ABSTRACT

A STUDY OF GOLD NANOPARTICLES FOR APPLICATION IN SEMICONDUCTOR CdS NANOSHEET BIOSENSOR DEVICES

By Nick Geitner

This thesis reports on work to study gold nanoparticles for application in semiconductor CdS nanosheet biosensor devices. We first characterize gold nanoparticle behavior and proceed to synthesize bipyramidal gold nanoparticles through a seed-mediated process. This process results in bipyramidal particles that are 89 nm long and have a tip radius of curvature of 3.6 nm. By examining the bipyramids’ absorbance spectra and TEM images we characterize their morphology and plasmonic resonant behavior. It is found that this synthesis is highly temperature dependent and can be tailored by adjusting the concentration of silver nitrate present in the growth solution. Higher silver nitrate concentration results in a higher growth rate and longer wavelength longitudinal spectral peak. As a final brief extension to this work, we attempt to functionalize these bipyramidal gold nanoparticles. Sensitivity tests find that the capture of target molecules results in a measurable spectral shift.
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Chapter I
Introduction

In a number of diverse situations, there are times when one needs to detect the presence of a very small quantity of some biological substance, whether it is a doctor scanning for cancer proteins, a government agent searching for biological weapons in a chemical plant, or an environmentalist checking a river for toxins, highly accurate and very sensitive detection of these biological molecules is imperative. While there are a number of biosensors in use today, the work described in this thesis includes the design, fabrication, and testing of a biosensor that offers greater sensitivity than those currently available or in the literature, as well as the ability to be packaged in hand-held housings.

Our device consists of a semiconductor (CdS) nanosheet placed between two metallic contacts and coated with gold nanoparticles, which are tiny gold crystals ranging in size from 5 nm – 100 nm, which is much smaller than the wavelength of visible light. The device’s sensitivity and ability to detect biological molecules arises from taking advantage of a property of small metallic particles known as localized surface plasmon resonance (LSPR). When light is incident on gold nanoparticles, the oscillating electric field of that incident light exerts a force on the free conduction electrons in the gold. This causes the cloud (or plasmon) of electrons to oscillate along the polarization direction of the oscillating electric field. Looking at Maxwell’s equations, it is the polarization of the electrons due to the incoming light which causes an enhancement of the incident electric field. The enhancement tends to be very close spatially to the surface of the nanoparticle. Every gold nanoparticle has a particular resonant wavelength based on its size and shape. When light at this resonant wavelength is incident on the nanoparticle, there will be much higher enhancement of the incident field. This can reach 100 or even over 1000 times enhancement. Fig. 1.1 is an illustration of this situation. Note that the electrons move in the opposite direction of the incident field due to their negative charge. Also due to their negative charge, the electric field due to the electrons themselves is in the same direction as polarization of the incident field.

In our device, Au nanoparticles are dispersed on the surface of the CdS nanosheet. Any incident laser light with energy above the band gap energy of CdS will induce a photocurrent in the CdS nanosheet, which can be measured in a straightforward way. The presence of gold

![Figure 1.1: Illustration of LSPR. Electrons feel a force from the incident E-field and their own E-field adds to or enhances the incident field.](image-url)
nanoparticles will enhance this photocurrent, if the exciting laser is near the AuNP’s resonant wavelength. Then, because of the enhancement of electric fields, light can penetrate farther into the CdS nanosheet, exciting more excitons and creating a higher photocurrent. Because we are above the band gap energy, 1 photon excites 1 exciton (electron-hole pair) in the nanosheet. If, however, the incident laser is below the band gap energy (but greater than half the band gap), exciting 1 exciton requires 2 photons. We would like to take advantage of this two-photon absorption because the photocurrent is sensitive to the power of the incident laser and is much higher due to the nonlinearity of the two-photon absorption proportionality. This will be discussed in greater detail in Chapter 2.

The nano device is capable of biosensing once the gold nanoparticles are functionalized, or coated with a receptor molecule. This receptor molecule is chosen specifically to bind strongly with the molecule one wishes to detect, the target molecule, while it will not bind with other molecules. This sort of lock and key mechanism is illustrated in Fig. 1.2, where the red shapes are the receptor molecules, target molecules are green, and other molecules that are not of interest are blue. Only the green target molecules will fit into the receptor molecules, representing highly specific chemical bonding. Finding the right receptor that is target molecule specific is also a challenge. We use well-known receptor/target molecule pairs to demonstrate the capability of our plasmon assisted semiconductor nanosheet biosensor device.

Figure 1.2: An illustration of a functionalized gold surface capturing only the target molecule. Receptor molecules are in red, target molecules in green, and other molecules not of interest in blue. Note that only the green target molecules will “fit” onto the receptor molecules, allowing for specific detection.

Because the resonant wavelength of gold nanoparticles is dependant not only on the size and shape of the particle, but also the refractive index of the surrounding material, capturing a molecule and therefore changing the local index of refraction, will shift the resonant wavelength of the coated particle. In this way, a device can be designed to shift either into or out of resonance with an incident laser once the desired molecules have been captured. This will cause a measurable change in the resulting photocurrent. This final device design is illustrated schematically in Fig. 1.3, showing first a bare nanosheet device (a), one coated with particles that have been functionalized with receptor molecule (green) (b), and finally one that has captured
the desired target or analyte molecule (red) (c). Upon capturing the analyte in Fig. 1.3(c) there is an increase in the measured photocurrent.

![Diagram of a biosensing device with three diagrams: (a) A bare CdS nanosheet device with low photocurrent, (b) A nanosheet device with functionalized gold nanoparticles with low photocurrent, and (c) A device that has captured analyte molecules with high resulting photocurrent.]

**Figure 1.3:** A bare CdS nanosheet (dark gray) device with low photocurrent (a), a nanosheet device with functionalized gold nanoparticles with low photocurrent (b), and a device that has captured analyte molecules with high resulting photocurrent (c).

There are several required steps in the design, construction, and testing of this biosensing device. First, Chapter 2 will more closely examine the general background and theory behind the device, as well as early device fabrication and electrical characterization. Chapter 3 describes the characterization of bare and functionalized gold nanospheres. In Chapter 4 we seek to synthesize and characterize bipyramidal gold nanoparticles, which are specially shaped nanoparticles that show great promise in this type of application. Finally, in Chapter 5 we attempt to functionalize these bipyramidal particles and perform sensitivity response experiments. Chapter 6 will conclude and summarize this work as well as recommend future work.
Chapter II
Background and Theory

2.1 Electromagnetic Theory

We will examine here the expected interaction between gold nanoparticles and an incident electromagnetic wave. We begin with a quasi-static approach and then expand to a time-dependent examination, following the method of Jackson [1].

The electric field, defined in terms of the local potential (1.1), using the Laplace Equation (1.2).

\[
\mathbf{E} = -\nabla \Phi \tag{1.1}
\]

\[
\nabla^2 \Phi = 0 \tag{1.2}
\]

Let us define the physical setup as shown in Fig. 2.1, which shows a small metallic sphere with dielectric constant \( \varepsilon(\omega) \) and radius \( a \) surrounded by a medium with \( \varepsilon = \varepsilon_m \). Because the sphere is a metal, the dielectric constant \( \varepsilon(\omega) \) must be complex. An electric field of amplitude \( E_0 \) and linear polarization is incident on the nanosphere along the \( z \) direction.

![Figure 2.1: Physical setup for examining the electric field at a point P some distance away from a small metallic sphere illuminated with an incident electric field.](image)

Because of the cylindrical symmetry of the physical setup, we use the well-known general solution (1.3) for distance \( r \) and angle \( \theta \) from the origin to point of interest and Legendre Polynomials \( P_l \) [1].

\[
\Phi(r, \theta) = \sum_{l=0}^{\infty} \left[ A_l r^l + B_l r^{-(l+1)} \right] P_l(\cos \theta) \tag{1.3}
\]

We now apply the boundary condition that the potential remains finite as \( r \to 0 \). This forces all \( B_l \) to be 0 inside of the sphere. Therefore, for the potential inside and outside of the sphere, we have 2 equations that we can solve using boundary conditions at infinity and our interface for normal and tangential components of the electric field.

\[
\Phi_{in} = \sum_{l=0}^{\infty} A_l r^l P_l(\cos \theta) \tag{1.4}
\]
\[ \Phi_{out} = \sum_{l=0}^{\infty} \left[ B_l r^l + C_l r^{-(l+1)} \right] P_l(\cos \theta) \] \hspace{1cm} (1.5)

**Boundary Conditions:**

1. Require that \( \Phi_{out} \rightarrow -E_0 r \cos \theta \) as \( r \rightarrow \infty \). Therefore, \( B_l = -E_0 \) and \( B_l = 0, l \neq 1 \)

