparticles can be conveniently assessed using a UV-Visible spectrophotometer. When an incident light of intensity \( (I_0) \) passes through a colloidal suspension of Au NPs with a path length of \( l \), the intensity decreases to \( I \).\textsuperscript{37} The attenuation of light can be written as \( \log \left( \frac{I_0}{I} \right) = \mu_{\text{ext}} l M_p \), where \( \mu_{\text{ext}} \) is the molar extinction coefficient \( (\text{L mol}^{-1} \text{ cm}^{-1}) \) of the colloidal suspension, \( l \) is the path length of the incident light \( (\text{cm}) \), and \( M_p \) is the molar concentration of the Au NPs \( (\text{mol L}^{-1}) \).\textsuperscript{37} The molar extinction coefficient is related to the extinction cross-section \( (C_{\text{ext}}) \) of an Au NP through \( \mu_{\text{ext}} = N_A C_{\text{ext}} \), where \( N_A \) is the Avogadro constant.\textsuperscript{37} For measurements of colloidal suspensions of Au NPs, the quantity measured by the UV-Visible spectrophotometer over a range of wavelengths is, therefore,
referred to as the extinction of the Au NPs by convention as it includes the absorbance and scattering properties of these materials. Spherical Au NPs exhibit a single LSPR band at ~ 520 nm in the visible region of the electromagnetic spectrum. For diameters in the range of 10 to 100 nm, an increase in particle size results in a red shift of the LSPR band. An increase of $\varepsilon_m$ also leads to a red shift of the LSPR, as well as an increase in the LSPR band intensity and bandwidth. Nanoparticles with diameters smaller than 10 nm experience dampened LSPR effects due to the transfer of absorbed energy from the surface plasmon to the excitation of individual electronic states of the free surface electrons of the nanoparticle. The chance of scattering of the incident light decreases as these nanoparticles decrease to a particle size of < 10 nm, because the diameter of the particle is much smaller than the wavelength of the incident light. On the other hand, nanoparticles with a size of >100 nm begin experiencing significant band broadening because of increased multipole modes from higher-order electron oscillations, as well as light scattering.

An interesting case of the LSPR of plasmonic nanoparticles is that of the rod-shaped Au NPs, or Au NRs, which have two extinction maxima in their extinction spectrum. Gold nanorods have one strong LSPR band in the NIR region from electron oscillations along the longitudinal axis, and one weak band in the visible region due to the transverse electron oscillations (Figure 2.1). The longitudinal band is particularly sensitive to changes among the factors mentioned above, and responds by shifting its spectral position rather dramatically in comparison to the LSPR responses from spherical Au NPs. For example, the longitudinal band of Au NRs can red shift up to 300 nm with an increasing aspect ratio from ~2.4 to ~8.5.
Figure 2.1 A typical extinction spectrum of gold nanorods (Au NRs) suspended in water.

2.2. Transmission Electron Microscopy

A transmission electron microscope uses the interactions between accelerated incident electrons and the sample to provide information on its morphological, compositional and crystallographic properties. The negatively charged electrons with their relatively low mass can be deflected while traveling past the electrons and nuclei of atoms in the sample. This phenomenon can be explained by Louis de Broglie’s description of the wavelength of physical particles, such as electrons:

$$\lambda = \frac{h}{p} = \frac{h}{mv} \quad (2.2)$$

where $\lambda$ is the wavelength of electrons in nm; $h=6.626 \times 10^{-34}$ J·s is the Planck constant; $p$, $m$, and $v$ are the momentum, mass, and velocity of the electron, respectively. When relativistic effects are neglected, the wavelength of electrons can be approximated according to this equation:

$$\lambda = \frac{1.22}{E^{1/2}} \quad (2.3)$$

where $E$ is the energy of the electron in electron volts (eV). At an accelerating voltage of 100 kV under vacuum, a heated filament can emit electrons at a wavelength of $\sim 4$ picometers (pm). The emitted electrons interact with nuclei and other electrons in the
sample, resulting in scattering and diffraction of these electrons. The electron scattering process is also called Coulomb or electrostatic interactions. These Coulomb (electrostatic) interactions of electrons and other charged atoms are the basis of transmission electron microscopy.  

The transmission electron microscope used to acquire images and related data in this thesis was operated at an electron accelerating voltage of 200 kV. This is considered to be an intermediate voltage. The motivation for using 200 kV accelerating voltage is that the higher the accelerating energy of electrons, the higher their momentum. As a result, a higher electron momentum is associated with a shorter wavelength of these electrons. The benefit of a shorter wavelength of electrons from the source is a reduced diffraction limit and improved spatial resolution. On the other hand, electron accelerating voltages higher than 300 kV are considered to be relatively high voltages and are only appropriate for thicker specimens (≥ 1 μm), because electrons of “ultra” high energy can penetrate deep into the samples without excessive scattering. Samples commonly analysed by the transmission electron microscope, including the nanoparticles studied in this thesis (diameters from ~20 to ~50 nm), have diameters much less than 500 nm. These samples will likely experience significant radiation damage, as well as knock-on damage (displacement of atoms from the crystal lattice) if exposed to an electron beam of 300 kV or higher accelerating voltages.

Transmission electron microscopy (TEM) images are generated from sending high-energy electrons from a source to interact with a sample held perpendicular to the path of the electrons in a vacuum column. The TEM images included this thesis were acquired using an electron microscope with a source consisted of a field emission gun made of Schottky emitters: a tungsten wire with a fine tip (radius < 0.1 μm) that is coated with a layer of zirconium oxide. The high energy electrons produced by the source are accelerated in a vacuum column, which is equipped with multiple electromagnetic and magnetic lenses, to generate a focused, collimated electron beam. This collimated electron beam is directed to interact with the samples, which are held on a piece of thin metal mesh commonly referred to as a TEM grid. The beam passes through an objective aperture and is directed to a detector located beneath the sample holder. Typical TEM grids are made of copper, nickel, or gold meshes. Support films of Formvar [poly(vinyl formal)] and carbon are used to coat these meshes so that the grids and samples can withstand the handling during sample preparation. The standard diameter of these grids
is 3.05 mm, and the thickness varies based on the type of metal that makes up the mesh and is in the range of 10 \( \mu \text{m} \) to 25 \( \mu \text{m} \). Following interactions of the incident electrons with the sample, the scattered and incident electrons that pass through the TEM grid are directed towards the detectors. An objective aperture positioned beneath the sample stage controls the collection angle of the scattered incident electrons. In particular, bright field (BF) TEM images are acquired when an objective aperture is positioned directly below the path of the original electron beam [Figure 2.2(a)], and dark field (DF) TEM images are acquired when the objective aperture is positioned away from the direct beam at an angle > 10 milliradian [Figure 2.2(b)].

![Figure 2.2](image)

Figure 2.2  A comparison between (a) bright field (BF) and (b) dark field (DF) imaging in the transmission mode of TEM, which is achieved by changing the position of an objective diaphragm. This scheme is a simplified illustration and is not drawn to scale.

A BF TEM image shows the samples as dark structures with a bright background, and a DF TEM image shows the samples as bright structures with a dark background. In BF TEM imaging, as the electron beam travels through the specimen plane and reaches the objective aperture, only those still traveling in the direction of the incident beam or nearly parallel to the incident beam direction will reach the BF detector, while most scattered electrons are blocked by the objective aperture.

The scattering of incident electrons by the sample forms the basis of contrast in TEM images. The incident electron wave can change in both amplitude and phase upon interacting with the sample and both types of changes can result in TEM image contrast. Amplitude contrast tends to be the dominate type and can be observed in both TEM and STEM modes, as well as in both BF and DF images. Amplitude contrast is generated when
variances in the mass (including atomic number, Z), thickness, or a combination of both occur in the sample. At thicker locations, incident electrons interact with more mass of the sample compared to thinner locations, hence more electrons will be potentially scattered and thus not be collected. Consequently, relatively more electrons will be ‘missing’ at thicker locations in the sample and the spatial distribution of relative intensity of incident electrons detected is reflected in the final image. A typical BF image contains dark structures with a contrast, in which darker regions represent a relatively greater sample thickness and lighter regions represent a smaller sample thickness at the corresponding locations.

One significant drawback of this technique is the projection-limitation of information. Transmission electron microscopy images are two-dimensional (2D) representations of three-dimensional (3D) specimens. The human eyes and brain can sometimes misinterpret artifacts from 2D images and, therefore, caution must be exercised when analyzing TEM results. One solution to counter this challenge is a technique called tilting. A series of TEM images can be acquired at various tilt angles of the sample with respect to the electron beam by rotating the sample holder and using post-imaging processing software to reconstruct a 3D image from the acquired 2D TEM images. As this method can be immensely labour intensive, time consuming, and costly, a simplified imaging analysis by tilting is included in Chapter 4.

High-energy incident electrons generate several different interactions with the sample (Figure 2.3). Many of these signals provide valuable information about the sample beyond the limits of imaging and can be utilized to form the basis of analytical electron microscopy (AEM). An example of an AEM technique, energy dispersive X-ray spectroscopy (EDS), will be discussed in the next section.
Figure 2.3 An illustration of the types of signals generated following the interactions between a beam of high-energy electrons and a thin specimen in an electron microscope. The signal directions are not always representative of the real directions of these signals, but show the general directions from where the resulting signals can be detected. The scheme is not drawn to scale.

2.3. Energy Dispersive X-Ray Spectroscopy

Energy dispersive X-ray spectroscopy is an AEM technique that combines imaging and spectroscopy in the electron microscope to provide an analysis by elemental mapping of the specimen. Energy dispersive X-ray spectroscopy is coupled with imaging in a scanning transmission electron microscopy (STEM) mode using a high-angle annular dark field (HAADF) detector. During the STEM image formation, an extremely focused electron beam, with a probe size of < 0.3 nm, is scanned across the specimen and the HAADF detector collects electrons scattered at angles > 50 milliradian (mrad). Figure 2.4 shows a simplified illustration of the positioning of the HAADF detector in the electron microscope, as well as the relative positioning of a BF detector and a conventional ADF detector. Following detection, the intensity of the electron signals is integrated and displayed as a function of the electron beam position. The advantage of using a HAADF detector is that only those electrons scattered at very high angles are detected. These high angles are highly dependent on the atomic number Z of the materials, hence this technique is sometimes also called Z-contrast imaging. The gold nanoparticles studied in this thesis can greatly benefit from using this imaging mode, as gold is a period 5 transition metal...
with a high atomic number of 79. The presence of gold in the sample can produce a high contrast in the HAADF-STEM imaging mode. As a comparison, the Formvar coatings on the TEM grids consist of mainly carbon and oxygen, which are both elements of relatively low atomic numbers of 6 and 8, respectively. Elements of lower atomic numbers (e.g., carbon, nitrogen, oxygen) produce a minimal contrast in HAADF-STEM images. In this way, the TEM grid will appear “transparent” in the microscope with only the copper grid frame visible.

![Schematic of detector setup](image)

**Figure 2.4** Schematic of detector setup for different types of imaging in a scanning transmission electron microscope. High angle annular dark field (HAADF) detector is positioned such that it collects scattered electrons at angles greater than 50 mrad. The positions of a conventional annular dark field (ADF) detector and a bright field detector are also shown for comparison. This scheme is drawn for illustration purposes and is not drawn to scale.

The other component of this AEM technique is the energy dispersive X-ray spectrometer. The output of this instrument are spectra of X-ray counts (intensity) plotted as a function of X-ray energies. There are two types of X-rays generated by the incident electrons (Figure 2.3), characteristic X-rays emitted by atoms upon ionization by the high-energy incident electrons, and a continuum of bremsstrahlung X-rays resulting from electrons being slowed by their interactions with the nuclei of elements within the sample. A typical EDS spectrum contains characteristic X-ray peaks, which are distinct
and Gaussian-shaped, superimposed on a continuous background of bremsstrahlung X-rays of lower intensity.

A characteristic X-ray is generated during the following process (Figure 2.5). First, high-energy incident electrons reach the atoms in the sample and penetrate through the outer conduction/valence bands to interact with the inner-shell electrons. Second, in the case where a minimum amount of energy or more is transferred from the incident electron to an inner-shell electron, the inner-shell electron is ejected and becomes a free electron. As this electron escapes the attractive field of the nucleus, an electron hole is created in the inner shell. The atom as a whole is now considered to be ionized. Third, the electron hole is filled with an electron from an outer shell, followed by emission of the excess energy in the form of an X-ray or an Auger electron. The remaining electron hole(s) are filled from electrons in the vicinity of the atom. If a material is an electrically insulating material, the charge neutralization can be limited, which leads to an apparent “charging” of the material. For conducting materials including noble metal nanoparticles, the filled electron hole(s) allow the atom to return to a neutral charge, and through the release of energy, the atom returns to its lowest energy or ground state. Importantly, both types of emissions are unique to the atom. The energy difference between two electron shells is characteristic and identifiable with respect to a specific atom number.100

As an alternative to X-ray emission, an ionized atom can return to its ground state by emitting Auger electrons instead (Figure 2.6). Following ejection of the inner shell electron, an electron from an outer shell fills the electron hole in the inner shell and the excess energy released by this electron is transferred to another outer shell electron of the same atom. Upon receiving the additional energy, the latter electron is no longer stable in its place and is subsequently ejected. The ejected electron here is an Auger electron, which carries an energy that equals to the difference between the incident electron energy and the binding energy of the outer shell from which the Auger electron is ejected.100
Figure 2.5  The ionization of an atom and generation of characteristic X-rays. Upon impact from a high-energy incident electron, an inner (K) shell electron is ejected, leaving an electron hole behind. An electron from an outer shell (i.e., L) fills the hole accompanied by characteristic X-ray emission (in this diagram, Kα). From these events, the incident electron loses some of its energy, but continues to travel through the sample.

Figure 2.6  The ionization of an atom followed by emission of an Auger electron. An inner (K) shell electron is ejected after interacting with an incident electron. An outer (L1) shell electron fills the electron hole left by the ejected electron and releases energy. The released energy is transferred to an outer (L3) shell electron, which is ejected as an Auger electron.
When an EDS measurement is performed in the STEM-HAADF mode, the information from HAADF imaging and the energy dispersive X-ray spectrometer are overlaid to create a map of the elemental distribution. Specifically, a map showing the locations of all elements detected in the sample can be displayed using false coloring to represent different elements. Furthermore, the spatial distribution of a single element with respect to its local concentration across the imaging area can also be displayed using a single color combined with varying levels of color intensity to differentiate signal intensity, or contrast. In this thesis, a metal chelation study on functionalized silica-coated gold nanorods utilized this AEM technique. The sensitivity and resolution of the instrument enabled an assessment of the potential chelation of calcium ions at the surfaces of silica-coated Au NRs, which will be discussed in Chapter 4.

2.4. Surface-Enhanced Raman Scattering Spectroscopy

Surface-enhanced Raman scattering (SERS) spectroscopy is a technique that utilizes the properties of plasmonic nanostructures. Similar to Raman scattering, SERS collects signals from the inelastic scattering events following the interactions between an incident monochromatic excitation and the sample. The electrons of Raman active analytes are excited from the ground state to the vibrational levels by the incident photons. A small fraction of these incident photons are scattered such that they experience an energy shift during the relaxation process and do not return to the same ground state energy level. These energy shifts correspond to particular vibrational modes of chemical bonds in the analyte. As the inelastic scattering process is rather inefficient, SERS spectroscopy requires a high energy light source. Lasers with excitation wavelengths from the UV to visible and NIR regions are standard excitation sources for SERS spectroscopy. As the LSPR of nanoparticles can generate large electric fields at their surfaces upon excitation with the incident light, the electric field can couple to the vibrational modes of the Raman active species in the sample and enhance the intensity of the characteristic Raman signals of the analyte, increasing the sensitivity of the Raman measurements. The most attractive aspects of this technique for the studies involved in this thesis are: (1) the minimal amount of sample required for a measurement (micromolar particle concentrations); and (2) straightforward sample preparation.

The mechanism of SERS is based on the electromagnetic properties of plasmonic nanoparticles. The electromagnetic enhancements of Raman signals can reach up to
Laser excitation from the Raman spectrometer triggers the LSPR effect, which are large electromagnetic fields localized at the surfaces of the nanoparticles. Meanwhile, the analyte (Raman active molecules) scatter the incident light and generate an intensity that is proportional to the square of the magnitude of the incident field ($E_0$). If the analytes are near the nanoparticles, the field can become enhanced by the LSPR,

$$|E_{out}|^2 = 4E_0^2 \left( \frac{\varepsilon_{metal} - \varepsilon_{env}}{\varepsilon_{metal} + 2\varepsilon_{env}} \right)^2$$

where $E_{out}$ is the electromagnetic field resulting from the incident excitation, $\varepsilon_{metal}$ is the dielectric constant of the nanoparticles, and $\varepsilon_{env}$ is that of the surrounding environment.

Another mechanism of SERS is the chemical or charge-transfer mechanism at the surfaces of plasmonic nanoparticles. Upon excitation, charge transfer can take place between the Fermi level of a metal nanoparticle and the lowest unoccupied molecular orbital (LUMO) of the adsorbed analyte at the nanoparticle surfaces. Additionally, this mechanism can also cause a change in the vibrational behavior of the analyte molecule due to potential alterations in its molecular symmetry, molecular orientation at the nanoparticle surfaces, as well as the polarization of light at the surfaces of the nanoparticles.

Surface-enhanced Raman scattering measurements were used in Chapter 3 to assess the progress of surface functionalization of silica-coated Au NPs by a microwave-assisted approach. The SERS band of the functional group on the molecule to be attached to the nanoparticles was monitored with respect to reaction time. Besides SERS, identification of organic functional groups on the surfaces of nanoparticles can also be indirectly accomplished by extinction spectroscopy measurements. Changes to the surface chemistry of nanoparticles during a surface modification reaction cause a shift in the nanoparticles’ LSPR in the spectrum. The choice of SERS technique was due to the fact that the SERS measurements can provide information on changes in surface chemistry, as well as the identity of the functional groups present on the surfaces of nanoparticles following these changes.
2.5. Fluorescence Spectroscopy

Fluorescence spectroscopy is a tool most commonly used in analytical chemistry and cell biology.\textsuperscript{109,110} The technique has been extensively developed to the stage of being capable to visualize biological processes and to detect specific biologically relevant molecules in tissues and living animals.\textsuperscript{109,111} A fluorescent molecule contains a reporter component or fluorophore for fluorescence emission, and can also contain a recognition unit for association to a region of interest. The absorption of a photon of appropriate energy results in a number of photophysical events that are summarized in Jablonski diagrams (Figure 2.7). Briefly, these events take place in three stages.\textsuperscript{111,112} First, an energy source irradiates the sample with photons of an energy $h\nu_{ex}$. Second, the fluorophore absorbs the incident photon energy and an electron is excited into an electronic singlet state ($S_1'$). The duration of the $S_1'$ state is on the time scale of femto- to picoseconds. In this excited state, the fluorophore undertakes conformational changes and experiences potential interactions with its molecular environment. These events result in a partial dissipation of energy that is associated with the $S_1'$ state, leading to a relaxed singlet excited state ($S_1$). The excited electrons of the fluorophore typically spend ~1 to 10 nanoseconds in the $S_1$ state. Third, fluorescence is emitted from the $S_1$ state, accompanying the fluorophore’s return to its ground state ($S_0$). It is important to note that this is not true for every excited electron. Some of them return to the ground state via other means, including collisional quenching, fluorescence resonance energy transfer (FRET), and intersystem crossing. The energy of one fluorescence emission event can be described as $h\nu_{em}$, which is lower than the incident photon energy $h\nu_{ex}$. The energy difference between $h\nu_{em}$ and $h\nu_{ex}$ is due to energy dissipation in the $S_1'$ state. Hence, the emission spectrum will exhibit a shift to longer wavelengths than that of the excitation spectrum.
The fluorescence lifetime, or the finite amount of time a fluorophore spends in the excited state before returning to the ground state, is a property of the fluorophore and can be determined from time-resolved photoluminescence measurements. The fluorescence lifetime of a fluorophore does not depend on the initial perturbation conditions such as the wavelength of excitation, whether it is a one- or multiphoton excitation process, and the duration to the incident light. Fluorescence lifetime is, however, critically dependent on the local environment surrounding the fluorescent molecule. In Chapter 3, this fluorescence spectroscopy technique was used to assess changes to the local environment of a fluorescent dye molecule when attached to the surfaces of silica-coated Au NPs. The goal of this study was to differentiate the surface-bound fluorescent molecules from those that were not bound because of the extreme sensitivity of fluorescence lifetime to external factors such as to changes in their microenvironment, polarity, temperature, and the presence of fluorescence quenchers.

Gold nanoparticles can absorb and scatter light at wavelengths that match with their LSPR, giving rise to strong electromagnetic fields at the surfaces of the nanoparticles due to photon confinement. As the strength of the electromagnetic field is the most intense at the surfaces of plasmonic nanoparticles, this property can be employed to electromagnetically couple the nanoparticles with fluorophores to generate additional de-excitation pathways following their excitation. This coupling is reflected in both the intensity of a steady-state fluorescence analysis and fluorescence lifetime analysis.
optimal outcome is enhanced excitation rates and/or radiative decay rates of the fluorophore, as well as fluorescence emission.\textsuperscript{114,115} The relaxation of excited fluorophores can, however, sometimes proceed through non-radiative energy transfer into the surface plasmon resonance, resulting in fluorescence quenching.\textsuperscript{116,117} The use of fluorescent molecules are commonly found in imaging and sensing studies for biological systems, but fluorescent molecules can also be challenging to work with due to their relatively low stability and quantum yield, as well as their sensitivity to photobleaching. These weaknesses are especially prominent in fluorophores emitting in the NIR region of the electromagnetic spectrum and have absorbance/emission profiles in the “water window” (700-1100 nm).\textsuperscript{114,118,119} As the LSPR of plasmonic gold nanoparticles can be tuned with ease within the NIR region, especially rod-shaped particles,\textsuperscript{54} plasmonic nanoparticle-coupled fluorophore systems are a potential solution to improve the usability and performance of such weak fluorophores.

A nanoparticle-fluorophore complex was produced by covalently linking fluorescent molecules to functionalized surfaces of silica-coated gold nanoparticles in Chapter 3. Fluorescence spectroscopy techniques were used to characterize the successful linkage between the two components and to compare these results with non-associated systems of nanoparticles and fluorophores.

Fluorescence spectroscopy studies, in combination with the rest of the characterization techniques discussed in this Chapter, allowed for the investigation and understanding of the properties of nanoparticles in this Thesis from multiple perspectives.
Chapter 3.

Alcohol Based Surface Functionalization of Silica-Coated Spherical Gold Nanoparticles

3.1. Notice of Permissions

A manuscript based on the work presented in this chapter is being prepared for publication. Mr. Rana Faryad Ali acquired the fluorescence decay data. The rest of the work presented here, including experiments, data acquisition and interpretation, and writing were performed by myself under the guidance of Dr. Byron Gates.

3.2. Introduction

This study demonstrates the surface modification of silica-coated Au NPs through a process that uses alcohol-based reagents. The post-synthesis surface modifications of Au NPs such as encapsulating them in thin (<30 nm) silica (SiO$_x$) shells provide these nanoparticles with enhanced stability, solubility in aqueous and in some organic solvents, and enables further surface functionalization using the silica shells as a platform. In this study, surface modified, silica-coated Au NPs were prepared as a platform for the attachment of a fluorescent species, CF647-amine, as a demonstration of the successful surface modifications of silica-coated Au NPs using alcohol-based reagents.

Gold nanoparticles are a class of the most extensively studied metallic nanomaterials for harnessing their light-matter interactions to develop a diverse range of probes and tools in biomedical applications. A selection of important applications for their respective subfields are summarized in Table 3.1. These applications are categorized into four types: (1) optical sensing; (2) cellular imaging; (3) loading and delivery of drugs; and (4) therapeutic uses. Specifically, optical sensing includes the detection of proteins, DNA, metal ions, and small molecules. The detection can be based on the photoluminescence of gold clusters, LSPR, SERS, and photoluminescence quenching. With respect to gold nanoparticles’ use in cellular imaging applications, the primary mechanisms include photoluminescence, elastic light scattering, inelastic light scattering, and photothermal conversion. In addition, immense research
concentration has also been focused on gold nanoparticles’ capability to load\textsuperscript{153,154} and control the release\textsuperscript{155–157} of drug molecules, as well as for use in cancer therapy.\textsuperscript{158–161} The diverse range of their applications originates from the vast amount of prior research in understanding their properties and gaining control in manipulating these properties and functionalities for specific needs in different applications.

Table 3.1  A summary of a selection of the biomedical applications of gold nanoparticles.

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<tr>
<th>type of applications</th>
<th>properties</th>
<th>references</th>
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The surface chemistry of Au NPs is the most important factor along with shape, size, and morphology that dictates the properties and functionalities of these nanomaterials. Common routes of preparing Au NPs result in surface capping groups that include citrate, CTAB, and PVP. Most of these surfactants are, however, not compatible with biological media and/or do not satisfy the requirements for particular biomedical applications. Post-synthesis modifications of the nanoparticle surfaces are, therefore, essential to enabling Au NPs for many applications.

Several properties of the as-synthesized Au NP can be changed by modifying their surface chemistry. First, water solubility of the as-prepared Au NPs can be manipulated from hydrophilic to hydrophobic, or reversibly from hydrophobic to hydrophilic.\textsuperscript{162} The ability to control the nanoparticles’ interactions with aqueous solvents is especially important in drug delivery applications, where the surface properties of the drug carrier, Au NPs, directly influence the effectiveness of the drug delivery process. The homogenous dispersion of nanoparticles in serum media requires hydrophilic surfaces, whereas hydrophobic nanoparticle surfaces can aid their own internalization into the cell by enhancing their affinity to the cellular membrane.\textsuperscript{163} Second, surface modifications can
improve the colloidal stability of the Au NPs. One of the common approaches to reduce aggregation under biological conditions of the as-synthesized spherical Au NPs is to coat their surfaces with a biocompatible polymer, such as poly(ethylene glycol) (PEG). The steric repulsion among the PEG chains provide increased colloidal stability to the Au NPs.165 Third, appropriate surface modifications are essential for preventing nonspecific protein adsorption and binding to the Au NPs during in vivo studies, and hence can prolong the nanoparticles’ circulation in the bloodstream and minimize the chance of clearance by the immune system to improve the chances for an efficient accumulation at the target site(s).164,166 Finally, surface modification is an important means to impart new functionalities to Au NPs that are not possible to directly achieve for the as-synthesized nanoparticles.

Using the ligand exchange method, the as-synthesized Au NPs can be modified to possess terminal functional moieties including alkyl halides, amines, azides, carboxylic acids, maleimides, phenols, alcohols, carbohydrates, amphiphilic polymers, amino acids, nucleic acids, peptides and proteins.1 It is worth noting that each of these ligand exchange reactions requires the presence of a thiol as one of the functional groups on the new ligand to utilize on the favorable thiolate-gold bond (40-50 kcal mol$^{-1}$) formation.2 An alternative surface modification route to establishing new functionalities to Au NPs is to first coat them with a thin layer of silica (SiO$_2$) with the goal of preserving their optical properties. The silica surfaces subsequently become a new platform on which more chemistry can be carried out via reactions with surface silanol groups.1 Importantly, there are three additional benefits from a thin silica shell: (1) silica coatings can improve the colloidal stability of the nanoparticles while preserving their optical properties;11 (2) encapsulating Au NPs in a silica coating also enhances the nanoparticles’ thermal stability, which is critical during their photothermal heating in applications such as drug release and cancer therapy;8,167 and (3) the biocompatibility of silica surfaces adds to the nanoparticles’ adaptivity to applications involving biological systems.168

The formation of self-assembled monolayers (SAMs) has been widely employed to modify and customize the surface properties of silicon oxide surfaces for a broad range of biomedical applications (among many other types of applications). These include drug and gene delivery, bioimaging and therapy, and biological interfaces.1,169 SAMs on silicon oxide surfaces are primarily formed using molecules containing reactive groups such as silanes1,170 and phosphonic acids,171,172 many of which are easily accessible and
commercially available from chemical distributors. The limitations of using silanes and phosphonic acids to prepare SAMs include extensive preparation procedures,\textsuperscript{173} the strength of their interactions with silicon oxide surfaces,\textsuperscript{171,172} the risk of side reactions and sensitivity to environmental factors such as humidity,\textsuperscript{174} and the potential to form multilayers.\textsuperscript{175} Alcohol reagents represent another class of reactive species to readily form SAMs on silicon oxide surfaces. The alcohol functional groups form a covalent silyl ether (Si-O-C, where the Si-O bond is 128 kcal mol\textsuperscript{-1})\textsuperscript{176} bonds with the surfaces of hydrogen-terminated silicon\textsuperscript{173,177} and silicon oxides.\textsuperscript{23,178} Alcohol functionalized reagents are widely available, monoreactive, and relatively insensitive to humidity.

The formation of SAMs using aliphatic alcohols on flat silicon oxide surfaces has been demonstrated using radiative heating using an oil-bath\textsuperscript{178} and dielectric heating using microwave radiation.\textsuperscript{23} The radiative heating method refers to both reflux at the boiling temperature of the alcohol reagent, as well as conventional heating using a temperature-controlled water or oil bath below the boiling temperature of the alcohol reagent. Radiative heating generally requires longer reaction times, on the order of hours to days. This long reaction time is due to the unfavorable energetics of the alcohol condensation reaction despite the elevated reaction temperature. Microwave radiation, on the other hand, can more effectively provide thermal energy to a reaction and more rapidly increase the reaction temperature than radiative heating methods.\textsuperscript{179} The first report of microwave-assisted alcohol condensation on silicon oxide surfaces used polished silicon wafers (~ 1 cm\textsuperscript{2}) with exposed native silicon oxide surfaces as substrates and 1-octanol as an model alcohol reagent.\textsuperscript{23} Amongst other findings, this study presented significantly shortened reaction times (10 min) from the use of microwave radiation. This time is much shorter compared to other methods such as UV illumination (2.5 h), radiative heating (2.5 h), and the use of metal catalysts (1 to 72 h). A variety of functionalized alcohol-based reagents, including those containing aldehydes, carboxylic acids, fluorocarbons, nucleic acids, quaternary ammonium cations and vitamins, have since been shown to successfully modify the surfaces of polished silicon wafers.\textsuperscript{180} Importantly, surface modifications of silicon oxides via the microwave-assisted alcohol condensation route have not been extended to the surfaces of nanoparticles.