2. \[-\frac{1}{a} \frac{\partial \Phi_{in}}{\partial \theta} \bigg|_{r=a} = -\frac{1}{a} \frac{\partial \Phi_{out}}{\partial \theta} \bigg|_{r=a} \]

3. \[-\varepsilon_0 \varepsilon_m \frac{\partial \Phi_{in}}{\partial r} \bigg|_{r=a} = -\varepsilon_0 \varepsilon_m \frac{\partial \Phi_{out}}{\partial r} \bigg|_{r=a} \]

Boundary condition 1 simply states that as we move very far from the sphere, the potential must approach what it would be if there were no sphere at all. Boundary conditions 2 and 3 are the continuity conditions for the tangential component of the electric field and normal component of the displacement fields, respectively. They force the fields to be continuous as they approach the interface between the inner and outer surfaces of the nanosphere. This requires that \( A_l = C_l = 0 \) for \( l \neq 1 \). We then solve for the remaining coefficients, resulting in the potentials (1.6) and (1.7) [1].

\[ \Phi_{in} = -\frac{3\varepsilon_m}{\varepsilon + 2\varepsilon_m} E_0 r \cos \theta \] \hspace{1cm} (1.6)

\[ \Phi_{out} = -E_0 r \cos \theta + \frac{\varepsilon - \varepsilon_m}{\varepsilon + 2\varepsilon_m} E_0 a^3 \cos \theta \] \hspace{1cm} (1.7)

By substituting \( r = a \) the solution is confirmed since \( \Phi_{in}(r = a) = \Phi_{out}(r = a) \). We now define the dipole moment of the metallic nanosphere in (1.8).

\[ \mathbf{p} = \varepsilon_0 \varepsilon_m \alpha \mathbf{E}_0 = 4\pi \varepsilon_0 \varepsilon_m a^3 \frac{\varepsilon - \varepsilon_m}{\varepsilon + 2\varepsilon_m} \mathbf{E}_0 \] \hspace{1cm} (1.8)

Here, \( \alpha \) is the *polarizability* of the particle, which is easily defined using (1.8):

\[ \alpha = 4\pi a^3 \frac{\varepsilon(\omega) - \varepsilon_m}{\varepsilon(\omega) + 2\varepsilon_m} \] \hspace{1cm} (1.9)

By simply substituting for the dipole of the particle and employing (1.1), we find the interior and exterior electric fields.

\[ \mathbf{E}_{in} = \frac{3\varepsilon_m}{\varepsilon(\omega) + 2\varepsilon_m} \mathbf{E}_0 \] \hspace{1cm} (1.10)

\[ \mathbf{E}_{out} = \mathbf{E}_0 + \frac{3\mathbf{n}(\mathbf{n} \cdot \mathbf{p}) - \mathbf{p}}{4\pi \varepsilon_0 \varepsilon_m r^3} \mathbf{p} \] \hspace{1cm} (1.11)

We see then that incident electric fields will polarize the nanoparticle, and that the second term of (1.11) is a clear enhancement term in the exterior electric field. A resonance condition is apparent, as the dielectric constant \( \varepsilon \) of the metallic nanosphere is frequency dependant (See Fig.
2.2). The polarizability, and thus the dipole moment, exterior potential, and exterior electric field will be a maximum when $|\varepsilon + 2\varepsilon_m|$ is a minimum. Thus, the resonance condition is given by (1.12), known as the Fröhlich Condition.

\[
\text{Re}\left[\varepsilon(\omega)\right] = -2\varepsilon_m
\]

(1.12)

The real part of the sphere’s dielectric constant is used in (1.12) because the dielectric constant of the medium is assumed to be entirely real. The enhancement terms and the polarization are prevented from blowing up to infinity because the imaginary part of $\varepsilon(\omega)$ is not 0.

![Figure 2.2: The wavelength dependence of the real and imaginary parts of the dielectric constant of gold [2]](image)

These results can be generalized beyond the electrostatic results by allowing $\mathbf{E}(\mathbf{r},t) = E_0 e^{-i\omega t}$ and $\mathbf{p}(t) = \varepsilon_0 \varepsilon_m \alpha \mathbf{E}_0 e^{-i\omega t}$. We can now find the associated scattering and absorption cross sections of the particle from the Poynting-vector [2].

\[
C_{\text{sca}} = \frac{k^4}{6\pi} \left|\alpha\right|^2 = \frac{8\pi}{3} k^4 a^6 \left|\frac{\varepsilon(\omega) - \varepsilon_m}{\varepsilon(\omega) + 2\varepsilon_m}\right|^2
\]

(1.13)

\[
C_{\text{abs}} = k \text{Im}\left[\alpha\right] = 4\pi k a^3 \text{Im}\left[\frac{\varepsilon(\omega) - \varepsilon_m}{\varepsilon(\omega) + 2\varepsilon_m}\right]
\]

(1.14)

By examining the power of the wave-number $k$ and radius $a$ in (1.13) and (1.14), we see that for particles with $a \ll \lambda$ (which is in the visible region of the spectrum) absorption will dominate over scattering. We can also combine these terms to arrive at the extinction cross section, $C_{\text{ext}} = C_{\text{abs}} + C_{\text{sca}}$ [2]. In other words, the intensity of incident light is reduced as it is both absorbed and scattered. Equation (1.15) is for a sphere of volume $V$ with dielectric function $\varepsilon = \varepsilon_1 + i\varepsilon_2$. 

6
This resonance we see in (1.15) for metallic particles is known as a *plasmon resonance*. On resonance, even very tiny gold nanoparticles can have a very high extinction cross section. Fig. 2.3 shows two metallic nanoparticles in an incident oscillating electric field. The polarization and dipoles induced by the externally applied electric field are clearly evident.

In Fig. 2.3, the electron clouds experience a force in the presence of an external electric field. Because gold is a good conductor it has a large number of free conduction electrons, allowing for the electron cloud to oscillate with an oscillating external electric field with little damping. This leads to a large polarization of the metallic particles. The separation of positive and negative charges creates an electric field in the same direction as the incident field, resulting in an enhancement of the incident electric field. Thus by using an exciting laser that matches the dominant absorption term for the AuNP, the incoming field is enhanced strongly.

2.2 Previous Modeling Results

We saw in Section 2.1 the electric field enhancement terms for metallic nanospheres. We now examine how metallic nanoparticles with different morphologies behave and what advantages they might offer for our application. First, Eqa. (1.9) must be generalized to allow for different geometries. This is done by incorporating a geometric factor, \( L_i \), a size parameter \( x = \frac{\pi a}{\lambda_0} \) (for principle particle axis dimension \( a \)), and two experimentally determined linear, real functions \( A(L) \) and \( B(L) \) which depend on the material the nanoparticles are composed of. Incorporating all of these factors, we arrive at the new polarizability: [4]

\[
\alpha \approx \frac{V}{\left( L + \frac{\varepsilon_m}{\varepsilon - \varepsilon_m} \right) + A\varepsilon_m x^2 + B\varepsilon_m^2 x^4 - i \frac{4\pi^2 \varepsilon_m^{3/2}}{3 \lambda_0^3} V}
\]  

(1.16)

This new function (1.16) allows one to carry out calculations for a great number of geometries. We will here be most concerned with spheres, rods, and bipyramids.

Liu *et al.* performed Finite-Difference Time-Domain (FDTD) calculations to predict E-field enhancement factors for three different gold nanoparticle shapes in 2007. In these
calculations, Maxwell’s equations are solved numerically. The physical setup is input into a computer program with initial conditions and small, finite steps in time are taken while space is divided into small, discrete portions. With each time step, a new E-field is calculated as a function of position, then a new time step is taken and new field calculated, etc. Typically smaller time steps and smaller grids result in more accurate calculations, but can require much greater computing power and time. Thus one must find the balance of the smallest time and grid sizes for results that no longer change. Using these calculations it was possible to determine the resonant wavelengths of the particles, demonstrate how these resonances depend on shape, and predict the magnitude of the enhancement of local electric fields [5].

Fig. 2.4 (a,b, and c) show the field enhancement results for spheres, nanorods, and bipyramids, respectively. Each of these calculations has been performed for a single nanoparticle suspended in pure water. Light is incident along the x axis and polarized along the z axis. The figure shows a snapshot in time, and color corresponds to the enhancement of the electric field \((E/E_0)\). The scale for enhancement is shown to the right. Note that each NP has a different \(z\) and \(y\) length scale.