Surface functionalized silica-coated plasmonic nanoparticles offer a flexible platform for practical applications including labeling with fluorescent dyes and surface enhanced Raman spectroscopy dyes, and immobilization onto reactive surfaces. One of
the most common techniques to link ligands covalently to a hydrophilic solid surface is through amine coupling reactions with activated carboxylic acids.\textsuperscript{181} CF\textsuperscript{®} fluorescent probes are commercially available cyanine-based water soluble fluorescent dyes that are commonly used for labeling antibodies, proteins, nucleic acids, and other biomolecules. According to the supplier, CF647-amine is a far-red fluorescent dye spectrally similar to Cy\textsuperscript{®}5 and Alexa Fluor\textsuperscript{®} 647 dyes, and its amine functional group is designed to be used in reactions with carboxylic acid groups to form covalent bonds. The chemical structure of CF\textsuperscript{®} dyes is confidential under patent protection laws.

In this study, we prepared silica-coated spherical Au NPs as a platform to demonstrate: (1) a microwave-assisted covalent surface modification of silica nanoparticle surfaces through a reaction of aliphatic alcohols and surface-bound silanols; and (2) their utility in attaching functional molecules such as fluorescent dyes to their surfaces. We sought to understand the impact of microwave radiation on the reaction between alcohols and silanol groups on the surfaces of nanoparticles of SiO\textsubscript{2}-based materials by comparing these results to those obtained from using a conventional temperature-controlled oil bath. More importantly, we sought to impart practical functionalities to these covalently modified nanoparticles by associating amine-functionalized fluorescent species to their surface using carbodiimide/N-hydroxysuccinimide (EDC/NHS) coupling chemistry, which is widely used for the formation of amide bonds. The synthesis and surface modification of Au NPs were monitored using extinction spectroscopy, TEM analysis, and SERS spectroscopy. The properties of the fluorescent probes covalently attached to the silica-coated Au NPs was monitored fluorescence spectroscopy techniques. Specifically, steady-state fluorescence analysis was used to determine the fluorescent intensity of the fluorophore, and time-resolved fluorescence measurements were performed to assess the fluorescence lifetime of the fluorescent species.

3.3. Experimental

All volumes of solutions were delivered using calibrated single channel mechanical pipettors [catalog numbers, 89079-974 (100 – 1000 μL); 89079-968 (10 – 100 μL); 89079-962 (0.5 – 10 μL); VWR International, Mississauga, ON, Canada]. The accuracy of the volumes from 0.5 to 10 μL was ± 4.0% (at 0.5 μL) to ± 0.5% (at 10 μL). The accuracy of each pipettor was determined by taking the average of three measurements of the weight.
of DI water at room temperature using an analytical balance. The accuracy for volumes ranging from 10 to 100 µL was ± 1.6% (at 10 µL) to ± 0.8% (at 100 µL), and for those volumes from 100 to 1000 µL was ± 1.6% (at 100 µL) to ± 0.6% (at 1000 µL).

3.3.1. Preparation of Glassware and Stir Bars

All glassware and stir bars were prepared using the following method prior to each synthesis. Glass Erlenmeyer flasks and Teflon® coated magnetic stir bars were immersed in a piranha solution for 1 h. The piranha solution was prepared by mixing sulfuric acid (H₂SO₄; purity 95-98%, Caledon Laboratory Chemicals, Georgetown, ON, Canada) and hydrogen peroxide (H₂O₂, 30% solution, VWR International, Mississauga, ON, Canada) in a 7:2 (v/v) ratio. **CAUTION: Piranha solution is a strong oxidizing agent and reacts violently with organic compounds. This solution should be handled with extreme care.** Following piranha cleaning, the flasks and stir bars were thoroughly rinsed with >500 mL of 18.2 MΩ-cm deionized (DI) water (Barnstead Nanopure Diamond Life Science water filtration system). The rinsed flasks and stir bars were further cleaned with an aqua regia solution prepared from a mixture of hydrochloric acid (HCl, 36.5 – 38.0% solution, VWR International, Mississauga, ON, Canada) and nitric acid (HNO₃, 68-70% solution, ACP Chemicals, Saint-Léonard, QC, Canada) in a 3:1 (v/v) ratio. **CAUTION: Aqua regia solutions are extremely oxidizing and corrosive. This solution should be handled with extreme care.** The glassware and stir bars were immersed in the aqua regia solution for 1 h, followed by rinsing with >500 mL of 18.2 MΩ-cm DI water. The glassware and stir bars were further soaked in DI water for an additional 12 h before drying in an oven at 110 °C for 15 min prior to each use.

3.3.2. Synthesis of Spherical Gold Nanoparticles

All reagents were used as received without further purification. The synthesis of spherical colloidal gold nanoparticles was adapted from the Turkevich method and recent modifications to the original method. The procedure involves the preparation of a stock solution of gold (III) chloride trihydrate (HAuCl₄•3H₂O) at least one day in advance of the synthesis. The stock solution of HAuCl₄•3H₂O (purity, 99.9%; lot # MKBW7819V, Aldrich Chemistry, St. Louis, MO, USA) was prepared by dissolving 100.67 mg of HAuCl₄•3H₂O in 50 mL 18.2 MΩ-cm DI water to achieve a concentration of 5.11 mM.
of gold nanoparticles, 6.58 mg of sodium citrate tribasic dihydrate (purity, 99%; batch # 018K0052, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 5 mL of DI water, and heated to 60 °C prior to use. Meanwhile, a 1.45 mL aliquot of the gold stock solution was diluted to a total volume of 50 mL using DI water and subsequently transferred to a 250 mL round bottom flask attached to a condenser. This mixture was brought to reflux with a constant and vigorous stirring using a magnetic stir bar. The heated solution of sodium citrate tribasic dihydrate (5 mL) was transferred into the refluxing gold salt solution through a single rapid injection using a syringe. The resulting solution was allowed to reflux for an additional 10 min before removal from the heat source and cooling to room temperature.

Gold nanoparticles were collected by centrifugation at 12,000 rpm (13443 × g) for 12 min using a Thermo Electron Corporation IEC Microlite microcentrifuge Model 120 with a rotor radius of 8.35 cm, followed by resuspension of the particles in DI water for subsequent silica coating reactions. Unless noted otherwise, the 55 mL of as synthesized gold nanoparticle solution was always concentrated to a total of 24 mL using DI water after their purification.

The solution of gold nanoparticles was analyzed using a Hewlett Packard 8543 Spectrophotometer to verify the characteristic extinction maximum at 520 nm and to assess changes in their concentration.\(^{182}\) The concentration of these spherical gold nanoparticles was calculated using the Beer-Lambert law and an extinction coefficient of \(\varepsilon = 8.78 \times 10^8 \text{M}^{-1} \text{cm}^{-1}\) (measured at 506 nm).\(^{183}\) The extinction value of each sample was measured at room temperature and held in a 4.5 mL disposable polystyrene cuvette (catalog number, 14955129, Fisher Scientific, Ottawa, ON, Canada) and filled to 1 mL for all measurements. The path length of the cuvette was 1 cm. Extinction spectra were recorded from 400 to 1000 nm with a resolution of 1 nm. All spectra were offset by their peak intensities at 400 nm for subsequent comparisons and analyses.\(^{54}\)

The dimensions and shapes of these nanoparticles were assessed from their electron micrographs obtained using an FEI Tecnai Osiris Scanning Transmission Electron Microscope (STEM) with the field emission source operating at 200 kV. To prepare the samples for imaging, a 10 \(\mu\)L aliquot of a gold nanoparticle solution was drop cast onto a copper grid coated with formvar supported carbon (300 mesh, catalog number: FCF300-CU-50, Electron Microscopy Sciences, Hatfield, PA, USA) and allowed to dry in a desiccator under vacuum at ~202 Torr (270 mbar) for 1h. The copper grid was handled
by a pair of Dumont Dumoxel tweezers (style, N4AC; catalog number: 72870-D, Electron Microscopy Sciences, Hatfield, PA, USA).

3.3.3. Silica Coating of Gold Nanoparticles

The silica coating on the surfaces of gold nanoparticles was optimized on a 3 mL scale based on a modified published procedure.\textsuperscript{18} First, tetraethyl orthosilicate (TEOS; purity, 99.999%, lot # MKBW9083V, Aldrich Chemistry, St. Louis, MO, USA) was diluted with anhydrous methanol (MeOH, purity 99.9%, lot # 157545, Fisher Chemical, Fair Lawn, NJ, USA) to a 20\% v/v TEOS solution. A 0.1 M solution of sodium hydroxide (NaOH; ACS grade, lot # 3094C508, British Drug Houses, Radnor, PA, USA) was prepared in 18.2 MΩ·cm DI water.

For the preparation of silica-coated spherical gold nanoparticles, all reactions were carried out in a temperature-controlled water bath (magnetic hotplate stirrer, model VMS-C7; electronic contact thermometer with PT1000 probe, model V5-5, VWR International, Mississauga, ON, Canada) at 25°C and kept away from light. In a 20 mL scintillation vial, 2 mL of purified, citrate coated spherical gold nanoparticles at 0.569 nM, 1 mL of 1 mM cetyltrimethylammonium bromide (CTAB, purity ≥96.0%, Lot #: BCBJ8048V, Fluka Analytical, St. Louis, MO, USA) and 100 μL of 0.1 M NaOH were combined and stirred for 15 min using a magnetic stir bar. The magnetic stir bar was treated with piranha and aqua regia prior to use according to the procedure described above. The pH of the mixture was within the range of 10 to 10.5. A 12 μL aliquot of the diluted TEOS solution was subsequently injected into the reaction mixture under continuous stirring. After 15 min, the stirring was stopped, and the reaction was left undisturbed in the water bath at 25°C for 20 h. The resulting silica-coated gold nanoparticles were separated from the reaction solution by centrifugation at 6,000 rpm (3361 × g) for 10 min, followed by an additional purification step using anhydrous ethanol (Commercial Alcohols, Brampton, ON, Canada) and the same centrifugation parameters. The purified products were resuspended in propylene carbonate (PC; purity, 99%, lot # MKBZ5654V, Sigma Aldrich, St. Louis, MO, USA) with the assistance of mild sonication for their subsequent surface functionalization.
3.3.4. Surface Functionalization of Silica-Coated Spherical Gold Nanoparticles Using a Microwave Reactor

The surfaces of silica-coated spherical gold nanoparticles were functionalized using 12-hydroxydodecanoic acid (12-HDA; purity, 97%, lot # SHBB3583V, Aldrich Chemistry, St. Louis, MO, USA). First, 0.1551 g of 12-HDA was dissolved in 5 mL PC at 80 °C in a temperature-controlled oil bath. Quickly transfer the dissolved 12-HDA solution into a capped glass microwave reaction vessel to which was added 200 uL of silica-coated spherical gold nanoparticles suspended in PC, obtained from section 3.3.3. The 200 µL of silica-coated gold nanoparticles was a result from concentrating four identical reactions of the previous section (3.3.3) after their purification to a total volume of 200 µL. The mixture was briefly agitated using a vortex mixer (Scientific Industries Vortex-Genie 2 Mixer, Bohemia, NY, USA) followed by a period of 3 h microwave irradiation using an Ethos Plus Microwave Labstation (model ATC-FO 300, Milestone Microwave Laboratory Systems, Shelton, CT, USA). The ramp time was programmed to 3 min with a target temperature of 80 °C. The maximum energy output was set as 500 W. After 3 h of microwave treatment, the sample was cooled for 10 min before adding 5 mL of isopropanol (ACS plus, Fisher Chemical, Fair Lawn, NJ, USA) was added to the reaction mixture to prevent its solidification. This mixture was transferred to microcentrifuge tubes and centrifuged at 7,000 rpm (4574 × g) for 7 min. The centrifugation was repeated once with the same speed and duration. The final nanostructures were redispersed in 1 mL of dimethyl sulfoxide (DMSO; purity, 99.9%, lot # 63809, Caledon Laboratories, Georgetown, ON, Canada) for the subsequent attachment of fluorescent molecules to the surfaces of these nanoparticles described in the next section.

Aliquots (300 uL) of the samples were taken from the reaction at various time points to assess the formation of monolayers on the surfaces of the nanoparticles by Raman spectroscopy. To prepare the samples, the aliquots were first purified by centrifugation at 7,000 rpm (4574 × g) for 7 min, and the purified particles were redispersed in 100 uL ethanol (EtOH, lot # 024194, Commercial Alcohols, Brampton, ON, Canada). Next, these solutions containing the samples were transferred to a microscope cover slip, which served as a substrate, by drop casting followed air drying these suspensions. This step was repeated 5 times to ensure an appropriate amount of nanoparticles were delivered to the cover slip for the Raman spectroscopy measurements. The Raman spectra were acquired with a Renishaw inVia confocal Raman microscope.
using a 50x long working distance lens (0.50 numerical aperture). The laser excitation wavelength was 514 nm. Each spectrum was acquired from an exposure time of 22 s at a 10% laser power (~ 42 mW/µm²) with 10 accumulations. The laser power was measured at the sample height using a Spectra-Physics laser power meter (model 407A) from Newport Corporation (Irvine, CA, USA).

### 3.3.5. Association of Fluorescent Dyes with the Surface Functionalized Silica-Coated Spherical Gold Nanoparticles

To demonstrate the functionality of the 12-HDA functionalized silica-coated gold nanoparticles, a fluorescent dye, CF®647-amine, was attached to these nanoparticles through carbodiimide crosslinking chemistry. Specifically, 1 mL of the purified 12-HDA functionalized silica-coated gold nanoparticles obtained from Section 3.3.4 was mixed with 13.6 µmol of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, lot # 040M17411V, Sigma Aldrich, St. Louis, MO, USA) dissolved in 1 mL of DMSO at room temperature for 1 h, followed by addition of 50.4 µmol of N-hydroxysuccinimide (NHS, lot # MKBX1364V, Aldrich Chemistry, St. Louis, MO, USA) dissolved in 1 mL of DMSO at room temperature for 4 h. Finally, an aliquot (20 µL) of a 2 mM solution of fluorescent dye CF647-amine (~969.0 g/mol, catalog number 92042, Biotium, Fremont, CA, USA) prepared in DMSO was added to the above mixture at room temperature with continued stirring for another 20 h. The mole ratio of CF647-amine to 12-HDA is approximately 1:18,000. To collect the dye associated particles, the sample was centrifuged at 7,000 rpm (4574 × g) for 10 min, and this centrifugation process was repeated for a total of four times. The collected particles were dispersed in EtOH and centrifuged for an additional four times using the same centrifugation parameters. The isolated and purified products were redispersed in EtOH for further characterization. The supernatants from each centrifugation step were also collected for analysis.

### 3.4. Results and Discussion

This study demonstrates the step-wise process of a microwave-assisted surface modification of silica-coated Au NPs using a functionalized aliphatic alcohol reagent, as well as their subsequent utilization as fluorescently tagged colloidal nanoparticles. A
schematic (Figure 3.1) details these steps and the important reagents involved in each step of the surface modification process.

Figure 3.1  Utilizing the alcohol condensation reaction to attach molecules to gold nanoparticles (Au NPs). A specific example is shown here for the attachment of a CF647-amine reactive dye to carboxylic acid functionalized Au NPs. These Au NPs were first coated with silica using tetraethyl orthosilicate (TEOS) followed by a microwave-assisted functionalization with 12-hydroxydodecanoic acid (12-HDA). The CF647-amine dyes was linked to the carboxylic acid-functionalized surfaces via a carbodiimide assisted cross linking method, and the coupling agents were N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS).