Figure 2.4: Showing electric field enhancement \(E/E_0\) for a sphere (a), nanorod (b), and bipyramid (c) [5]

Simply by examining the scales for each figure, we can see that the nanorod has much higher enhancement than the sphere, and the bipyramid has much higher enhancement than either of the other two. The spheres reach a maximum local field enhancement of 6, approximately 80 for the nanorods, and approximately 300 for the bipyramids. Thus, field enhancement becomes stronger for sharper tips at the ends of the bipyramids. The region of highest enhancement is also three times larger for the bipyramids than the nanorods. So, we can see that bipyramids offer somewhat higher maximum enhancement and this enhancement fills a significantly larger volume. Liu also observed that a sharper tip at the end of the particles results in a redshift in the LSPR resonant wavelength [5].

These FDTD results from Liu et al. suggests bipyramids may be a promising candidate for further study, but we are ultimately concerned with how a particle will perform when deposited on a semiconductor substrate, not simply suspended in water. In 2008-2009, See performed FDTD calculations for several nanoparticle shapes. In these calculations, a
A nanoparticle is deposited onto a 3 x 5 μm CdS nanosheet and light is incident onto the particle normal to the plane of the sheet (see Fig. 2.5) and is polarized in the plane of the nanosheet [4].

**Figure 2.5:** An E-field incident along the z direction on a gold nanosphere on top of a CdS nanosheet

See first found that when light is incident on a nanosphere normal to the plane of the nanosheet (NS), just outside of the CdS NS the maximum enhancement is 5.1, in very good agreement with Liu et al. However, there is little or no penetration of the field into the CdS NS. Results were better when the electric field was incident at 45º to the CdS NS as some penetration into the NS occurred. However, such a physical setup is highly impractical to implement.

A nanotriangle was then examined. The isosceles gold triangle had a base of 50 nm and height of 75 nm. It was found that the maximum field enhancement outside of the nanosheet occurs at the sharp tip of the triangle and has a value of 13.5, more than twice the enhancement observed with the sphere. Just inside of the sheet (< 1 nm) the enhancement is 5.6 and it falls to 1/e of that value in 6 nm [4].

These triangles are then placed into a dimer configuration, in which two triangles are placed 20 nm apart, tip to tip (see Fig. 2.6). This configuration was found to have an enhancement of 20.5 just outside of the nanosheet (Fig. 2.7(a)) and 3.5 at 5 nm into the nanosheet (Fig. 2.7(b)). Once again the peak enhancement occurs at the tips of the triangles. The enhancement reaches its 1/e value at approximately 10 nm into the sheet. As seen in Fig. 2.7(c), at 40 nm into the sheet, there is still a field strength of 0.62 E₀, which is considerably larger than one would expect from light incident on the nanosheet in the absence of gold nanoparticles.
Figure 2.6: Two gold triangles on a CdS nanosheet in a dimer configuration [4]

Figure 2.7: Field enhancement $E/E_0$ for a triangle dimer at the CdS nanosheet surface (a), 5 nm into the nanosheet (b), and 40 nm into the sheet (c). Note that the strongest enhancement occurs at the sharp tips of the triangles, but this enhancement merges together into one large region by the time you reach the back of the nanosheet. [4]

This nanotriangle was then altered to form a “block-tip,” which is a triangle with a square rather than a sharp tip (see Fig. 2.8). These were then placed into a dimer configuration similar to that of the triangle dimer, separated again by 20 nm. This block tip dimer had a maximum enhancement of 7.8 just outside of the nanosheet, much lower than the triangular dimer configuration. These peak enhancements occur at the front edges and corners of the block tip, as seen in Fig. 2.9(a). At 5 nm into the sheet the peak enhancement is just 0.7 (Fig. 2.9(b)), but even 40 nm into the sheet it has fallen to just 0.6 (Fig. 2.9(c)). This suggests that while the overall enhancement is noticeably lower than other configurations, it dies off much more slowly inside of the nanosheet. This is presumably because of the larger size and effective area of the particle [4]. As we move to 5 nm and 40 nm into the sheet, we see the sharp, narrow regions of
enhancement merge together into larger regions of enhancement, resulting in one large relatively uniform enhancement region 40 nm into the sheet.

Figure 2.8: Block-tip dimer configuration, on a CdS nanosheet. Note that the dimensions (other than size of tip) are the same here as in the triangle dimer [4]

Figure 2.9: Enhancement from block-tip dimer at the surface of the CdS NS (a), 5 nm into the nanosheet (b), and 40 nm into the sheet (c). Again, strongest enhancement occurs at sharp edges and tips at the surface of the sheet, merging into larger regions of enhancement of smaller magnitude the farther one advances into the sheet. [4]

In summary, we see the largest enhancement from a triangular dimer, and this large enhancement occurs at the very sharp tips. We then see lower enhancement, but better nanosheet
penetration from the “blunt” block-tip dimer. This suggests that for our device we would like a particle with very sharp points but also a relatively large cross-sectional area, as this could potentially maximize both enhancement and the penetration of this enhancement into the nanosheet. With these considerations in mind, a bipyramidal shaped nanoparticle seems to be a likely candidate.

2.3 The Semiconductor CdS Nanosheet Device Without Au Nanoparticles

The proposed semiconductor biosensor employs changes in induced photocurrent to detect the presence of the desired analyte (or captured target molecule). The biosensor our research group has designed and fabricated consists of a CdS nanosheet between electric contacts, coated with prepared and functionalized gold nanoparticles, and illuminated by a laser. This laser excites excitons (electron – hole pairs) in the NS, which are separated by a bias voltage applied across the contact pads. These charged particles are then mobile, resulting in a photocurrent. An image of a nanosheet between contacts on a Si, SiO$_2$ substrate can be seen in Fig. 2.10. Note via the 20 μm scale bar that the nanosheet has an exposed area of approximately 3 μm x 5 μm in the Gap region.

![Image of nanosheet](image)

**Figure 2.10**: The basic biosensor device where the CdS nanosheet is just visible at the center of the image (shown by arrow) between two metallic contacts.

These devices can be prepared with either Schottky or Ohmic contacts. A key difference between these two contact types is the size and location of the photosensitive region. When under a positive 10 V bias, the two types show significantly different behavior, shown in Fig. 2.11. For Schottky contacts, the photosensitive region is highly localized near the reverse bias contact. This indicates that the electrons are much more mobile than holes, allowing electrons to diffuse across the device before being collected at the forward bias contact, while the holes are confined to the reverse bias contact. In contrast, the Ohmic contact device shows a photosensitive region evenly distributed across and centered at the middle of the sheet. This indicates that both electrons and holes have similar mobilities and that the average distance before recombination is greater than the distance between the contacts. This suggests that an Ohmic device will be better suited for this application, allowing greater versatility, functionality, and ease of use [6].
The device can be illuminated to induce a photocurrent in two ways. The first is by simply using a laser with energy above the band gap, thus giving every incident photon the ability to excite an electron-hole pair, resulting in a photocurrent. This single photon absorption photocurrent is described by equation (1.17), exhibiting a linear dependence on the power, $P$, of the laser. The other possibility is to use a laser with energy less than the band gap energy, but greater than half the band gap. In this situation, two photons are required to excite an exciton (electron-hole pair) above the band gap. This two-photon absorption induced photocurrent is a nonlinear optical process described by equation (1.18) and exhibits a quadratic dependence on laser power [6].

$$I_1 \propto \frac{P}{\hbar \nu}$$  \hspace{1cm} (1.17)

$$I_2 \propto \frac{P^2}{2\hbar \nu}$$  \hspace{1cm} (1.18)

The bare devices (without a coating of gold nanoparticles) were tested by our group at the University of Cincinnati. Fig. 2.12(a,b) shows the results for a Schottky device and Ohmic device, respectively, for both 800 nm (black squares) and 488 nm (red circles) excitation. The 488 nm excitation is above the band gap energy, allowing for single photon absorption, while 800 nm excitation is below band gap, but above half of the band gap. For CdS the band gap is 2.50 eV (496 nm), so half the band gap would be 1.25 eV (992 nm). Both plots are on a logarithmic scale [6].
Figure 2.12: Photocurrent vs laser power plots for a Schottky device (a) and Ohmic device (b). 800 nm (sub-band gap) shown in black squares and 488 nm (above band gap) excitation shown in red circles. Red data points correspond to red axes on right and top, black points with black axes on left and bottom. We see 2-photon absorption for higher powers in the Schottky device and at low powers in the Ohmic device [6]

In both the Schottky (a) and Ohmic (b) device results, we see that the 488 nm excitation results in a linear dependence of current on power with a fitted slope of 1. Note that the photocurrent saturates at ~100 nA in the Ohmic device for laser powers above 4 nW. Noting the logarithmic scale of the plots, this implies a linear dependence of current on laser power, which indicates single photon absorption. For the Schottky device, at low powers of 800 nm excitation we see the same slope of 1 at low powers, but then a shift at higher powers to a slope of 2, indicating a dependence of $P^2$. This is a more complex power dependence than expected. However, 2-photon absorption is observed in this device at higher powers. The Ohmic device exhibits this 2-photon absorption behavior at low powers. At higher powers there seems to be a decrease in this slope, suggesting a saturation of excitons in that region of the nanosheet [6].

The choice between a single-photon absorption and 2-photon absorption configuration is made clear by the introduction of gold nanoparticles into the system. As seen several times in this chapter, gold nanoparticles exhibit strong enhancement of local incident electric fields when in resonance. Also note that changes in this resonance wavelength can be caused by changing the dielectric constant of the medium surrounding the particle, which can be achieved by capturing an analyte molecule. A device can thus be designed such that capturing a molecule of interest will cause the particles to shift closer to or farther from resonance, in turn increasing or decreasing enhancement of electric fields penetrating into the nanosheet, respectively. One would like for this enhancement to cause as large a shift in photocurrent as possible, making a
quadratic dependence on power much more desirable than a linear one. See Fig. 2.13 for a visual reminder of the fully operational plasmon-assisted biosensor’s functionality. Fig. 2.13(a) shows a laser incident on a bare CdS nanosheet, resulting in low photocurrent. Functionalized nanoparticles, represented as green circles in Fig. 2.13(b) (nanoparticles not to scale), also result in low photocurrent in this particular setup.

Only when we capture the analyte in question, as in Fig. 2.13(c) with nanoparticles that have captured an analyte molecule shown in red, does the LSPR of our particles shift into strong resonance with the incident laser to obtain the related strong enhancement of the photocurrent. Thus the Au plasmon-assisted biosensor becomes a device which senses the capture of target molecules with an almost TTL-like signal.

2.4 Au Bipyramidal Nanoparticle Synthesis

Through the course of this work a seed-mediated growth process is employed. It requires the growth first of very small spherical gold nanoparticles. These are then used as a “seed”, upon which larger particles of varying shapes and sizes can be grown.

The growth of gold nanoparticles, both seeds and bipyramids, is based on the supersaturation and precipitation of a solution of atomic gold. Chloroauric acid ($HAuCl_4 \cdot 3H_2O$) dissolved in water results in Gold (III) ions. In the presence of a strong reducing agent these ions are reduced to atomic gold, the aqueous saturation point for which is very nearly 0 [7]. So, the atomic gold precipitates out of solution and tends to crystallize. Stopping this growth quickly and capping off the nanoparticles results in a seed solution of very small nanoparticles. Left unhindered, these crystals will continue to aggregate (grow larger and
combine with other gold crystals) until they are visible to the naked eye, given enough gold is present. So, to produce uniform nanometer-scale particles, a stabilizing agent is required.

There are a number of stabilizing methods in use today to prevent the aggregation of particles. The most common is the use of trisodium citrate. This serves two functions: to control the size of the nanoparticles and to prevent them from aggregating once growth is complete. Trisodium citrate dissolves into sodium and citrate ions in water. The sodium remains unused and the citrate accomplishes both of our goals. First, to control particle size, citrate serves as a “capping agent.” It bonds to the surface of gold nanoparticles, slowing and eventually halting their growth. Depending on the concentration of citrate and the solution temperature, this results in particles ranging from about 3 – 20 nm which can be finely tuned for highly homogenous particle size [8]. Citrate also stabilizes the solution, preventing aggregation, by imparting a negative charge to the otherwise neutral nanoparticles. Because all of the particles are then negatively charged, they repel each other and will tend not to aggregate. Prepared this way, particles are stable for 1-2 months [8].

The other common method of controlling nanoparticle growth is hexadecyltrimethylammonium bromide (CTAB). This is used in the growth of non-spherical particles. Much like sodium citrate, CTAB bonds to gold crystal faces and halts growth on that face. However, CTAB bonds much more readily to some facets than others, therefore halting growth in some directions while allowing it to continue elsewhere. CTAB tends to bond to more stable crystal faces, such as the {100} face [9] (see Figs. 2.14(a,b)).

![Figure 2.14: The crystalline faces of gold nanoparticles. CTAB will bond preferentially to the {100} face (a). The only space available for Au growth is along {111} (b) [8].](image)

This preferential bonding results in elongated particles, such as nanorods and bipyramidal structures. The aspect ratio of nanorods and finer shape control to achieve bipyramids and other similar structures can be directly controlled by varying the concentration of CTAB present [8,9]. The Au bipyramidal nanoparticle synthesis processes will be revisited in detail in Chapter 4, but we first experimentally examine the spectral behavior of spherical gold nanoparticles.
Chapter III
Characterization of Gold Nanospheres

3.1. Methods

In order to gain a greater understanding of the behavior of gold nanoparticles and the role of functionalization (as necessary for the plasmon enhanced biosensor), time was spent examining the spectra of gold nanospheres. We examined 3 samples – bare gold nanoparticles, particles that have been functionalized with Streptavidin, and functionalized particles that have captured Biotin. Streptavidin and Biotin are chosen because they are readily available and have very strong, well-known, and specific binding. All samples are placed in 1 cm path length optical plastic cuvettes and sealed with a paraffin film. Bare spherical nanoparticles are provided by Ted Pella Inc. while functionalized spherical particles are provided by BBInternational. When biotin is introduced to functionalized samples, the sample is mixed in a sonicator for 10 minutes before any spectra are taken. This ensures bonding and capture of the biotin by the streptavidin molecules. When diluting samples, de-ionized water is used (1 MΩ-cm).

For white light excited spectra, a fiber optic illuminator is used. It is shone through a neutral density filter, a linear polarizer, a narrow iris, and finally a convex lens to focus the beam. This focused beam is then incident on the sample cuvette. This setup can be seen in Fig. 3.1 and schematically in Fig. 3.2. For absorption spectra the spectrometer is in line with the excitation beam (blue ray). This allows us to collect a transmission spectrum, from which an absorption spectrum can be calculated. Scattering spectra are separated out by placing the spectrometer in line with the red ray, which is at 90º to the incident light line. This allows you to observe light scattering from the NPs while avoiding any direct incident light from entering the spectrometer’s fiber. All spectra are taken using a fiber optic Ocean Optics USB 4000 spectrometer with 1200 mm⁻¹ grating (0.11 nm resolution) and are processed using the Spectra Suite software package.

Figure 3.1: A photo of the setup used to measure bulk solution spectra of spherical gold nanoparticles.
For the white light transmission spectral measurements, the integration time was set to 8 ms and data was averaged over 500 scans. From these transmission spectra, absorption ratios are calculated using equation (1.19) where $trans$ is the transmission intensity and $excitation$ is the intensity of the incident excitation light at each respective wavelength.

$$abs = \frac{excitation - trans}{excitation} = 1 - \frac{trans}{excitation}$$  \hspace{1cm} (1.19)

Based on this calculation, a value of 0 is no absorption and a value of 1 is complete absorption at a given wavelength.

3.2. Results and Discussion

Figure 3.3 shows the transmission spectra for all 3 types of gold nanoparticle samples as well as the white light source through de-ionized water (black line). This is obtained by observing the blue output ray, which traverses the entire cuvette in Fig. 3.2 with a spectrometer. The sample was diluted to 4 parts deionized water : 1 part nanoparticle solution. Here, AuNP denotes bare particles (green line), AuNP + Streptavidin the functionali zed (red line), and AuNP + Streptavidin + Biotin the functionalized particles which have captured Biotin (blue line).
Figure 3.3: The white light source and the corresponding transmission spectra of all 3 gold nanospheres samples, diluted to a 4:1 ratio. White light source (black line), bare particles (green line), functionalized (red line) and biotin-captured (blue line).

Note that the loss of intensity in all samples is across the entire observed wavelength range with the largest loss due to absorption below 575 nm, and the remainder due to scattering. The transmitted light intensity from the Streptavidin sample is nearly 0 from 500 nm to beyond 550 nm, thus this sample is clearly not dilute enough to use for calculation and characterization of the nanospheres. So, the samples were then diluted to a 7:1 (water : nanoparticle) concentration. The transmission results for this concentration are shown in Fig. 3.4.

Figure 3.4: Transmission spectra for all 3 spherical nanoparticle samples. White light source (black line), bare particles (green line), functionalized (red line) and biotin-captured (blue line).
The spectra in Fig. 3.4 are now suitable for analysis. While the spectra of all solutions follow the white light excitation spectrum over much of the long wavelength range, we can even infer that the bare AuNP will have an absorption peak somewhere between 500 and 550 nm which will be more narrow than the other 2 resonance peaks. The streptavidin functionalized and biotin samples clearly have higher absorption, which extend much further into longer wavelengths. We can also see that the Streptavidin sample is highly absorbing. Absorption spectra are calculated using Eqn. (1.19).