The Au NPs were synthesized on the basis of the Turkevich method, in which citrate ions serve the roles as reducing agent, surfactant, and pH mediator in turning gold salt (e.g., HAuCl₄) into monodispersed spherical Au NPs. The resultant Au NPs were separated from the excess sodium citrate reagent by repeated centrifugation and removal of the supernatants, followed by incubation with a 1 mM aqueous solution of CTAB. The adsorbed (negatively charged) citrate surfactants were subsequently replaced by CTAB through a ligand exchange process, and CTAB formed a layer covering the surfaces Au NPs. This layer served as an ordered template for the eventual deposition of silica oligomers. Following their purification as described in Section 3.3.2, the shape, size, monodispersity of these Au NPs were examined by extinction spectroscopy, electron...
microscopy, and dynamic light scattering techniques (Figure 3.2) to ensure their batch-to-batch quality and reproducibility. The solution of Au NPs obtained after their incubation with a CTAB solution exhibited an extinction maximum centered at 520 nm (Figure 3.2a), which is indicative of the LSPR wavelength of Au NPs and confirmed the formation of spherical shaped colloidal nanostructures. Transmission electron microscopy (TEM) imaging (Figure 3.2a inset) further confirmed the shape and monodispersity of these Au NPs. Finally, the average hydrodynamic diameter of the Au NPs was measured to be 31.2 ± 0.5 nm with a log-normal fit of the size distribution (Figure 3.2b). The larger average hydrodynamic diameter compared to that indicated from the TEM image is mainly due to the presence of CTAB surfactant layer (and possibly a minimal amount of citrate residue), which is weakly adsorbed on the Au NPs and can be distributed non-uniformly over the surfaces. This characterization of the nanoparticles is important to monitor both the success of subsequent surface modifications, and the reproducibility of the process as outlined in Figure 3.1.
Figure 3.2 Characterization of Au NPs prepared using a citrate reduction method. (a) Extinction spectrum of Au NPs suspended in water after purification. Inset is a representative transmission electron microscopy (TEM) image of these Au NPs. The scale bar is 50 nm. (b) Histogram of the Au NP size distribution measured by dynamic light scattering techniques and the corresponding log-normal fit of the distribution.

The silica coating procedure is based on the Stöber method, performed in the presence of CTAB surfactant. The formation of the silica coating took place overnight at 25 °C, and the resulting silica-coated Au NPs were isolated from solution by centrifugation and decanting of the supernatant. Next, these silica-coated Au NPs were transferred from the aqueous phase into polar aprotic propylene carbonate (PC) for surface functionalization with alcohol-based reagents.

The choice of solvent for the subsequent reactions is an important aspect of the surface modification processes. In this study, the solvent must be capable of facilitating solvation and transport of alcohol molecules to the surfaces of the silica-coated nanoparticles, as well as driving the byproduct, water molecules, away from the surfaces of these nanoparticles. In general, polar aprotic solvents are suitable choices of solvents.
for alcohol condensation reactions because they are capable of dissolving both the alcohol-based reagents as well as water produced during the formation of the silyl ether (Si-O-C) bonds.\textsuperscript{178} Propylene carbonate is a polar aprotic solvent that has recently been demonstrated as a suitable solvent for the formation of alcohol-based monolayers during the surface modification of silicon oxide substrates.\textsuperscript{180} Propylene carbonate has a relatively low melting point of -55 °C and a high boiling point of 242 °C.\textsuperscript{184} Cyclic organic carbonates including propylene carbonate each have two types of electrophilic sites: one carbonyl carbon, and two alkyl carbons. “Hard” nucleophiles including aliphatic alcohols can potentially react with the carbonyl carbon from the cyclic carbonate via a transesterification reaction.\textsuperscript{185} This reaction requires the presence of a catalyst (e.g., metal oxides and tungsten catalysts)\textsuperscript{186,187} or high temperatures (>200°C) and high pressures (≥30 bar) in the absence of a catalyst.\textsuperscript{188} In the current study, neither a catalyst nor a combination of high temperatures and pressures was involved. As a result, a potential competition from PC for the alcohol reagents should be considered minimal and negligible. These properties discussed here enable PC to accommodate a wide range of reaction temperatures for various types of alcohol-based reagents. The relatively low reactivity and toxicity of PC, as well as accessibility and low cost are also factors that contributed to the choice of PC as the solvent for this study.

Following the solvent exchange of the silica-coated Au NPs into PC (pH 7), they were combined with a functionalized aliphatic alcohol, 12-hydroxydodecanoic acid (12-HDA) in a microwave reaction vessel. The 12-HDA (in a solid powder form) was first dissolved in PC prior to the reaction. The reaction vessel was placed in a microwave reactor and exposed to microwave radiation in a closed chamber (Figure 3.1). A Milestone microwave reactor was used because of its ability to safely and reliably monitor and control the reaction temperature of the solvent, and the reaction vessels were used to minimize solvent evaporation.\textsuperscript{189} To maintain a consistent reaction temperature, the microwave power is automatically adjusted throughout the reaction with respect to the programmable target temperature.

The reaction mixture was treated by microwave radiation and investigated at different lengths of time to evaluate the influence of the total dose of microwave radiation on the formation of 12-HDA SAMs on the surfaces of the silica-coated Au NPs. Previously, the influence of the duration of microwave exposure on the formation of 1-octanol SAMs was investigated on four types of polished silicon substrates using techniques that
included water contact angle (WCA) measurements. These WCAs reached ~110° from an initial ~25° within 10 min of treatment with microwave radiation, indicating the successful formation of the 1-octanol SAMs on these surfaces. The high WCAs also remained consistent across all substrates with prolonged microwave treatment (e.g., 30 min), further confirming the covalent modification of the silica surfaces. The rapid reaction rate was partially attributed to the efficient elimination of surface adsorbed water molecules as a result of dielectric heating. We hypothesize that this property can extend to nanoparticles of silicon-oxide and could assist in the modification of their surfaces with alcohol-based reagents.

The primary alcohol on one end of the aliphatic chain will interact with the silica surfaces on Au NPs by undergoing an alcohol condensation reaction to form silyl ether bonds with these surfaces. The functional group on the other end of the hydrocarbon chain is exposed toward the surrounding solvent and, therefore, becomes the new functional surface chemistry of the nanoparticles. The resulting SAMs on the silica surfaces will also experience van der Waals forces that aid in their favorable close packing at the surfaces. The choice of a 12-carbon alcohol reagent with a carboxylic acid functional group, 12-HDA, was based on: (1) the ease for further attaching functional molecules to the nanoparticles through the carboxylic acid surface chemistry; (2) the suitable length of the alkyl chain for efficient formation of the monolayers; and (3) the low cost of the reagent relative to thiol- and silane-based carboxylic acid functionalized reagents.

Carboxylic acid is an important type of surface functional group because it provides a convenient site for biomolecules to be bound to the surfaces of inorganic materials. Nanoparticles functionalized with carboxylic acids can be covalently attached to a wide range of biomolecules. These biomolecules include amino acids, peptides, proteins, antibodies, and other nanoparticles that possess primary or secondary amino groups via a simple carbodiimide coupling chemistry. Previously, carboxylic acid functional groups were added to the surfaces of Au NPs using a ligand exchange method with thiolated carboxylic acids, and on the surfaces of silica using carboxylic acid functionalized alkoxysilanes or halosilanes functionalized with carboxylic acids through a process of hydrolysis, substitution, and condensation reactions.

The time-based microwave-assisted covalent modification of silica-coated Au NPs with 12-HDA was monitored by SERS spectroscopy. Strong vibrational bands are
associated with carboxylic groups.\textsuperscript{190–192} As the carboxyl group is the primary functional group present in the system, vibrational spectroscopy techniques such as Raman and infrared (IR) spectroscopy are suitable for assessing the progress of the surface modification. Surface-enhanced Raman scattering spectroscopy was the more appropriate choice for this study because it requires a significantly smaller amount of sample for each measurement in comparison to that required for IR spectroscopy measurements (i.e., micromolar vs. millimolar particle concentrations, respectively).

Figure 3.3a shows a series of Raman spectra for the samples, acquired after 0 min, 20 min, 1 h, 3 h, and 24 h of exposure to microwave radiation. Prior to the Raman measurement, each aliquot of sample was first purified carefully to remove non-covalently bound, or residual 12-HDA species. The purification process contained two times of centrifugation at 7,000 rpm (4574 x g) for 7 min. This combination of centrifugation parameters is only sufficient to cause the functionalized silica-coated Au NPs to pellet on the bottom of the centrifugation tubes. Smaller gold nanoparticles (e.g., non-silica-coated Au NPs) and molecular species (free 12-HDA molecules) cannot be obtained with the centrifugation speed and time used here. From samples except the sample with 0 min microwave treatment, a single broad peak spanning from $\approx 1520 \text{ cm}^{-1}$ to $\approx 1650 \text{ cm}^{-1}$ was present in the spectra. The intensity of this broad peak progressively increased as the reaction progressed to 24 h. We attributed this peak to the stretching vibrations of carboxyl groups (COOH) of the surface-bound 12-HDA molecules.
Figure 3.3  Monitoring the microwave-assisted process of functionalizing silica-coated Au NPs with 12-HDA. (a) Surface-enhanced Raman spectra (λₑₓ = 514 nm) of the samples were recorded after 20 min (magenta trace), 1 h (black trace), 3 h (red trace), and 24 h (blue trace) of microwave radiation. These reactions were carried out with a target temperature of 80°C using a maximum microwave power of 500 W. The control spectrum is the 0 min spectrum (green trace), which represents that of the silica-coated Au NPs without the attachment of 12-HDA. The spectra are vertically offset for clarity. (b) The calculated area of the Raman peak around 1600 cm⁻¹ from each spectrum as a function of reaction time. The dotted curve represents an exponential fit using the equation shown in the graph with an indicated R² value.
According to Raman spectroscopy studies of unbound aliphatic carboxylic acids with increasing alkyl chain lengths up to 7 carbons (heptanoic acid), the C=O stretching vibration follows a general trend towards lower energy with increasing alkyl chain length. For example, the C=O stretching band was reported at 1661 cm\(^{-1}\) for formic acid,\(^{191}\) 1657 cm\(^{-1}\) for acetic acid,\(^{190}\) 1658 cm\(^{-1}\) for propanoic acid,\(^{190}\) and 1647 to 1644 cm\(^{-1}\) for pentanoic, hexanoic, and heptanoic acids at progressively decreasing wavenumbers.\(^{192}\) Following this trend, the Raman C=O stretching vibration for 12-HDA should further shift towards 1600 cm\(^{-1}\). Additionally, the Raman C=O stretching band and carboxyl band for surface-bound species with a carboxylic acid functional group have been reported at 1656 cm\(^{-1}\) and 1600 cm\(^{-1}\), respectively.\(^{193,194}\) Thus, the broad band observed in Figure 3.3a is most likely the result from the carboxyl group of the 12-HDA.

The featureless, broad characteristic of the band could be the result of agglomeration of the nanoparticles, formed during drop casting and drying the samples on microscope cover slip substrates. Islands of agglomerated particles, which were large enough to be visible by optical microscopy at a 50x magnification, were intentionally selected for these SERS measurements to achieve a high signal-to-noise ratio. Agglomerated nanoparticles are, however, known to easily develop noncovalent intermolecular interactions, commonly through hydrogen bonds and dipole-dipole forces, which lead to variations in their surface molecular conformations.\(^{191,193}\) Broad distributions of molecular conformations, as well as the nanoparticle assemblies in turn trigger variations in the intramolecular mode coupling behaviors between the nanoparticles. These interactions result in the broadening and shifts in the vibrational frequencies of these functional groups.\(^{193}\) In addition, hydrogen bonding may also contribute to the observed broadening effect.

Another important piece of information from the time-based SERS measurements is the progressively increasing intensity of the broad band (Figure 3.3a). From a qualitative perspective, this is an indication of the degree of completeness of the surface modification process. The broad Raman band began to appear after 20 min of exposure to microwave radiation, suggesting that a significant amount of 12-HDA was covalently bound to the surfaces of the silica-coated Au NPs. The intensity of the band was approximately 7,000 counts after 20 min of microwave treatment. At 1 h, the intensity increased to \(~12,000\) counts and continued to grow to \(~18,600\) counts after 3 h of microwave radiation. The final Raman spectrum was recorded after 24 h of microwave treatment, and the intensity of the
band reached ~19,600 counts, which implied that the rate of change in the intensity of the band was progressively decreasing. This progressive increase is clearly depicted by Figure 3.3b, in which the integrated peak area of the SERS band was plotted as a function of microwave treatment time. This peak area profile of the band over the 24 h microwave irradiation process exhibited a trend that is described by an inverse exponential decay model \( y_0 = 1.75 \times 10^6, A = -1.8 \times 10^6, x_0 = -0.091, \text{ and } t = 1.15 \). This curve suggests a relationship between the amount of free 12-HDA molecules in the reaction mixture and the duration of microwave irradiation such that the larger the quantity of 12-HDA molecules, the more rapidly the area of the SERS band will increase. The area of the SERS band is a portrayal of the SERS intensity originated from the carboxylic acid functional group of the 12-HDA. Hence, the larger the quantity of available 12-HDA, the more rapidly these molecules can attach to the silica-coated Au NPs through the alcohol condensation reaction, giving rise to the intensity of the SERS band. The maximum theoretical number of 12-HDA that can be covalently bound to a silica-coated Au NP with a radius of 28 nm is ~45,319 (see Appendix C for further details). In summary, the time-based SERS measurements of the microwave-treated samples provided insights to the kinetics of the attachment process of the 12-HDA to the silica-coated Au NPs.

Although unbound 12-HDA molecules may also be present in the Raman samples, their concentration was determined to be negligible because: (1) specific peaks for the carboxyl groups of the unbound species would appear in the Raman spectra; (2) the 0 min spectrum was the result from purifying an aliquot obtained from the reaction mixture containing both the silica-coated Au NPs and 12-HDA prior to its microwave exposure, indicating the ability to effectively purify this sample; and (3) each sample was purified at least twice by a process of centrifugation at a low speed (7,000 rpm) prior to being drop cast on the Raman substrates. As discussed above, this centrifugation speed was only effective for collecting nanoparticles of a similar size to that of the silica-coated Au NPs, and was not sufficient to isolate even the smaller nanoparticles (i.e., Au NPs without a silica coating) from the solvent. Hence, a negligible amount of excess, non-covalently bound 12-HDA molecules would have been collected in the pellet following centrifugation.

Another experiment was performed using a temperature-controlled oil bath as the source of energy for initiating the alcohol condensation reaction between the surfaces of silica-coated Au NPs and the 12-HDA. The purposes of this experiment were to: (1) further confirm the validity of the alcohol-condensation reaction to modify the surfaces of silica-
coated Au NPs; and (2) provide a comparison of the effectiveness of microwave-assisted alcohol condensation reactions and those achieved using the radiative heating methods. The oil-bath-assisted reactions were carried out at the same temperature (80°C) as those using the microwave-assisted approach. Aliquots of samples were collected at the same time points (0 min, 20 min, 1 h, 3 h, and 24 h) and prepared in the same way as the microwaved reactions for the SERS spectroscopy measurements. Prior to each Raman measurement, an aliquot of these sample was first purified by two times of centrifugation at 7,000 rpm (4574 × g) for 7 min and removal of the supernatant solutions to remove residual 12-HDA species.