Figure 3.5 shows the resulting absorption spectra for all 3 samples, and Table 3.1 gives the peak positions for each sample.

![Absorption Spectra](image)

**Figure 3.5**: Absorption spectra for white light excitation, 40 nm AuNP. Bare in green, functionalized in red, biotin captured in blue.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Peak (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuNP</td>
<td>527</td>
</tr>
<tr>
<td>AuNP+S</td>
<td>530</td>
</tr>
<tr>
<td>AuNP+S+B</td>
<td>537</td>
</tr>
</tbody>
</table>

**Table 3.1**: LSPR Peaks for 3 samples from Fig. 3.1

We can see that the addition of each “layer” of molecules to the outside of our spherical nanoparticles results in a redshift of the LSPR peak. The sample which has captured biotin has a much more broad spectrum, with a tail extending past 800 nm. This is to be expected because we now have two molecules bonded together on the surface of the gold. These two molecules bonded together have a large number of vibrational modes with widely varying energies. Also note that should we use these particular particles in our biosensor device, we would expect a 7 nm shift in resonance in the presence of our target molecule versus the bare AuNP. This redshift is not surprising. By examining the Fröhlich Condition (1.12), we can see that, as $\epsilon_m$ increases, there will be a corresponding redshift in the particle’s resonance. Recall that $\epsilon_m$ is the material that the NPs are surrounded by. The $\epsilon_m$ for a bare nanoparticle is that of water, or $\sim 1.15$. So, adding additional molecules to the coating around the particle will increase $\epsilon_m$ and therefore
cause a redshift in resonance. Also note the long tail into the red for the AuNP which has captured biotin. This is expected due to the long, chained organic molecules which have many rotational and vibrational modes with low energy that can be excited by the incident white light and although streptavidin is a reasonably long (~5 nm) molecule, up to 8 biotin molecules can be captured by a single streptavidin molecule.

We can also examine the particles’ scattering spectra by observing the red ray in Fig. 3.2 with a spectrometer. The spectrometer was set for an integration time of 60 seconds and 15 scans were averaged for each sample. Because the intensities from the scattering of these particles are very weak when observing with the small cross-section of a fiber-optic spectrometer, the resulting spectra grow noisier with lower intensity (see Fig. 3.6 – 3.8). However, it is still possible to identify the scattered spectra through additional analysis. Due to their asymmetry, these spectra were fit very closely with a dual-Gaussian fit, in which the curves were allowed to be fit by a different Gaussian on either side of the peak. These fits are shown in Fig. 3.9.

![Fitting AuNP Scattering to Gaussian](image1)

**Figure 3.6:** Raw scattering data for bare AuNP (green) and corresponding dual-Gaussian fit (black) with peak at 618 nm

![Fitting AuNP+S Scattering to Gaussian](image2)

**Figure 3.7:** Raw scattering data for streptavidin functionalized AuNP (red) and dual-Gaussian fit (black) with peak at 742 nm.
Figure 3.8: Raw scattering data for biotin-captured AuNP (purple) and dual-Gaussian fit (black) with peak at 720 nm.

Figure 3.9: The Gaussian fits for the scattering spectra of all 3 nanosphere samples.

We can see in Fig. 3.9 that the sample that has captured biotin has the strongest scattering intensities, followed by the functionalized sample, and finally the bare particles have the weakest scattering intensity. We see a large redshift when we go from bare to functionalized particles. This corresponds to an increase in $\varepsilon_m$. There is then a slight blue shift when the particles capture
biotin. It is also important to note that the wavelength positions of peaks are significantly redshifted, reflecting the input light’s spectrum and how much remains to scatter after absorption. As noted in Chapter 2, it is expected that absorption will dominate over scattering in the extinction behavior of gold nanoparticles when their size is much smaller than the incident wavelength, which is certainly the case here. So, in the examination of the behavior of our gold nanoparticles, we will concern ourselves primarily with the absorptive behavior of the particles, as it will serve as a matching descriptor of their resonance for enhancement of the E-field we want to excite the charge carriers in the CdS nanosheets.

We have seen in this chapter that gold nanospheres have distinct spectral behavior specific to their size and coating. We also saw in Chapter 2 that a gold nanoparticle with sharp points and a LSPR peak near 800 nm is ideal for our semiconductor biosensor application which would require extremely large Au nanospheres. We now therefore turn our attention to bipyramidal gold nanoparticles, which may satisfy both of these conditions. We will first need to synthesize these bipyramidal particles, characterize them, and finally functionalize them in preparation for application.
Chapter IV
Synthesis of Bipyramidal Gold Nanoparticles

4.1. Introduction

We saw in Chapter 2 that there are several characteristics we would like to have in an ideal nanoparticle for our biosensor application. First, due to the CdS band gap, the NP should have a resonance near 800 nm, allowing for 2-photon absorption on the CdS nanosheet. Second, it should have sharp points, causing much higher enhancement of electric fields. Finally, we have seen that larger particles allow deeper penetration into a CdS nanosheet which will allow more excitons to be excited before saturating the device. An excellent fit for all of these criteria is a gold bipyramidal nanoparticle. Bipyramidal particles refer to the nanoparticles that have a long, diamond-like shape and sharp tips. A diagram is shown in Fig. 4.1(a) and an example TEM in Fig. 4.1(b).

Figure 4.1: A diagram characterizing a bipyramidal gold nanoparticle (a) and an example TEM of a bipyramidal nanoparticle at 800 kx magnification (b).

Recently, bipyramidal gold nanoparticles (AuBPs) have been of great intrinsic interest for their physical characteristics, as well as broad interest because of their immunoassay applications. AuBPs have localized surface plasmon resonance (LSPR) peaks with high sensitivity to changes in the surrounding index of refraction. They are also preferred because they exhibit higher plasmon enhancement than the comparable gold nanorods, because of their sharp tips [10]. AuBPs have a wide range of potential LSPR peaks, ranging from 700 nm – 1100 nm depending on their length and tip sharpness [11]. This makes them nanoparticles of choice for immunoassay and plasmon enhanced semiconductor applications (like our biosensor) due to their strong plasmon enhancement, which assists in exciting extra charge carriers, and wavelength resonance tenability, which is desirable for different semiconductor materials [5]. Here we wish to chemically synthesize and investigate highly stable AuBPs with a LSPR peak in the 800-850 nm range. These AuBPs are approximately 80 nm long and ~ 30 nm wide at their thickest point.

Several groups have grown AuBPs in the presence of silver ions and high concentrations of the surfactant CTAB [9, 10, 12]. These nanoparticles are penta-twinned crystals of relatively
high uniformity and small radius of curvature at both longitudinal tips. Growths resulted in bipyramids rather than nanorods based on the crystalline nature of the seed particles used in growth, e.g. penta-twinned seeds result in penta-twinned bipyramids, while single crystalline seeds result in nanorods, cubes, octahedrons, and other particle shapes [12]. The presence of Ag(I) in the growth solution results in a slowing of the growth process, but also allows for the formation of bipyramidal particles [12]. While several groups have studied Ag-mediated growth of bipyramids, a detailed study of AuBP spectral evolution and morphology as a function of growth time, growth temperature, and silver nitrate concentration has not been reported.

As seen in Chapter 2, the size and shape of gold nanoparticles affects their LSPR peaks and thus their extinction spectra [3]. LSPR also results in strong enhancement of local electric fields [3,13]. For AuBPs, theoretical calculations show a correlation between the length and tip radius of curvature of bipyramids and the resulting resonance peaks [5]. We report here a detailed examination of the evolution of bipyramidal gold nanoparticles both during and after the primary growth process using a consistent seed solution, as well as the dependence of the growth kinetics on Ag(I) concentration. We also present an experimental correlation between the LSPR resonance peak wavelength and both the length along the longitudinal axis and the radius of curvature of the bipyramids. The results provided here form a strong foundation for fine-tuning the growth of Au bipyramids to prepare them for specific applications where particular enhancement properties of the nanoparticles are essential.

4.2. Synthesis of Seed Solution

4.2.1 Methods for CTAB and Citrate Terminated Seed Growths

Our synthesis of AuBPs closely follows the protocol presented by Senapati et al. with a few modifications [9]. See Appendix A for details on preparing individual reagent solutions. The first step of this process is the synthesis of a seed solution, which is a solution of small spherical gold particles. Our seed particles were synthesized by first dissolving 5 mL of 0.2 M CTAB instead of the 0.1 mM tri-sodium citrate suggested by Senapati et al. CTAB is not easily dissolved in water. This is achieved by sonicating the CTAB in water for 30 minutes, until the solution goes from milky white to clear. The process is facilitated by raising the solution temperature slightly, just above room temperature (25º C).

We then add 5 mL 0.5 mM HAuCl₄ under vigorous stirring at room temperature. This is a critical step in the synthesis. This chemical comes in powder form and is stored at 4º C and is highly hydroscopic, meaning that when exposed to air it will rapidly pull water out of the atmosphere. If this continues too long, the salt-like powder turns to a yellow sludge. Even before that occurs, however, pulling water from the atmosphere makes any mass measurements of the powder much less accurate. To avoid this, one must measure by mass difference rather than measure the mass directly. First (once it has come to room temperature), take the mass of the vial of salt. Then open the vial, quickly take a small amount in the end of a glass pipette and dissolve this immediately. This method means the vial is open for only a few seconds for any measurement, and allowing the vial to come to room temperature prevents condensation on the interior of the vial once the vial is opened. This vial of powder must be refrigerated for long-term storage, as it will slowly decompose at temperatures at or above room temperature.
The solution now consists of dissolved CTAB and free Au(III) ions, which must be reduced. Our reducing agent is NaBH$_4$, which, at room temperature, reacts violently with water either in solution or even from the air. When ice cooled, however, it is inert. So, we ice cool the stock NaBH$_4$ solution and the water required to bring it to the necessary concentration of 0.01 M. This reducing agent is then kept ice cooled until added to the growth solution within an hour after dilution.

While stirring, 0.60 mL of 0.01 M ice cooled NaBH$_4$ is slowly introduced into the room temperature seed growth solution. The solution is stirred gently for 2 minutes and then covered tightly and stored in the dark at 30º C overnight to allow for the reduced Au in solution to precipitate into small CTAB terminated spherical crystals, which is a very slow process at these concentrations. This results in 10 mL of CTAB-terminated seed solution.

Another type of seed solution was prepared for comparison to our CTAB-terminated seed. The above process was repeated identically except that the CTAB was replaced with sodium citrate, the final concentration of which was 0.1 mM. This results in 10 mL of citrate-terminated gold seed particles. Both of these processes result in spherical gold nanoparticles, 50% of which are face-centered-cubic crystals, and 50% are penta-twinned crystals.

4.2.2 Measurements to Determine Best Terminating Molecule for Seed Solution

Figure 4.2 shows the spectra for both the CTAB (blue) and citrate (red) terminated gold seeds. We can see that their absorbance peaks fall at nearly identical wavelengths (525 for citrate-terminated seeds, 526 for CTAB terminated seeds), with the citrate-terminated seed having the slightly narrower peak. It was found that the CTAB seed proved more stable over time and with changing temperature (particularly at room and slightly elevated temperatures). By more stable, we mean that during and after the seed growth process the particles more reliably stay suspended in solution without precipitation.

![AuNP Seed Spectra](image)

**Figure 4.2:** The absorbance spectra for a citrate-capped seed solution (red line) and a CTAB-capped seed solution (blue line).
Once a stable Au nanoparticle seed solution was prepared and characterized, we could then move on to synthesizing the bipyramidal nanoparticles. All AuBP synthesis described from here onward, unless otherwise noted, was prepared using this CTAB terminated seed solution.

4.3. Synthesis of Bipyramidal Particles from Seed Solution

4.3.1 Methods

The synthesis of gold bipyramids was carried out at $30.0 \pm 0.2 \, ^\circ C$. Initial studies of AuBP growth indicated that one of the important factors in the AuBP growth was the temperature of growth. Studies at $33^\circ C$, $30^\circ C$, and $27^\circ C$ showed that a temperature of $30^\circ C$ exhibited the best morphological specifications after synthesis.

In brief the AuBP synthesis was performed first by combining 4.25 mL of 0.4 M CTAB, dissolved as described above, with 200 µL of 10 mM HAuCl$_4$, 30 µL of 10 mM AgNO$_3$ and 32 µL of 0.1 M ascorbic acid, all under gentle stirring. Here ascorbic acid is our partial reducing agent and the seed particles will complete the reduction of gold ions in solution. Stirring is then immediately stopped and we add 50 µL of our previously prepared seed solution. This differs from the protocol presented by Senapati et al. where vigorous stirring is used and is not halted before the addition of gold seed [9].

Some growths proceeded from the seed solution inside of a temperature-controlled spectrometer in order to monitor the initial growth and successive temporal kinetics via their spectral evolution (see Sec. 4.3.2), while bulk preparations were synthesized as above simply using a constant dry temperature bath during growth. After the seed solution is added, the solution is inverted once to ensure reagent homogeneity. A small volume is removed to a 4 mm path length cuvette and placed in the spectrometer. Temperature is maintained by a heated cuvette holder, provided by circulating water in thermal contact with the sample mount. During and after the growth, TEM samples are drawn from the dry temperature bath solution for imaging. TEM slides are prepared by pipetting a small droplet of AuBP solution directly onto a TEM grid, which dries quickly and halts any growth in the droplet. Samples grown both in the dry bath and wet bath cuvette produced spectra that varied little when sampled at the termination of the initial fast growth of the temporal evolution study.

Following this overall protocol (Sec. 4.2 to Sec. 4.3.1) usually results in a successful growth. However, one occasionally ends up with an unsuccessful growth. There are several ways to tell whether the growth has been successful and should be used for further study. Fig. 4.3 (a,b,c) shows the 3 indicators for an unsuccessful growth – the color of the growth solution, the UV-vis-NIR spectrum, and a TEM image (with 800 kx magnification), respectively.
Figure 4.3: Showing the solution color (a), absorbance spectrum (b), and a TEM sample (c) for an unsuccessful bipyramidal growth. Note the distinct blue color of the solution, the single very broad peak in the spectrum, and the small, oddly and inconsistently shaped nanoparticles.

First in Fig. 4.3 (a), the resulting solution is dark but a very vivid blue color. This color is first visible at approximately 30 minutes into the growth and then simply intensifies for the remainder of the growth period. Fig. 4.3 (b) shows the corresponding absorbance spectrum where the peak is at 624 nm and there appears to be a “shoulder” near 720 nm. The entire spectrum consists of a single extremely broad peak, indicating a range of particle sizes, but not much asymmetry or homogeneity. An asymmetrical NP such as a AuBP or nanorod would exhibit 2 spectral peaks: one for the narrow portion, or diameter of the particle, and one for the long axis. The TEM in Fig. 4.3 (c) bears this out. This growth exhibits several particles, each of which is very irregular in shape with no two quite alike. Clearly these are not the desired, well-formed bipyramidal gold nanoparticles exhibiting the expected two different resonance peaks. A well-formed, successful growth is shown in Fig. 4.4 (a,b,c).
Figure 4.4: Showing the solution color (a), absorbance spectrum (b), and a TEM sample (c) for a successful bipyramidal growth. Note the vivid purple solution color, the 2 distinct absorbance peaks in the absorbance spectrum, and the high population of bipyramidal gold nanoparticles in the wide-field TEM image.

It is immediately evident that distinct differences exist between Fig. 4.4 and Fig. 4.3. Fig. 4.4 (a) shows a vivid purple color. This color is again visible at approximately 30 minutes of growth and continues to intensify during the growth process. Identifying this color is an excellent way to very quickly determine the probability that growth is going to be successful. However, while this purple color does not guarantee a successful growth, any other color (or a lack of color) in the growth solution indicates a growth that will certainly not be successful.

The absorbance spectrum in Fig. 4.4 (b) shows 2 distinct absorbance peaks, corresponding to the longitudinal (827 nm) and transverse (570 nm) LSPR peaks of bipyramidal particles. Non-bipyramidal particles also contribute to the large 570 nm peak. It is the presence of the 827 nm peak in particular that indicates the presence of bipyramidal gold nanoparticles.