Similar to the results from the microwave-assisted reactions, a single broad band was observed from ~1520 cm⁻¹ to ~1650 cm⁻¹ (Figure 3.4a). A progressive increase in the intensity of this band was observed, but it changed at a significantly slower rate when compared to that of the microwave-treated sample. The integrated peak area under each of the SERS band shown in Figure 3.4a was plotted as a function of oil bath heating time (Figure 3.4b). Similar to that of the microwave-treated sample, the peak area profile of the band over the 24-h oil bath heating process also exhibited a trend that can be described by an inverse exponential decay model. The slope of a part of the curve for the peak areas from 0 to 3 h appeared less steep when compared to the same part of the curve from the microwave-treated sample (Figure 3.3b). The smaller slope suggested a slower increase of the peak area, hence a slower rate of attachment of the 12-HDA to the silica-coated Au NPs under the oil bath heating conditions. In addition, the peak areas associated with the oil-bath-treated samples were also much smaller when compared to those of the microwave-treated samples. As the peak areas are the portrayals of the intensity of the SERS band, a comparison of the intensities of the band between the microwave-treated and oil-bath-treated samples will be discussed next.
Figure 3.4 Monitoring the oil bath-assisted process of functionalizing silica-coated Au NPs with 12-HDA. Surface-enhanced Raman spectra ($\lambda_{ex} = 514$ nm) of the samples were recorded following 20 min (magenta trace), 1 h (black trace), 3 h (red trace), and 24 h (blue trace) of heating using a temperature-controlled oil bath at 80°C. The spectrum of the 0 min control sample (green trace) represents that of the silica-coated Au NPs without the attachment of 12-HDA. The spectra are vertically offset for clarity. (b) The calculated area of the Raman peak around 1600 cm$^{-1}$ from each spectrum as a function of reaction time. The dotted curve represents an exponential fit using the equation shown in the graph with an indicated $R^2$ value.

A direct comparison of the intensity of the SERS broad band is depicted by Figure 3.5, in which the intensities of the band for both the microwave- and oil bath-treated
samples were plotted as a function of time. Raman active species bound to the surfaces of plasmonic nanoparticles can increase the Raman cross-section by up to several orders of magnitude, due to the enhanced electromagnetic fields on the surfaces of plasmonic nanoparticles upon excitation.\(^\text{195}\) The relative Raman intensity can be used as a rough estimate of the concentration of surface bound SERS active species, such as the 12-HDA. For the oil-bath facilitated condensation reactions, the intensity of the broad band (from \(\sim1520\text{ cm}^{-1}\) to \(\sim1650\text{ cm}^{-1}\)) did not become apparent until after 1 h. Specifically, the Raman spectrum of the sample at a 20 min reaction time was nearly identical (<1,000 counts) to that acquired prior to the surface modification reactions and in the absence of 12-HDA (Figure 3.4a, 0 min). This broad band became distinguishable after 1 h of reaction, with an intensity of \(\sim2,000\) counts, indicating the presence of surface bound 12-HDA. The band intensity had, however, increased to only \(\sim5,000\) counts after 3 h and at the end of the 24 h period the intensity reached \(\sim6,000\) counts.

In contrast, after 20 min of microwave radiation, the same band had already reached an intensity of \(\sim7,000\) counts, higher than that achieved over 24 h of heating in the oil bath. The final intensity of the SERS band of the sample obtained from the microwave-assisted method was nearly 3 times the intensity of that from the oil-bath treated sample. This trend is clearly observed in Figure 3.5, suggesting a significantly higher rate of reaction achieved \textit{via} microwave radiation and, consequently, more 12-HDA molecules were condensed onto the surfaces of the silica-coated Au NPs at each time point for the reactions. The higher rate of reaction by the microwave-assisted method could be due to the more efficient removal of the reaction by-product (i.e., water) from the surfaces of the nanoparticles because of the dielectric heating of both the solvent and the Au NPs.\(^\text{179}\)
The SERS intensities of the broad peak centered around 1600 cm\(^{-1}\) from the spectra of 12-HDA functionalized silica-coated Au NPs prepared using microwave radiation and oil bath heating as a function of the duration of either treatment.

The carboxylic acid functionalized surfaces of the silica-coated Au NPs can become a platform for which molecules with primary amine functional groups can attach to those surfaces. One type of such molecules is fluorescent probes. Following the surface modification of silica-coated Au NPs with a carboxylic acid terminated alcohol (12-HDA), these functionalized nanoparticles were transferred into dimethyl sulfoxide (DMSO) for surface modification with the amine-terminated fluorescent dye CF647-amine (Figure 3.1).

Previous literature has reported that the presence of fluorophores near plasmonic nanoparticles can cause deviations in their fluorescence excitation and emission properties in comparison to those unbound fluorophores.\(^{11,118,196}\) These changes can include an increase or decrease fluorescence intensity, as well as to the fluorescence decay rate. An altered fluorescence decay rate can also lead to changes to the fluorescence lifetime of the fluorophore. At this stage of the surface modifications, we aimed to demonstrate a successful coupling of a fluorescent dye to the surfaces of the carboxylic acid functionalized nanoparticles as a first step. The fluorescent properties of these fluorescently labeled nanoparticles were assessed, but a further investigation of enhancements to the properties of these nanoparticles was beyond the scope of the present study.
A primary amine can be covalently linked to an activated carboxylic acid via EDC/NHS coupling chemistry. The proposed cross-linking mechanism is shown in Figure 3.6. The EDC reacts with the carboxylic acids on the surfaces of the silica-coated Au NPs to form an α-aclylisourea intermediate. In polar solvents, α-aclylisourea is a labile species and is prone to displacement by nucleophiles, such as water molecules, which can deactivate EDC by cleaving the intermediate and releasing isourea. Hence, NHS is often used to help improve the coupling efficiency. The addition of NHS stabilizes the α-aclylisourea intermediate by converting it to an amine-reactive ester, that subsequently forms a stable amide bond through a nucleophilic attack by the primary amine from the CF647-amine fluorescent dye. In this study, the coupling reaction was also carried out in DMSO to further minimize the hydrolysis of EDC in contrast to reactions in aqueous systems.

![Diagram of EDC/NHS assisted cross linking](image)

**Figure 3.6** The EDC/NHS assisted cross linking of carboxylic acid functionalized silica-coated Au NPs with the fluorescent species CF647-amine.

The preservation of the overall structure and shape of the nanoparticles is an important aspect for their stability and plasmonic properties. After the attachment of the fluorescent dye to the silica-coated Au NPs, they were first characterized by TEM imaging (Figure 3.7) to assess any changes to the overall structure and shape of the nanoparticles. A comparison was made between the silica-coated Au NPs prior to microwave radiation for the 12-HDA condensation reactions (Figure 3.7a) to those NPs after both modification with 12-HDA and the association of CF647-amine through the EDC/NHS crosslinking (Figure 3.7b). Multiple regions of the TEM grid were examined by TEM for both types of samples to ensure that these images were representative of each sample. The shapes
and average sizes of the Au NPs and their silica shells were monitored for any significant changes during the multistep surface modification process. Specifically, the Au NPs remained the same size of ~20 nm throughout the process, from 20.5 ± 2.1 nm (before the silica coating process) to 20.3 ± 2.4 nm (after fluorescent dye attachment). The average diameter of the silica-coated Au NPs was also relatively consistent and measured to be 52.1 ± 2.6 nm and 56.6 ± 3.1 nm in Figures 3.7a and 3.7b, respectively. Each value reported herein was the average of 100 independent measurements from their corresponding TEM images, and the uncertainties were reported to one standard deviation from the mean values. The slight increase in the average diameter of the silica shells could be due to a further condensation of the residual TEOS precursors during the microwave-based dielectric heating. The unreacted TEOS could adhere to the silica surfaces if the purification process was not sufficient to remove these species from the suspension. Following this characterization of the morphology and size of the silica-coated Au NPs, their optical properties were assessed by extinction spectroscopy.
Figure 3.7  Comparing the shape and size of the silica-coated Au NPs before and after the surface modifications: (a) TEM image of silica-coated Au NPs prior to their surface functionalization with 12-HDA; and (b) the same particles after both 12-HDA attachment via microwave radiation and CF647 amine association through the EDC and NHS crosslinking approach.

Changes in the optical properties of the Au NPs can suggest changes to the dielectric constant of their surrounding environment such as that of the solvent and/or surface coating. The multistep process for surface modification was monitored by extinction spectroscopy measurements (Figure 3.8). Each spectrum corresponds to one step of the processes outlined in Figure 3.1. The extinction maximum for the as-synthesized Au NPs was centered at 520 nm. Following the silica coating procedure, this peak red-shifted 13 nm to 533 nm. This shift is attributed to an increase in the local refractive index \( n \) around the Au NPs, changing from water \( (n = 1.332) \) to silica \( (n = 1.456) \).\textsuperscript{201,202} Upon surface modification with 12-HDA, the LSPR peak further red-shifted by 4 nm to 537 nm, followed by another 4 nm shift to 541 nm after the final association between the silica-coated Au NPs and the CF647-amine fluorescent dyes. These spectral
shifts were also the result of changes to the local refractive index at the surfaces of the Au NPs. Similar red-shifts have been previously observed following the adsorption of molecules onto the surfaces of silica-coated Au NPs.\textsuperscript{30,203} In addition, peak broadening was observed at the final step of the reaction process (Figure 3.8, CF647-silica-Au NPs). The appearance of the shoulder to the right of the extinction maximum suggests the absorption from the fluorescent molecules, which has a maximum absorption at 647 nm. The deconvoluted spectrum (Figure B1, Appendix B), obtained by subtracting the extinction spectrum of 12-HDA-silica-Au NPs from that of the CF647-silica-Au NPs, further confirms the absorption in the sample (CF647-silica-Au NPs) arising from the addition of the CF647-amine within the anticipated spectral range (i.e., from ~570 to ~700 nm). The overall shape of the LSPR peak is preserved throughout these reactions. This observation suggests that the silica coating and subsequent surface modifications did not fundamentally reshape the spherical morphology or induce aggregation of the Au NPs.

![Figure 3.8](image)

**Figure 3.8** The processes for surface modification of spherical Au NPs as monitored by extinction spectroscopy. Shown in this figure are the extinction spectra of the (i) as-synthesized Au NPs (black trace), and spectra following each of the subsequent modifications to their surfaces: (ii) silica-coated Au NPs (blue trace); (iii) 12-HDA functionalized silica-coated Au NPs (red trace); and (iv) CF647-amine fluorescent dye associated silica-coated Au NPs (green trace).

The fluorescent properties of the fluorescently labeled Au NPs were assessed using fluorescence spectroscopy. The fluorescence intensity of CF647-amine when bound
to the silica-coated Au NPs was measured using steady-state measurements (Figure 3.9). The absorption and emission peak for the CF647-amine is 647 and 665 nm, respectively (Figure A1, Appendix A). The spectral range of the absorption and emission profiles of the CF647-amine is distinct from the extinction properties of the silica-coated Au NPs. The non-overlapping spectral locations of the fluorescent probe and the nanoparticles allow for a clearer distinction of the fluorescence activities of the CF647-amine molecules from the extinction of the nanoparticles during fluorescence measurements of the samples. The samples were excited at the absorbance maximum of this fluorescent molecule (647 nm), and its fluorescence emission was assessed between 650 nm to 760 nm. The CF647-amine fluorescent probe was specifically chosen for its reactive amine and the spectral position of its maximum absorbance, which is distinct from that of the silica-coated Au NPs (533 nm, Figure 3.8). We aimed to minimize the overlap between this extinction peak and the absorbance peak from the fluorophore. Any fluorescence emission observed would be, therefore, due to activity of the CF647-amine species without direct contributions from or interference with the Au NPs. The excitation/emission profile of the CF647-amine demonstrated that it was an ideal candidate because it is well-separated from the extinction peak of the Au NPs while still in the visible region of the electromagnetic spectrum. The significance of being within the visible region becomes apparent for these fluorophore-bound nanoparticles’ future applications in bioimaging, as the most common emission filters in fluorescence microscopes cover the visible region.

Following the EDC/NHS cross linking of the CF647-amine to the carboxylic acid terminated nanoparticles, the particles were purified by a purification process of centrifugation at 7,000 rpm (4574 × g) for 10 min and removal of the supernatants. The centrifugation process was repeated four times. The redispersed sample was assessed by its fluorescence activity using steady-state measurements. An emission maximum for the purified sample was observed at 669 nm, which is 4 nm red-shifted from the reported emission maximum of the free CF647-amine at 665 nm. This shift would be an indication of changes to the microenvironment of the fluorescent molecules. The observation of a slightly shifted emission peak from the peak position of the free fluorescent molecules suggests the successful attachment of these molecules to the surfaces of the silica-coated Au NPs. A spectral shift of a similar magnitude to the fluorescence emission have been reported in a study involving the association of fluorescently labeled molecules to the surfaces of gold nanoparticles.204 This study reported a 2 nm red-shift of the fluorescence
emission peak of an enzyme, lipase (contains intrinsic fluorophore, tryptophan), after its cross-linking to amine functionalized Au NPs through EDC/NHS covalent coupling.

![Fluorescence spectra of CF647-amine covalently attached to silica-coated Au NPs. Control samples include 12-HDA functionalized silica-coated Au NPs incubated with the CF647-amine dye, in the absence of coupling agents (control). The excitation wavelength for each of these measurements was set to 647 nm.](image)

To further confirm the formation of a covalent bond between the CF647-amine and the surfaces of nanoparticles, a control spectrum was also obtained using the same excitation process. The control contained a mixture of CF647-amine and silica-coated Au NPs, which were functionalized with 12-HDA, in the absence of EDC and NHS. (Figure 3.9). Specifically, the control sample was obtained by incubating the CF647-amine with 12-HDA functionalized silica-coated Au NPs under the same conditions (i.e., at room temperature for 20 h) as for the 12-HDA functionalized silica-coated Au NPs in the presence of EDC and NHS. The spectral analysis of the control sample was measured after performing the same procedures for the sample purification. This control experiment suggest that no measurable amounts of fluorescent species remained in the purified sample for the control. This result implied that, in the absence of EDC and NHS, the CF647-amine did not become covalently attached to the surfaces of 12-HDA functionalized silica-coated Au NPs and that the majority of unbound CF647-amine molecules were effectively separated from the nanoparticles using the purification method. The same purification was used for the sample following the EDC/NHS assisted process.
to couple the CF647-amine to the 12-HDA functionalized silica-coated Au NPs. For convenience, these nanoparticles will be referred to as CF647-silica-Au NPs in the following discussion.

In addition, each supernatant solution removed during the sample purification was collected and analyzed to further assess the efficiency of the purification process. The fluorescence spectra of the supernatants were obtained following the purification process that included four times of centrifugation at 7,000 rpm (4574 × g) for 10 min (Figure 3.10). As a reference, for Au NPs without a silica coating, a centrifugation speed of 12,000 rpm (13443 × g) was required to collect these nanoparticles from the solution phase. From the resulting series of fluorescence spectra, broad peaks were observed for the first three supernatants, indicating the continued presence of CF647-amine in these solutions. The fourth supernatant exhibited no observable signal in comparison to the spectrum for the solvent, suggesting that the concentration of this fluorescent molecule was below the detection limits of the fluorescence spectrometer. In addition, a particularly broad peak in the spectrum of the first supernatant indicated a possible emission signal saturation, as part of the peak appeared to be flat with a constant intensity. Overall, these fluorescence measurements of the supernatants indicated that a purification process with four rounds of centrifugation is sufficient to remove the unassociated fluorescent species from the solution.
Figure 3.10 Fluorescence spectra of supernatants obtained during the purification of the silica-coated Au NPs after attachment of the CF647-amine fluorescent molecule. The purification process removed the non-covalently linked molecules by isolating the nanoparticles using centrifugation, followed by decanting of the supernatant and resuspension of the nanoparticles in a fresh solvent solution. This process was repeated for a total of four times. These spectra were vertically offset for clarity, and each spectrum is labeled with the corresponding step of the purification process.

The absorption spectra were also measured for the supernatants obtained from the purifying the CF647-silica-Au NP products (Figure 3.11). The pattern for the observed change in intensity mirrored that of the fluorescence measurements for these products. The spectra from the first three supernatants exhibited distinguishable spectral features while the fourth spectrum resembled that of the solvent. The broad peak centered at 650 nm in each of the first three spectra is assigned to the absorption of the unbound CF647-amine molecules, and the shoulder around 530 nm resulted from the plasmon resonance from a small amount of silica-coated Au NPs. The reason these nanoparticles were present in the supernatant was from the transfer of the supernatant out of the centrifuge tube. Some nanoparticles from the isolated fraction were likely disturbed and brought back into solution during the pipetting step. In addition, another shoulder peak centered at 614 nm was observed in the spectrum of the first supernatant. The shape and position of this peak closely resembled the shoulder peak of free CF647-amine molecules.
Figure 3.11  Extinction spectra of the supernatants obtained from the purification of the silica-coated Au NPs after covalent attachment of the CF647-amine molecules. The supernatants contained residual CF647-amine, unattached to the particles, and a trace quantity of particles. The fourth supernatant indicated that any remaining, unconjugated molecules were below the detection limits.

The fluorescence of CF647-silica-Au NP products can be distinguished from that of the free CF647-amine molecules through assessing their fluorescence lifetimes using time-resolved photoluminescence. Fluorescence lifetime ($\tau$) is the average duration a fluorophore is found in the excited state prior to its relaxation to the ground state.\textsuperscript{110} The decay of sample fluorescence intensity is related to the fluorescence lifetime by the following equation:\textsuperscript{11}

$$I(t) = Ae^{-t/\tau} \quad (3.1)$$

in which intensity ($I$) is expressed as the product of the initial fluorescence intensity ($A$), the time point of interest ($t$), and the fluorescence lifetime of the molecule ($\tau$). Using $k$ to represent the decay rates of all the relaxation pathways, the fluorescence lifetime can be expressed in the following manner:\textsuperscript{11}

$$\tau = \frac{1}{k_{rad} + k_{nr}} = \frac{1}{k} \quad (3.2)$$
where $k_{rad}$ and $k_{nr}$ are the radiative and nonradiative decay rates, respectively. The nonradiative decay of a free fluorophore in solution is a result of multiple processes including intersystem crossing, internal conversion, and collisional quenching.\textsuperscript{110}

In the case of a fluorophore that is bound to the surfaces of a plasmonic metal nanoparticle, its relaxation dynamics can be more complex.\textsuperscript{11} The surfaces of the metal nanoparticle present a continuum of states to which fluorophores can couple. Upon coupling, both radiative and nonradiative decay rates of the fluorophore are subject to change. These potential changes are due to the emergence of new decay pathways as a result of the coupling. The altered emission dynamics lead to a different fluorescence lifetime ($\tau'$) and a change in the total decay rate ($k'$). A new expression of the fluorescence lifetime can thus be written:\textsuperscript{11}

$$\tau' = \frac{1}{k_{rad}+k_{nr}+k_{rad}'+k_{nr}'+k_{s}+k_{dd}} = \frac{1}{k'}.$$  \hfill (3.3)

Here, the additional terms are from a surface-plasmon-induced enhancement of the radiative rate ($k_{rad}'$), the nonradiative interactions of the fluorophore and metal nanoparticle ($k_{nr}'$), the interactions of the fluorophore with the nanoparticle surfaces ($k_s$), and the fluorophore-fluorophore fluorescence coupling of species in proximity to the surfaces of the nanoparticles ($k_{dd}$).\textsuperscript{11}

The surface-plasmon-induced enhancement of the radiative rate ($k_{rad}'$) is related to the radiative decay rate of the free dye $k_{rad}$ in the following way:\textsuperscript{11,205}

$$\frac{k_{rad}'}{k_{rad}} \approx 1 + \frac{3}{2} \text{Im} \frac{\mu \cdot E(\vec{r}, \omega_0)}{|\mu|^2 k^3}$$ \hfill (3.4)

in which the second term on the right-hand side of the equation is the imaginary part of dipolar coupling; $\mu$ is the dipole momentum, $E(\vec{r}, \omega_0)$ is the electric field at the atom position $\vec{r}$ with the frequency of its transition $\omega_0$, and $k$ is a field free space wavevector. The complete analytical solution to this equation requires a full multipole expansion of the electric field.\textsuperscript{11,205}

Despite the complexity of fluorescence of molecules coupled to plasmonic NPs, two dynamic observables from this system can be deduced from a solution: (i) a complex radiative contribution that uses the Drude model of metallic electrons; and (ii) a plasmon-
induced nonradiative contribution from the Förster energy transfer process. Overall, the radiative contribution is dependent on the polarizability of the total system and its associated impact on the transition dipole, and the nonradiative component is the result of energy dissipation from the fluorophore to the metal nanoparticle through dipole-dipole couplings.\textsuperscript{11}

Time-resolved photoluminescence of the purified CF647-silica-Au NPs and the control samples were measured at room temperature (Figure 3.12). The sample that contained CF647-amine, 12HDA-silica-Au NPs, without the EDC and NHS (red trace), was measured without a further purification of the nanoparticles after their incubation with CF647-amine. The goal was to evaluate the difference between this measurement and that of the free CF647-amine. The fluorescence decay curves for both the free CF647-amine (black trace), and a mixture of the 12-HDA modified silica-coated Au NPs and the CF647-amine without the addition of the EDC and NHS (red trace) showed almost identical trends. The close resemblance of these two decay curves implied that the free CF647-amine, when mixed with the nanoparticles, exhibits the same fluorescence decay kinetics. These results further confirmed that the CF647-amine species did not associate with the silica-coated Au NPs without the crosslinking reaction, even when carboxylic acids were present on the surfaces of the nanoparticles.
Figure 3.12  Fluorescence decay curves of CF647-amine associated with the Au NPs. All of the Au NPs were coated with silica and subsequently surface-functionalized with 12-HDA prior to their introduction to the fluorescent species. The CF647-amine was added in the presence (blue trace) and absence (red trace) of the crosslinkers EDC and NHS. The sample that contained CF647-amine, but not EDC and NHS, was measured directly without purification.

The fluorescence decay curve of the CF647-silica-Au NPs (blue trace) is different from that of the free CF647-amine (black trace). The rate of decay is slower for the covalently linked fluorescent molecules than that of the free CF647-amine. It has been observed that for a system of fluorescent molecules covalently coupled to silica-coated gold nanorods, the rate of decay became faster compared to that of the free fluorescent molecules because of a dominant contribution from the surface-plasmon-induced radiative decay rate.11 In the same report, the authors found that the fluorescence decay rate increased as the thickness of a silica shell on the nanorods decreased from 26 nm to 11 nm. As the fluorophore-gold core distance increased to ≥ 22 nm, the fluorescence decay dynamics appeared to be consistent with the free fluorescent species. In our study, the average silica shell thickness was 25.7 ± 3.0 nm, which is outside of the range previously observed for a fluorescent decay rate enhancement due to the presence of additional decay pathways in a coupled system. The apparent differences in the decay rate between the CF647-silica-Au NPs and free CF647-amine observed in Figures 3.12 was, however, still a valid indication of the altered fluorescence dynamics due to the fluorescent molecules’ linkage to the silica-coated Au NPs.
The difference in the fluorescence decay dynamics is reflected in the fluorescence lifetime of these samples. The fluorescence lifetime is calculated from the time-resolved photoluminescence measurements. An isolated molecule decays from the excited state to the ground state exhibit a single exponential decay. This expression does not, however, fit the decay curve of the CF647 amine when covalently attached to the 12-HDA functionalized silica-coated Au NPs. An equation that describes a simplified biexponential decay is a better model for the fluorescent molecule-nanoparticle coupled systems:\(^{11}\)

\[
I(t) = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2}
\] (3.5)

In this equation, \(A_1\) and \(A_2\) are the initial fluorescence intensities each corresponding to the “fast” and “slow” components of the decay, respectively. Here, \(\tau_1\) and \(\tau_2\) correspond to the fluorescence lifetimes of the “fast” and “slow” processes. The biexponential decay fit of the decay curve indicates that there are multiple pathways of decay implied by Equation 3.3. The fluorescence lifetimes were calculated from the data in Figure 3.12 and are listed in Table 3.2.

**Table 3.2** Fluorescence lifetimes of the CF647-amine before and after attachment to the silica-coated Au NPs.

<table>
<thead>
<tr>
<th></th>
<th>A intensity</th>
<th>(\tau) (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF647-amine</td>
<td>5.28 ± 0.02</td>
<td>1.99 ± 0.01</td>
</tr>
<tr>
<td>carboxylic acid modified particles + dye without EDC&amp;NHS</td>
<td>1.27 ± 0.02</td>
<td>2.16 ± 0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>(A_1) intensity</th>
<th>(\tau_1) (ns)</th>
<th>(A_2) intensity</th>
<th>(\tau_2) (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>carboxylic acid modified particles + dye + EDC&amp;NHS</td>
<td>4.71 ± 0.01</td>
<td>1.57 ± 0.01</td>
<td>0.72 ± 0.02</td>
<td>4.73 ± 0.01</td>
</tr>
</tbody>
</table>

\(^{*\tau_1} = \text{fluorescence lifetime associated with fluorescence intensity } A_1; \text{ and } \tau_2 = \text{fluorescence lifetime associated with fluorescence intensity } A_2.\)

The lifetime of the free CF647-amine without the presence of any nanoparticles is 1.99 ± 0.01 ns from a single exponential decay fit using Equation 3.1. The measurement of the control experiment containing a mixture of non-covalently attached CF647-amine and 12-HDA functionalized silica-coated Au NPs was also fit using the single exponential decay model. In the control sample, a relatively small amount of the CF647-amine could be in close proximity to the nanoparticles in the suspension during the measurement. As mentioned in Section 1.2, the fluorescence decay mechanisms of fluorescent species can
be affected by being near plasmonic nanoparticles, both with and without bonding to the nanoparticles. The fluorescence decay dynamics of the CF647-amine in the control sample was predicted to be similar to, but not identical to, that of the free CF647-amine in the absence of the nanoparticles. The amount of the CF647-amine in close proximity (<20 nm) to the nanoparticles in the control sample was due to random events and should be much lower when compared to those that are covalently attached to the nanoparticles. Hence, the fluorescence decay dynamics of the control sample was expected to be different from both that of the free CF647-amine and the covalently bound CF647-amine.

The differences in the fluorescence decay dynamics between the three samples were identified by comparing both the calculated intensity and lifetime values. The calculated lifetime for the free CF647-amine is 1.99 ± 0.01 ns, which is similar to that of the control sample, 2.16 ± 0.03 ns. For the CF647-amine that were covalently attached to the nanoparticles, the fast component (τ₁) of their lifetime is 1.57 ± 0.01 ns and the slow component (τ₂) is 4.73 ± 0.01 ns. The magnitude of the signal intensity (A₁) associated with the lifetime of the fast component (4.71 ± 0.01) for the covalently-bound CF647-amine was much higher than the intensity associated with the slow component (0.72 ± 0.02). Overall, the covalently attached fluorescent molecules exhibited a much different set of fluorescent lifetimes from that of the free CF647-amine and non-covalently linked control sample. The fast component has a faster lifetime and the slow component has a slower lifetime for the covalently bound CF647-amine. These results suggest that the fluorescence decay dynamics of CF647-amine were altered when covalently attached to the 12-HDA functionalized silica-coated Au NPs. The presence of functionalized silica-coated Au NPs alone did not, however, significantly influence the fluorescence decay dynamics of the non-covalently attached CF647-amine species.
3.5. Conclusions

The surfaces of silica-coated gold nanoparticles were modified using a carboxylic acid functionalized alcohol through microwave-assisted alcohol condensation processes. The synthesis of spherical gold nanoparticles and their subsequent surface modifications, including silica coating and functionalization with an alcohol-based carboxylic acid, 12-hydroxydodecanoic acid, were characterized using a number of techniques. Further confirmation of the successful surface modification with the carboxylic acid species, as well as a demonstration of the utility of these functionalized silica-coated gold nanoparticles, were simultaneously demonstrated through the association of an amine-functionalized fluorescent molecule, CF647-amine, to the surfaces of these nanoparticles. The covalently linking the CF647-amine to the carboxylic acid functionalized silica-coated gold nanoparticles was enabled by the use of carbodiimide/N-hydroxysuccinimide (EDC/NHS) coupling chemistry. A combination of steady-state fluorescence measurements and time-resolved photoluminescence measurements revealed that the fluorescence lifetimes of the CF647-amine-silica-Au NPs increased relative to that of the free CF647-amine. This study demonstrated an alternative route to modifying the surfaces of silica-based nanoparticles. These processes can be used for attaching molecular probes, such as fluorophores, using alcohol-based reagents coupled to the silica surfaces through a microwave-assisted alcohol condensation reaction.
Chapter 4.

Alcohol Based Surface Functionalization of Silica-Coated Gold Nanorods

4.1. Notice of Permissions

A manuscript based on the work discussed in this chapter is being prepared for publication. All the work presented here, including experiments, data acquisition and interpretation, and writing were performed by myself under the guidance of Dr. Byron Gates.

4.2. Introduction

This study demonstrates the surface modification of silica-coated gold nanorods (Au NRs) through a process that uses alcohol-based reagents. The post-synthesis surface modification of Au NRs, such as encapsulating them in thin (<30 nm) silica (SiO₂) shells, can effectively enhance their stability and solubility, and meanwhile enable further surface functionalization using the silica shells as a platform. Section 1.2 of this thesis provided a more detailed discussion on the preparation and benefits of encapsulating the surfaces of gold nanoparticles with a layer of silica.

The surface modification of silica-coated Au NRs utilized the same microwave-assisted approach discussed in Chapter 3. Instead of a carboxylic acid functionalized aliphatic alcohol, an alcohol-functionalized aromatic chelating agent, 2-hydroxymethyl-18-crown-6 (referred to herein as HO-crown ether) (Figure 4.1), was used in this study to functionalize the surfaces of the silica-coated Au NRs. Crown ethers are cyclic molecules that consist of a ring of ether groups. The size of the cavity within this ring-like structure determines the types of cations that can be effectively chelated by the crown ether. The HO-crown ether used in this study is best for selectively binding to Group II metal cations. Furthermore, it has been shown that immobilized crown ethers on the surfaces of silica gel exhibit selectivity during the separation of mixed salts such that they can be used as the stationary phase in a chromatography column. A crown ether species was selected for this study with the following reasons: (1) a chelating agent represents an
important class of functional species for attaching to the surfaces of nanoparticles; (2) a ring-like molecular structure which possesses a terminal alcohol group adds to the diversity of chemical structures of alcohol-based reagents that are available to modify the surfaces of silica-based nanoparticles; (3) in contrast to using chelating agents freely dispersed in the solution phase, this work demonstrates a method to achieve localized chelation effects by immobilizing the chelating agents on the surfaces of the silica-based nanoparticles. One of the benefits for immobilizing a chelator species on the surfaces of nanoparticles is that these functional nanoparticles can be isolated from solution with ease by a purification process such as centrifugation or filtration. In addition, these localized chelation effects can be directly characterized using electron microscopy imaging techniques.