Once the solution color and absorbance spectrum indicate the presence of bipyramidal particles, a TEM provides the final confirmation. Figure 4.4 (c) shows such a TEM. This 800 kx magnified image shows many particles, about half of which are bipyramidal particles. These bipyramids are all very similar to each other in shape and size, have the desired elongated structure with sharp tips, and are suitable for further study.
After the correct time for the growth process was determined, each sample believed to be successful at that point was centrifuged in order to remove all excess CTAB and reagents, as well as to separate the larger bipyramidal particles from the smaller irregular particles. The process serves to stabilize the particle suspension as well as to halt the growth process. Once the excess CTAB is removed, there is still a thin layer of CTAB coating the particles, which ensures their long-term stability in water. Samples were centrifuged 3 times for 5 minutes each time. After each spin, a small volume of particles collected at the bottom of the vial. The supernatant is then removed and replaced with an equal volume of deionized water. The supernatant contains excess CTAB, smaller particles with less mass, and trace amounts of other reagents. This process is repeated three times, until the absorbance near 200 nm due to CTAB is greatly reduced and foaming of the solution does not occur when shaken. The ratio of the long \( \lambda \) longitudinal to the short-wavelength peaks is calculated from absorption spectra before and after the sample has been centrifuged and resuspended in deionized water 3 times. A higher ratio indicates a higher percentage of bipyramids in the final solution. Before centrifugation, we find a typical ratio of approximately 0.5. Tests were run at 6 different centrifugation speeds with radial centrifugal forces (RCF) ranging from 800g – 3800g. From the resulting absorbance spectra we found an optimal post-centrifugation ratio of 1.5 near an RCF of 3200g. This result can be seen in Fig. 4.5, where the spectrum before spin (green line) exhibits a high peak at ~560 nm compared to the peak near 800 nm while the spectrum after the spin (green line) has a higher peak at ~800 nm than the peak near 560 nm.

![Sample Purification](image)

**Figure 4.5**: Absorbance spectra of gold bipyramids before (green) and after (red) purification in centrifuge.

In examining TEM images from each spin speed, no significant difference was found in the ultimate size of the bipyramids with spin speeds. This indicates that the only measurable difference is the resulting fraction of bipyramids in the final suspension. Once resuspended in deionized water, the AuBPs exhibit consistent spectra for months, meaning the final AuBP solution was stable. In all of the experiments described in which purification and stabilization
has been performed, the RCF of 3200g was used to maximize the number of AuBPs with longitudinal axis of ~90nm.

4.3.2 Growth Spectra Measurements

Once we identified a growth process that successfully produced nanosized bipyramids with the desired longitudinal resonance peak, we examined the stability and growth kinetics of the particle solutions during the growth process and beyond. This is accomplished by monitoring the sample growth and lifetime inside of a temperature controlled spectrometer. Spectra of a sample are taken periodically (at 5 minute intervals during the initial growth process, and at 20 minute intervals after the growth has saturated) to observe bulk AuBP characteristics as a function of time. Note that because these spectra are taken over time during the growth process in a spectrometer, they are necessarily taken before any centrifugation has taken place.

Figure 4.6 includes 3 absorbance plots for 3 different growth solutions Growth A, B and C, where optical spectra are measured periodically over an extended period of time. Growth A was observed for ~240 minutes and Growth B was observed for ~ 160 minutes. In Fig. 4.6(a) of Growth A, the longitudinal LSPR spectral peak (that associated with the long axis of the AuBPs) starts at 681 nm and grows in intensity while red shifting to 803 nm. The short-wavelength peak starts at 521 nm and increases to 553 nm as the short axis, or AuBP width, grows thicker. Growth B, Fig. 4.6(b), shows a mode of growth where the wavelength of peak absorbance for the longitudinal mode also starts at 680 nm, but after reaching 730 nm it blue shifts until it joins the transverse peak. An additional temporal behavior is shown in Growth C (Fig. 4.6(c)) where the longitudinal peak grows briefly and then decays, merging into an increasing background. Growth A in Fig. 4.6(a) represents the type of growth we would like to consistently reproduce because of its strong, relatively narrow, and stable LSPR peak near 800 nm. All growth types A, B, and C are obtained using solutions prepared at 30 °C and with the concentrations and volumes described in section 3.1 of this chapter. However, type A growths are consistently obtained when all growth solutions are used directly after preparation from solid compounds. This ensures that each component maintains the desired active concentration, composition, and thus behavior during the AuBP synthesis. For instance, ascorbic acid is unstable in water, oxidizing to form dehydroascorbic acid within 3 h when held at 30º C. It then spontaneously degrades into diketogulonic acid. This degradation process occurs more quickly above room temperature [14], and can be further accelerated by the presence of trace elements found in water and even on the surface of most glassware [15]. In addition, silver nitrate is known to begin decomposing when exposed to light [16]. We have already spoken about the gold salt being highly hydroscopic and the importance of the technique used in measuring and dissolving that salt to ensure correct concentrations and prevention of degradation. Using the protocol in Sec. 4.3.1 results in a type A growth ∼90% of the time. Using reagents that are several hours or older, will almost always result in type B or C growths.
Figure 4.6: (a), (b) and (c). Absorbance spectra of bipyramidal growth solutions plotted over time. The transverse peak (~500nm) appears and is stable over time, while the longitudinal peak (~800nm) exhibits stable behavior in Growth A (a), a transient blue shifting behavior in Growth B (b), and transient absorbance while maintaining peak position followed by loss of longitudinal peak in Growth C (c).
The peak wavelength (triangles) and absorbance (squares) for the longitudinal peaks from Fig. 4.6 are plotted in Fig. 4.7 (a, b and c), providing a more detailed view of the temporal evolution of the AuBPs from Fig. 4.6. In all three cases, an initial growth phase is seen where both peaks shift towards longer wavelengths and absorbance increases. This is followed by a slower decay phase (for Growths B, C) where the opposite trend for absorbance and peak position are observed for bipyramidal growth. In Growth A, the longitudinal peak position changes by $(1.3\pm0.2)$ nm min$^{-1}$ and the peak intensity increases at a rate of $(8.5\pm0.3)x10^{-3}$ a.u. min$^{-1}$ (Fig. 4.7(a)). The longitudinal wavelength reached a maximum of 805 nm at 50 minutes while the absorbance maxima of 0.65 a.u occurred at 145 minutes. At 50 minutes the growth saturates to a steady state. Figure 4.7(b) shows an absorbance value which increases slowly for 50 minutes and then slowly increases at a different rate, while the peak position never fully reaches the ~800 nm mark and stops growing along the long axis at only 30 minutes. Thereafter, it rapidly drops to join the transverse peak, dropping to near 620 nm at the end of the run. In Fig. 4.7(c), the growth begins following the kinetics of the sample in Fig. 4.6(a) peaking at a wavelength of 820 nm at around 50 minutes, while its absorbance peaks at a later time. However, the absorbance never reaches the level seen in Fig. 4.7(a); it decreases to merge with the background. Note that Growths B and C have max absorbance values near 0.17 and 0.3, respectively, while Growth A exhibits an absorbance maximum of ~0.65. We have found that growths similar to those in Figs. 4.6 and 4.7(a) are optimum and can be best reproduced by carefully maintaining the conditions discussed above.
Figure 4.7: (a, b and c). Wavelength (triangles) vs. time and absorbance (squares) vs. time showing the growth of the long axis of AuBPs for growth A, B and C respectively.

In addition to the spectra that were recorded during growth in the temperature controlled spectrometer, TEM images were both taken periodically to complete the picture of the AuBP growth. This provides the data to correlate the physical dimensions of our particles with their corresponding spectra. Figure 4.8(a) shows particles, at 800 kx magnification, representative of growths from Growth A at 20 minutes, 40 minutes, and after a steady state has been reached. Figure 4.8(b) shows the spectra corresponding to these times during growth. This time frame corresponds to the initial rapid growth process seen in Fig. 4.7(a) and the subsequent steady state. We can see that at 20 minutes the nanoparticles have a longitudinal LSPR peak at 775 nm with particle length of 58 nm and radius of curvature at the tip of 2.7 nm. At 40 minutes the AuBPs have already redshifted to 795 nm with length 71.4 nm and radius of curvature 3.6 nm. By the time the growth reaches a steady state, the final AuBPs have redshifted with longitudinal peaks at 803 nm, have a particle length of 82.7 nm, and tip radius of curvature 3.6 nm.
We now perform a similar analysis for a Type B growth, shown in Fig. 4.9. We have TEM images of representative particles at 50, 65, and 100 minutes of growth in Fig. 4.9(a) with corresponding spectra in Fig. 4.9(b) taken from Fig. 4.6(b). This full growth process can also be seen in Fig. 4.7(b).

At 50 minutes our particles have a length of 62.2 nm and radius of curvature 3.76 nm and LSPR peak of 722 nm. At 100 minutes we now see a length of 54.9 nm and radius of 6.62 nm, almost a two-fold increase. Over the corresponding time period, the spectra have blueshifted from a longitudinal LSPR peak of 722 nm to 630 nm. So, we see in the AuBPs an increase in radius of curvature and decrease in length, creating a markedly blueshifted longitudinal resonance peak.