Figure 4.1 The chemical structure of 2-hydroxymethyl-18-crown-6.

4.3. Experimental

All volumes of solutions were delivered using single channel mechanical pipettors [catalog numbers, 89079-974 (100 – 1,000 μL); 89079-968 (10 – 100 μL); 89079-962 (0.5 – 10 μL); VWR International, Mississauga, ON, Canada]. The accuracy of the volumes delivered from 0.5 to 10 μL was ± 4.0% (at 0.5 μL) and ± 0.5% (at 10 μL), the volumes ranging from 10 to 100 μL was ± 1.6% (at 10 μL) and ± 0.8% (at 100 μL), and the volumes from 100 to 1000 μL was ± 1.6% (at 100 μL) and ± 0.6% (at 1,000 μL).

4.3.1. Synthesis of Colloidal Gold Seeds for Gold Nanorods

Gold nanorods were synthesized using a modified seed-mediated method. A solution of gold seed was prepared using an acid cleaned, 50 mL round bottom flask held at 30°C in a temperature controlled water bath. First, 5 mL of 0.5 mM HAuCl₄•3H₂O (purity, 99.9%; lot # MKBW7819V, Aldrich Chemistry, St. Louis, MO, USA) and 5 mL of 0.2 M
cetyltrimethylammonium bromide (CTAB; purity ≥96.0%, lot #: BCBJ8048V, Fluka Analytical, St. Louis, MO, USA), each prepared in 18.2 MΩ·cm DI water, were pipetted into the round bottom flask under continuous stirring at 1,200 rpm. Next, a 0.6 mL aliquot of an ice-cold aqueous solution of 0.01 M sodium borohydride (NaBH₄; purity, 99%, lot # SHBD4770V, Aldrich Chemistry, St. Louis, MO, USA) was diluted to 1 mL with 18.2 MΩ·cm DI water and introduced all at once into the reaction mixture in the round bottom flask. The reaction mixture quickly changed from a bright yellow to a brown-yellow color, indicating the formation of ligand-substituted anions, such as [AuCl₃Br]⁻ and CTA-AuCl₄⁻ complexes. The mixture was stirred for an additional 2 min at 1,200 rpm and subsequently held at 30°C without stirring until their addition into the growth solution for the gold nanorods within 30 to 40 min.

4.3.2. Synthesis of Gold Nanorods Using a Surfactant Mixture of CTAB and Salicylic Acid

The gold nanorod growth solution was prepared by dissolving 0.9 g of CTAB and 0.05525 g of salicylic acid (purity, ≥99.0%, lot # W29A022, Alfa Aesar; Ward Hill, MA, USA) in 25 mL of 18.2 MΩ·cm DI water within a 250 mL Erlenmeyer flask under magnetic stirring (1,000 rpm) while maintaining this solution at a temperature of 65°C using a temperature-controlled water bath. After 4 to 6 h, all of the reagents had dissolved and the solution was cooled to 30°C before proceeding. A 0.9 mL aliquot of freshly prepared 4 mM silver nitrate (AgNO₃; purity, ≥99.0%, lot # MKKBH5454V, Sigma-Aldrich, St. Louis, MO, USA) dissolved in 18.2 MΩ·cm DI water was added to the reaction mixture under magnetic stirring (1,000 rpm). The resulting mixture was left undisturbed for 15 min at 30°C. Next, 25 mL of 1 mM HAuCl₄ and 200 µL of HCl were added to the mixture together and stirred for an additional 15 min, followed by the addition of 0.2 mL of a freshly prepared 0.064 M solution of ascorbic acid (purity, ≥99.0%, lot # SLBH4617V, Sigma Life Science, St. Louis, MO, USA) under vigorous stirring (1,500 rpm). After reaching a colorless state, 80 µL of the colloidal gold seed solution prepared following the procedure outlined above was added to this solution. The resulting mixture was stirred for an additional 2 min at 1,200 rpm and left undisturbed and kept away from light to allow for nanorod growth for the next 20 h at 30°C. The resulting Au NR solution was centrifuged at 13,500 rpm (17014 × g) for 15 min (Thermo Electron Corporation IEC Microlite microcentrifuge, model 120, rotor radius, 8.35 cm), followed by the removal of the supernatant, to collect the Au NRs. Two additional times of purification using the same centrifugation parameters were performed.
afterwards. A 1 mM solution of CTAB was used to resuspend the Au NRs. Unless stated otherwise, the ~50 mL of as-synthesized gold nanorod solution was always concentrated to a total of ~6 mL using 18.2 MΩ·cm DI water after their purification. These Au NRs had two extinction bands measured by a Hewlett Packard 8543 Spectrophotometer. These bands corresponded to the transverse and longitudinal surface plasmon resonances of the Au NRs centered at 520 nm and 886 nm, respectively. The size of the Au NRs was assessed by TEM techniques using an FEI Tecnai Osiris Scanning Transmission Electron Microscope.

4.3.3. Silica Coating of Gold Nanorods

A silica coating of the Au NRs was also optimized on a 3 mL scale based on modifications to a published procedure. First, tetraethyl orthosilicate (TEOS; purity, 99.999%, lot # MKBW9083V, Aldrich Chemistry, St. Louis, MO, USA) was diluted with anhydrous methanol (purity 99.9%, lot # 157545, Fisher Chemical, Fair Lawn, NJ, USA) to achieve a 20% v/v TEOS solution. A 0.1 M solution of sodium hydroxide (NaOH; ACS grade, lot # 3094C508, British Drug Houses, Radnor, PA, USA) was prepared in 18.2 MΩ·cm DI water.

The preparation of silica-coated gold nanorods followed a similar approach to that for the spherical gold nanoparticles. All reactions were carried out in a temperature-controlled water bath at 30°C and shielded from light. In a scintillation vial, 100 μL of purified gold nanorods suspended in a 1 mM CTAB solution, 3 mL of additional 1 mM CTAB, and 100 μL of NaOH were combined and stirred for 15 min using a piranha and aqua regia washed magnetic stir bar. The pH of the mixture was confirmed to be in the range of 10 to 10.5. A 12 μL aliquot of the diluted TEOS solution was injected rapidly into the mixture under vigorous stirring. After 15 min, the stirring was stopped, and the reaction allowed to proceed for 20 h. Purification of the resulting silica-coated gold nanorods was the same as that of the silica-coated spherical gold nanoparticles, and the purified products were suspended in PC for further surface modifications.

4.3.4. Surface Functionalization of Silica-Coated Gold Nanorods

Silica-coated gold nanorods were functionalized with 2-hydroxymethyl-18-crown-6 (purity, 95%, lot # MKBJ5325V, Aldrich Chemistry, St. Louis, MO, USA). A 100 μL aliquot
of 2-hydroxymethyl-18-crown-6 was added to 1 mL of the purified and concentrated silica-coated gold nanorods dispersed in PC. An additional 4 mL of PC was added to bring the total volume of the mixture to an appropriate level for the microwave treatment. The amount of silica-coated gold nanorods was a result of concentrating four identical reactions of the silica coating procedure as described in Section 4.3.3. The reaction mixture was briefly agitated on a vortex mixer prior to transferring to a capped glass microwave reaction vessel. This glass vessel was placed in a secondary sealed vessel and treated with microwave radiation for 3 h, holding the solution at a target temperature of 80°C using a maximum energy output of 500 W. Following the microwave treatment, the sample was cooled down for 10 min. The mixture was purified by two times of centrifugation at 7,000 rpm (4574 × g) for 7 min and removal of the supernatants. The final products were redispersed in 1 mL of MeOH for the subsequent chelation tests.

4.3.5. Chelation Tests with Surface Functionalized Silica-Coated Gold Nanorods

The covalent modification of the silica surfaces with 2-hydroxymethyl-18-crown-6 was verified through performing a chelation test using calcium cations. Crown ether compounds are known for their strong interactions with metals ions through the lone pairs of their oxygens. Calcium nitrate (0.50 M) was dissolved in MeOH prior to mixing with the crown ether functionalized silica-coated Au NRs. Upon addition of calcium nitrate to the Au NRs at room temperature, the mixture was placed on a stirring plate for 1 h. The Au NRs were subsequently isolated and purified twice by centrifugation at 7,000 rpm (4574 × g) for 10 min and removal of the supernatants.

4.4. Results and Discussion

This study demonstrated a step-wise process for a microwave-assisted surface modification to silica-coated Au NRs using a functionalized alcohol reagent, as well as their subsequent utilization to chelate calcium cations. A schematic (Figure 4.2) outlines these steps and the important reagents involved in each step of the surface modification process. Gold nanorods were synthesized in the solution-phase and coated with a silica shell. Next, the surfaces of the silica-coated Au NRs were functionalized with a chelating agent via an alcohol condensation reaction using a microwave-assisted method. The purified and functionalized silica-Au NRs were subsequently tested for their ability to
capture calcium ions from solution to demonstrate the successful attachment of the functional chelating agent.

Figure 4.2 The preparation of gold nanorods (Au NRs) and their surface modification via an alcohol condensation reaction. An example of this process is given using a microwave-assisted approach in the surface modification step with 2-hydroxymethyl 18-crown-6. This schematic diagram is not drawn to scale.

The Au NRs were synthesized using the seed-mediated method in the presence of a surfactant mixture consisted of CTAB and SA. Next, these Au NRs were coated with a silica shell following the same procedure for coating the Au NPs that was described in Chapter 3. The purified silica-coated Au NRs were transferred from aqueous solution into
PC, the same solvent that was used in the functionalization of the silica-coated spherical Au NPs with 12-HDA in Chapter 3. The chelating agent 2-hydroxymethyl 18-crown-6 (HO-crown ether) was introduced to the solution of silica-coated Au NRs in PC and mixed thoroughly. The well-mixed solution was treated with microwave radiation for 3 h using the same settings as those in Chapter 3 (target temperature, 80°C). Following the microwave treatment and sample purifications, the crown-ether-O-silica-Au NRs were transferred into methanol for a subsequent evaluation of ability to chelate calcium ions. The successful attachment of HO-crown ether to the surfaces of silica-Au NRs can be confirmed by performing this evaluation. The process described above was monitored by extinction spectroscopy (Figure 4.3), and a spectrum was measured following the completion and purification of each step outlined in Figure 4.1.

Figure 4.3 The surface modification of Au NRs as monitored by extinction spectroscopy. Shown in this figure are the extinction spectra of the as-synthesized Au NRs (black trace), silica-coated Au NRs (red trace), and silica-coated Au NRs functionalized with 2-hydroxymethyl-18-crown-6 (HO-crown ether) (blue trace), as well as HO-crown ether functionalized silica-Au NRs in the presence of calcium ions. All spectra except for that of the as-synthesized Au NRs were measured using methanol as a solvent. The spectrum of the as-synthesized Au NRs was measured using water as a solvent.

The longitudinal LSPR band of the Au NRs showed a spectral shift following every surface modification step (Figure 4.3). The as-synthesized Au NRs exhibited a longitudinal
LSPR band centered at 886 nm. The silica coating process of these Au NRs resulted in a spectral red-shift of the longitudinal LSPR band to 895 nm due to an increase of the local refractive index of the Au NRs from the CTAB bilayer (1.41)\textsuperscript{215} to silica (1.45).\textsuperscript{216}

The next spectral shift following the surface modification of silica-coated Au NRs with HO-crown ether was a blue-shift of approximately 20 nm to 874 nm. The refractive index of free HO-crown ether is \(\sim1.48\), which is higher than that of silica. The spectral blue-shift cannot, therefore, be explained by simply comparing the refractive indices of the HO-crown ether and the silica. The spectral blue-shift could be an indication of the change in the local environment of the silica-Au NRs through the covalent attachment of the HO-crown ether via microwave-assisted alcohol condensation reactions. The attached HO-crown ether on the surfaces of the silica-coated Au NRs were likely to have assembled into SAMs.\textsuperscript{23} The density of the crown ether SAMs were, however, expected to be much lower than the density of the silica shell, which has an estimated average thickness of \(\sim10\) nm (Figure 4.4). In general, a decrease in the density of molecules on the surfaces and an increase in the local concentration of water can be related to a decrease in the local refractive index of the silica-coated nanoparticles.\textsuperscript{216} A spectral blue-shift in the extinction spectrum also indicates a decrease in the local refractive index of the nanoparticles. Hence, the observed blue-shift could be the result of a decrease in the density of molecules on the surfaces of the Au NRs as a result of HO-crown ether attachment. An additional spectral blue-shift of 17 nm was observed in the next step, when calcium ions (from dissolving calcium nitrate) were introduced to the purified solution of HO-crown ether functionalized silica-Au NRs in MeOH.

As stated above, the chelating agent, 2-hydroxymethyl-18-crown-6, has a strong selectivity for Group II metal cations.\textsuperscript{211} Calcium cations were specifically selected for this study. We hypothesized that the spectral blue-shift from 874 nm to 857 nm was due to the binding of calcium ions by the surface-bound HO-crown ether molecules (Figure 4.3). The chelation of metal cations by crown ethers was proposed to result in a further decrease in the refractive index of the crown ether monolayer on a silica surface.\textsuperscript{210} Following binding of a cation by a crown ether, the solvation of the crown ether motif changes.\textsuperscript{217} The cation in the crown ether’s cavity can, however, remain solvated with solvent molecules in the presence of solvated counter anions.\textsuperscript{218} These solvent molecules and counter anions cause an increase of the steric bulk of the crown ether-metal cation complexes when compared to uncomplexed crown ether molecules and, therefore, a decrease in the
density of the monolayer. In addition, the presence of solvent molecules (MeOH), which have a lower refractive index (1.32)\textsuperscript{219} than that of the silica and water, in proximity to the complexed crown ether molecules can further lower the effective refractive index of the crown ether monolayer.\textsuperscript{210} The electrostatic repulsion among the overall positively charged crown ether-metal cation complexes can contribute to the decrease of the monolayer density and lead to a lower refractive index. Hence, the spectral blue-shift upon introduction of the calcium cations was most likely from a lowered refractive index around the Au NRs from the rearrangement of the surface-bound crown ether molecules upon their complexation with the calcium cations.

Energy dispersive X-ray spectroscopy coupled with STEM-HAADF imaging was employed to further confirm the binding of calcium cations to the surface-bound HO-crown ether species. The spatial resolution of STEM imaging is < 1nm. The choice of calcium cations over the other Group II metal cations was for the identification of chelated cations with a high degree of certainty during EDS analyses. The energies of the characteristic X-rays of the highest intensity for magnesium (Mg), calcium (Ca), strontium (Sr), and barium (Ba) are 1.253 keV (K\textsubscript{\alpha}), 3.690 keV (K\textsubscript{\alpha}), 1.806 (L\textsubscript{\alpha}), and 4.465 (L\textsubscript{\alpha}), respectively.\textsuperscript{100} Beryllium (Be) was not in our consideration due to its low atomic mass and an extremely low energy characteristic X-ray (K\textsubscript{\alpha} = 0.110 keV) that is beyond the detection limit of the EDS detector. Radium (Ra) was also not considered for this study because it is a radioactive substance. Among Mg, Ca, Sr, and Ba, the characteristic X-ray energy of Ca appears in a region where no other energies associated with the other elements contained in the samples would be observed. For Au NP-related samples, the most “populated” regions of an X-ray energy spectrum are from 0 to ~2.5 keV and ~8.0 to ~12.0 keV. As energies of the characteristic X-rays of Mg and Sr are also found in the 0 to ~2.5 keV region of the X-ray energy spectrum, their signals overlap with many of the other elements in the sample. The deconvolution of these signals and assignment of peaks can, therefore, become complicated. Finally, Ba was eliminated because of its lower stability when complexed with the HO-crown ether, in comparison to that of the Ca-crown ether complex.\textsuperscript{211}

The EDS analysis was accompanied by a tilt-series analysis by TEM techniques. A BF TEM image, a DF STEM image, and an EDS map were each acquired at tilt angles of +28°, 0°, and −28° for the crown-ether-O-silica-Au NRs. Here, tilt angles refer to the angle between the sample plane and the incident beam. A 0° tilt indicates that the sample
plane is positioned perpendicular (90°) to the electron beam, and a +/−28° tilt indicates that the sample plane has a 28° deviation from the perpendicular position relative to the beam. Figure 4.4 shows the tilt series acquired in the BF TEM mode. The tilting of the sample plane can be easily observed by following the red arrow, which points to the same Au NR at all three angles. The Au NRs in these images are purified samples of calcium treated HO-crown ether functionalized silica-coated Au NRs.

Figure 4.4 A tilt series of HO-crown ether functionalized silica-coated Au NRs in the presence of calcium cations. These images were acquired in the bright field (BF) TEM mode at tilt angles of (a) +28°, (b) 0°, and (c) -28°. The red arrow in all three images points to the same Au NR.

The distribution of the species in the sample imaged in Figure 4.4 was examined by elemental analysis (Figure 4.5). Each of the Au NRs (blue) was coated relatively uniformly with a thin layer of silica (yellow). This uniform silica coating appeared to be more evident for the circular structure in the middle of Figure 4.5a. Because of its nearly perpendicular orientation relative to the incident electron beam at a tilt angle of +28°, a nearly perfect “top view” of this particular Au NR was captured by EDS analysis. A relatively uniform distribution of calcium (shown in magenta) was also observed from the elemental analysis. The tilting of the sample also demonstrated that the calcium cations present were not the result of adsorption upon drying of the samples. Specifically, adsorption by drying refers to artifacts generated during TEM sample preparation, which involves drop-casting and drying a suspension of nanoparticles on a TEM grid. Upon drying, the nanoparticles and any free molecular species and free ions contained in the droplet, tend to concentrate into isolated patches at the perimeter of the dried droplet (the “coffee ring” effect).220 The molecular species and ions can, therefore, appear to be associated with the nanoparticles in the resulting TEM analyses. If this was the case for the current study, the elemental maps acquired at different tilt angles would appear less
uniform in terms of the distribution of the calcium cations. Instead, a uniform distribution was observed at all three tilt angles. The appearance of some “free” calcium ions in the top left corner of Figure 4.5c is likely due to non-specific interactions of the ions with the TEM grid. Overall, these results show the first demonstration of alcohol-based chelating agents immobilized on the surfaces of silica-coated nanoparticles achieved through an alcohol condensation reaction.

Figure 4.5  Energy dispersive X-ray spectroscopy mapping of Au, Ca, and Si from a tilt series of the HO-crown ether functionalized silica-coated Au NRs in the presence of calcium cations. These images were acquired in the dark field (DF) STEM mode at tilt angles of (a) +28°, (b) 0°, and (c) -28°. Magenta indicates the signals from Ca atoms, yellow indicates the signals from Si atoms, and blue indicates the signals from Au atoms.

The spatial distribution of calcium from the tilt series was also examined by overlaying their intensity map and the corresponding HAADF image (white underlying Au NR structures in the background) as shown in Figure 4.6. The colour gradient shows the relative local concentrations of Ca. These images further demonstrated that the majority of the Ca signals were observed in close proximity to the Au NRs. The highest concentration of Ca was on the Au NRs, confirming the successful chelation of these cations at the nanoparticle surfaces.
Figure 4.6  Energy dispersive X-ray spectroscopy mapping of the spatial distribution of Ca overlaid on the corresponding HAADF images from a tilt series of the OH-crown ether functionalized silica-coated Au NRs in the presence of calcium cations. These images were acquired in the dark field (DF) STEM mode at tilt angles of (a) +28°, (b) 0°, and (c) -28°. The y-axis of each image is the relative intensity from 10% [11% for (b)] to 98%.
Additional confirmation of the presence of calcium at the surfaces of HO-crown ether-bound silica-coated Au NRs was gathered through analysis of the EDS spectra of the sample. These spectra (Figures 4.7, 4.8, and 4.9) were acquired from the corresponding EDS maps in Figure 4.5. A distinctive calcium signal (shown in magenta) at ~3.7 keV was observed in all three spectra, along with signals from the other elements contained in the sample (i.e., Au, C, O, and Si). The presence of copper (Cu) signals was from the copper TEM grid. The aluminum (Al) (Figure 4.9) signals could be due to residual chemicals that remained on the TEM tweezers and sample holder from previous uses. The associated quantifications of the relative amount of Au, Ca, and Si within each spectrum are also included in Tables 4.1 and 4.2. On average, the calcium cations chelated at the surfaces of crown-ether-silica-Au NRs showed an atomic percent of ~8.9, relative to the sum of Au, Ca, and Si species. The relatively lower atomic percentage of calcium was expected because the bulk of the nanorod consisted of a gold core (length, 63.7 ± 2.9 nm; width, 19.3 ± 2.2 nm) with a silica shell thickness of ~10 nm. Hence, the highest signal intensity was expected for the Au followed by Si, and the results matched this expectation. In conclusion, the successful chelation of calcium cations at the surfaces of silica-coated Au NRs can be confirmed from these analyses. In addition, the attachment of HO-crown ether molecules on the silica-coated Au NRs can also be inferred from the localization of calcium cations at the surfaces of the NPs.
Figure 4.7  An EDS spectrum of calcium bound to HO-crown ether functionalized silica-coated Au NRs acquired from a +28° tilt angle.

Figure 4.8  An EDS spectrum of calcium bound to HO-crown ether functionalized silica-coated Au NRs acquired from a 0° tilt angle.
Figure 4.9  An EDS spectrum of calcium bound to HO-crown ether functionalized silica-coated Au NRs acquired from a -28° tilt angle.

Table 4.1  The quantification of relative amount of gold, calcium, and silicon in the purified calcium treated silica-coated Au NRs functionalized with OH-crown ether acquired at +28°, 0°, and -28° tilt angles.

<table>
<thead>
<tr>
<th>Element</th>
<th>Series</th>
<th>+28° tilt</th>
<th>0° tilt</th>
<th>-28° tilt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Norm. at.%*</td>
<td>Norm. at.%*</td>
<td>Norm. at.%*</td>
</tr>
<tr>
<td>Au</td>
<td>L-series</td>
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<td>49</td>
</tr>
<tr>
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<td>8.7</td>
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<tr>
<td>Si</td>
<td>K-series</td>
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<td>40</td>
<td>41</td>
</tr>
</tbody>
</table>

*Norm. at. % refers to normalized atomic percent.

Table 4.2 The errors associated with the quantification in Table 4.1.

<table>
<thead>
<tr>
<th>Element</th>
<th>Series</th>
<th>+28° tilt</th>
<th>0° tilt</th>
<th>-28° tilt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Error (3 sigma) at.%</td>
<td>Error (3 sigma) at.%</td>
<td>Error (3 sigma) at.%</td>
</tr>
<tr>
<td>Au</td>
<td>L-series</td>
<td>26*</td>
<td>26*</td>
<td>26*</td>
</tr>
<tr>
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<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Si</td>
<td>K-series</td>
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<td>0.4</td>
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</table>

*The number of significant figures was kept as two for clarity
A control experiment was performed by mixing the same concentration of calcium cations with a solution of silica-coated Au NRs, which were not functionalized with the HO-crown ether, was performed to further support the above conclusions. Following treatment with calcium cations, these silica-coated Au NRs were purified following the same method as for those functionalized with the HO-crown ether. The spatial distribution of calcium was mapped using an EDS analysis (Figure 4.10). Similar to Figure 4.6, the associated DF HAADF image was overlaid with the elemental map for ease of interpretation. A minimum amount of Ca signal from this analysis suggested that Ca cations were not binding to the surfaces of the silica-coated Au NRs in the absence of chelating agent, HO-crown ether. The signal could be from residual Ca cations following purifications of the Au NRs. In addition, the associated EDS spectrum also showed no significant Ca signal around 3.70 keV (Figure 4.11). The atomic percent of Ca was 0.87 relative to the sum of Au, Ca, and Si species (Table 4.3). This control experiment further confirmed that attachment of HO-crown ether molecules on the surfaces of silica-coated Au NRs was necessary for observing the large calcium signals in Figures 4.5 and 4.6.

![Image](image_url)

Figure 4.10 Energy dispersive X-ray spectroscopy mapping of the spatial distribution of Ca overlaid on the corresponding HAADF images from silica-coated Au NRs treated with calcium cations. These images were acquired in the dark field (DF) STEM mode at a tilt angle of 0°. The y-axis of the image is the relative intensity from 10% to 98%.
Figure 4.11 An EDS spectrum of the calcium treated silica-coated Au NRs acquired based on Figure 4.10.

Table 4.3 The quantification of relative amount of gold, calcium, and silicon in the purified calcium treated silica-coated Au NRs that were not functionalized with HO-crown ether acquired at a 0° tilt angle.

<table>
<thead>
<tr>
<th>Element</th>
<th>Series</th>
<th>Norm. at.%*</th>
<th>Error (3 sigma) at. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au</td>
<td>L-series</td>
<td>49</td>
<td>26**</td>
</tr>
<tr>
<td>Ca</td>
<td>K-series</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Si</td>
<td>K-series</td>
<td>49.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Norm. at. % refers to normalized atomic percent. ** The number of significant figures was kept as two for clarity.

4.5. Conclusions

This study demonstrated the surface modification of silica-coated gold nanorods using an alcohol-functionalized chelating agent, 2-hydroxymethyl-18-crown-6, through a microwave-assisted alcohol condensation process. The synthesis, surface modification, and ion chelation tests were performed and characterized using extinction spectroscopy and electron microscopy techniques. A tilt series of TEM imaging and elemental analyses demonstrated that the chelation of calcium cations was largely localized on the surfaces of the silica-coated Au NRs with a distribution of calcium over the surfaces of the nanorods. A control experiment of silica-coated Au NRs that were not functionalized with the chelator,
but were treated with calcium cations, showed no significant calcium signals in their energy dispersive X-ray spectroscopy analyses. These results further confirmed that the successful surface modification with 2-hydroxymethyl-18-crown-6 was necessary to achieve calcium signals observed in the tilt-series analysis by TEM techniques. This study enables an alternative method to modify the surfaces of silica-based nanoparticles for capturing metal ions from solution using alcohol-based reagents anchored to the silica through a microwave-assisted alcohol condensation reaction.
Chapter 5.

Summary and Outlook

This thesis described a method to modify the surfaces of silica-coated gold nanoparticles via a microwave-assisted alcohol condensation reaction. Alcohol-based reagents have been demonstrated for the first time to covalently attach to the surfaces of silica-coated gold nanoparticles. Surface modifications of as-synthesized gold nanoparticles have been largely reported based on gold-thiol chemistry and silane chemistry. Challenges associated with these methods include the stability of gold-thiol bonds, the extensive steps required to achieve a target surface functionality through gold-thiol chemistry or silane-based chemistries, the toxicity, and the reaction time, and the cost of reagents. In general, alcohol-based reagents possess relatively low toxicity, and they can be purchased with relative ease. The diversity of alcohol-based reagents is also a benefit for the surface functionalization approach described here. The work presented in this thesis provides an alternative method for the covalent surface modification of gold nanoparticles and silica-based nanoparticles through alcohol condensation reactions.

Chapter 1 reviewed the syntheses and surface modification methods of gold nanoparticles of both a spherical and a rod-like shapes, as well as silica-based nanoparticles. The mechanisms involved in these processes and applications of these nanoparticles are discussed. In Chapter 2, a discussion of the characterization tools and techniques used during the thesis work was presented. These techniques include extinction spectroscopy, transmission electron microscopy, energy dispersive X-ray spectroscopy, surface-enhanced Raman spectroscopy, and fluorescence spectroscopy. The specific advantages of selecting each tool and technique were also included. Chapter 3 demonstrated the synthesis and surface modification of spherical gold nanoparticles, which were first coated with silica followed by a functionalization with 12-hydroxydodecanoic acid using a condensation reaction initiated by microwave radiation. Next, these carboxylic acid functionalized nanoparticles were used to attach a fluorescent molecule, CF647-amine, onto their surfaces via carbodiimide/N-hydroxysuccinimide (EDC/NHS) coupling chemistry. The fluorescence properties of the purified nanoparticles were examined by steady-state fluorescence measurements and time-resolved photoluminescence measurements. In Chapter 4, gold nanoparticles with rod-like shapes
were used to demonstrate a different use of the alcohol-based surface modification reactions. Here, silica-coated gold nanorods were treated with microwave radiation in the presence of a hydroxyl-terminated chelating agent, 2-hydroxymethyl-18-crown-6. These crown ether molecules attached onto the surfaces of silica-coated Au NRs through an alcohol condensation reaction. Subsequent chelation tests with calcium cations using purified nanoparticles after their surface modification with crown ether further confirmed the successful attachment of both the crown ether species and calcium cations.

Overall, the surface modification methods demonstrated in this thesis can be utilized to tune the surface chemistry of silica-coated nanoparticles. Further studies on diversifying the types of functionalities that can be achieved using alcohol-based reagents through the same approach can be pursued. For example, functional groups including amine, alkyl halogen, azide, and other reactive groups can be explored next. Other types of underlying materials besides gold nanoparticles can also be pursued. These materials include plasmonic nanoparticles, such as silver and copper nanoparticles. Other types of oxide-based nanoparticles can also be investigated for their surface modification using the alcohol condensation reaction. In addition, nanoparticles of non-spherical shapes are another type of particles that can benefit from using the alcohol condensation to achieve surface modification and functionalization. Another aspect of future studies can be the examination and optimization of the quality of surface-bound alcohol reagents through analytical approaches. By maximizing the surface coverage of functional groups on silica-based nanoparticles, the efficiency of these functionalized silica-based nanoparticles in further applications can be improved.
References


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Appendix A.

The Absorption/Emission Profile of CF647-amine

Figure A1  The absorption and emission profiles of the fluorescent molecule CF647-amine obtained from Biotium, Inc.
Appendix B.

Deconvolution of the Absorption of CF647-amine from the Extinction Spectrum of CF647-silica-Au NPs

Figure B1  Deconvoluted UV-Vis absorption spectrum of the CF647-amine. The spectrum was obtained by subtracting the extinction spectrum of the 12-HDA-silica-Au NPs from the spectrum of CF647-silica-Au NPs; both orginals are shown in Figure 3.9.
Appendix C.

Calculation of the Maximum Surface Coverage of 12-HDA Molecules on the Surfaces of Silica-Coated Au NPs

1) Assume the 12-HDA-silica Au NPs have an average radius of: $r = 28$ nm

2) The calculated surface area per nanoparticle is: $A = 4\pi r^2 = 9,852 \text{ nm}^2$

3) Assuming the area per silanol (OH) group on the surfaces of the silica coated Au NPs is $4.6 \text{ nm}^2$.

4) The maximum theoretical number of silanol groups = $9,852 \text{ nm}^2 \times 4.6 \text{ nm}^2 = 45,319$ per NP.