If we compare these two modes of growth using Figs. 4.7, 4.8, and 4.9, we notice that these growths are distinctly different right from the initiation of the growth process. While both longitudinal peaks start at 680 nm (see Fig. 4.7(a,b)) Growth B reaches a peak wavelength of 730 nm in roughly the same time it takes Growth A to reach 775 nm. But, by the time we reach the 50 minute mark for Growth B, the blueshift in the spectral peak has begun, Growth A in contrast is above 95% of its steady state wavelength of 803 nm. So, if one is observing the spectra during growth, it is clearly evident whether you have a successful growth from very early times. Even
if one does not perform the growth in a spectrometer, distinct differences are evident in the color of the bulk solution. As discussed in Sec. 4.3.1, a successful growth will have a vivid purple color even at 30 minutes of growth, and it simply becomes more opaque with time. A Type B growth will remain more red/brown, or blue, and will never reach the opacity of a Type A growth.

The blueshift (and loss of longitudinal peak) observed in Type B and C growths is likely due to scenarios where the tips of the bipyramidal particles are degraded and additional growth occurs at the waist of the AuBP. Jana et al. performed reactivity experiments with bipyramidal gold nanoparticles. Upon the introduction of cyanide into bipyramidal solutions, they find blue shifts nearly identical to those shown in Figs. 4.5(b) and 4.6(b). Their TEM images reveal a transition in shape similar to that seen in our Fig. 4.8(a) [17]. They found that bipyramids are highly reactive, particularly at their sharp tips and that these tips can become oxidized and dissolve in the presence of cyanide.

We, however, see this same effect without the introduction of any additional substances into the solution. We conclude that the highly reactive AuBP tips reacted with a component of the growth solution in an unintended way, resulting in the dissolution of their tips. It is likely that because of the high curvature of the tips of the AuBPs, the surface density of CTAB is lower in some growths and thus the tips are less well protected from reagents in solution, allowing for this change in morphology to occur. We also find, as mentioned above, that solution concentration and growth temperature are very important to producing good, stable growths similar to Type A.

4.4. Silver Nitrate Study

4.4.1 Methods

Silver nitrate concentration studies were performed during the synthesis following the same methods described in section (Sec. 4.3.1) except that the AgNO₃ concentration was varied. These syntheses were performed in an attempt to “fine tune” the growth of AuBP and to examine the role of silver nitrate in the growth process, as it is not yet well understood.

The CTAB, gold salt and ascorbic acid were mixed, and then a small aliquot of AgNO₃ was added. This was then transferred to 3 wells of a 96-well plate. To increase the concentration, additional aliquots of AgNO₃ were added to the bulk solution and samples of each new concentration were transferred to the well plate for study. The resulting well plate consisted of a 3 by 5 matrix of wells, representing 5 different concentrations of AgNO₃. Seed solution was added directly to the individual wells using a multi channel pipette just before placing the plates in the spectrometer. The plates were shaken for ten seconds to mix the solution, and then spectra were recorded at regular time intervals in the temperature-controlled spectrometer.

4.4.2 Results

Two sets of experiments were conducted with 3 solutions at each of 5 different concentrations. The mean values of all of the measured quantities for the 3 samples at each of 10 concentrations are then used in the calculations and analysis to follow. From the spectra, we find that the absorbance of the longitudinal peak increased directly with the AgNO₃ concentration in the growth solution. The change in absorbance over time for the long axis, during the rapid
growth phase (0-50 min), versus the concentration of AgNO$_3$ used in the growth is plotted in Fig. 4.10. Data points are taken from the ~800 nm peak every 5 minutes until this peak saturates – this time ranges from 50 minutes to 2.3 hours, with the longest times for the lowest concentrations of AgNO$_3$. The data shows mean growth rates for values over a range of from 44 $\mu$M to 159 $\mu$M of AgNO$_3$. Measurements of higher concentration were not performed because of the resulting very low yield of AuBP. The lowest concentration tested, 22 $\mu$M, shown in Fig. 4.11, produced a single spectral peak near 500 nm and thus was not included in Fig. 4.10.

**Figure 4.10:** The plot of initial growth rate versus the concentration of silver nitrate from 2 separate experiments (stars and squares) and a linear fit of combined data.

The two different experimental runs are represented by different shaped data points, with Run 1 being red stars and Run 2 being black squares. The slope of the linear fit, with all data points in the graph included, is $26 \pm 5$ a.u. min$^{-1}$ M$^{-1}$. However, looking at the data on the plot we see that the relationship between AgNO$_3$ concentration and growth rate increases with concentration, but is not monotonic in one portion of the concentration region tested. A region of more rapid growth is seen near a concentration of 75-82 $\mu$M, and this result occurred with both solution sets and all 3 measurements at those concentrations. Note the error bars are not large for these points, indicating little variation in growth rate at those concentrations.

Individual spectra of bipyramidal growth with increasing AgNO$_3$ concentrations are shown in Fig. 4.11. Absorbance spectra have been normalized to the minimum absorbance values between the two peaks. The arrow indicates increasing AgNO$_3$ concentration from the bottom spectrum to the top. We can see that the 22 $\mu$M solution, shown in black in Fig. 4.11 (bottom spectrum), did not produce a longitudinal peak. From there, an increase in concentration monotonically yields a red shift in longitudinal peak, as well as increased absorbance. Additionally, the longitudinal axis absorbance peak for the high concentrations red shifts more than in the original growths (where the AgNO$_3$ concentration was 80 $\mu$M).
Figure 4.11: Absorbance spectra of bipyramidal growth with varying concentrations of AgNO₃. Concentrations of AgNO₃ are, from lowest to highest absorbance, 22 μM, 44 μM, 82 μM, 117 μM, and 159 μM. The spectra are from the time at which the longitudinal peak wavelength reaches maximum value. Absorbance is normalized at minimum between peaks (dotted line).

Liu et al. studied the growth of bipyramidal nanoparticles compared to that of other particles [12]. They observed slower growth with non-zero AgNO₃ compared to growths with zero AgNO₃ (which results in nanorods), reporting that it took 2 h until the end of the growth, with 92 μM AgNO₃ in their final growth solution. So, while the initial introduction of AgNO₃ slows the growth, we find that in the range of 44 – 159 μM (which encompasses the concentrations most often used in the synthesis of gold bipyramids) the rate of growth increases with the concentration of Ag(I) ions.

4.5. Gold Bipyramid Yield, Morphology, and Behavior

4.5.1 Yield and Average Morphology

We now use high-resolution TEM images to measure both AuBPs yields and the morphology of the AuBPs, along with how the morphology affects the absorbance spectra. With the TEM set between 100 kx – 200 kx magnification, the TEM images had a field of 715 nm by 715 nm which typically encompassed ~50 particles. The larger field provided as many particles as possible in which the particles of various shapes could still be identified, providing a yield count of AuBPs just after synthesis. An example of such a wide-field TEM used for statistical analysis is shown in Fig. 4.12. Only particles that are clearly visible and do not overlap with other particles are counted. Approximately 25 images similar to those in Fig. 4.12 were used in this survey, resulting in ~500 particles counted in each growth for yield analysis. Additional
TEM images can be found in Appendix B. We find we consistently have a yield of 49-50% bipyramidal nanoparticles.

This morphological yield is not surprising, as Esparza et al. found that seed solutions used in growth protocols such as the one presented here result in 2 different crystalline structures. They find that face-centered-cubic crystals make up 50% of the particles, while the other 50% of the particles are penta-twinned crystals [18]. It is only the penta-twinned seeds that will result in bipyramids, which are also penta-twinned in structure. The face-centered-cubic seeds result in particles that are cubic, octahedral or more irregular shapes. Thus our 50% yield of bipyramids is what is statistically expected given the nature of our seed mediated process.

![Figure 4.12: Wide-field TEMs, 200 kx magnification](image)

A 50% yield is not, however, ideal for practical application. We therefore purify our samples as described in Section 3.1 of this chapter using a RCF of 3200 g (see Fig. 4.5) Note that the ratio of longitudinal / short-axis peak height goes from 0.72 before purification (upper line) to 1.6 after purification (lower line), indicating a much higher percentage of bipyramids in the sample. The overall decrease in intensity indicates a decrease in the total number of particles, though TEM images indicate that the vast majority of the particles lost are smaller, non-bipyramidal particles.
To focus on fewer AuBPs at a time, we now use TEM images with higher magnification and resolution – 800 kx magnification at 0.13 nm/pixel. This creates TEM images of high enough resolution to distinguish and measure with high accuracy (± 0.2 nm) the dimensions of our nanoparticles resulting from the best growth conditions.

![Image](image_url)

**Figure 4.13:** Measurements performed on bipyramids

The length $L$, width $W$, tip radius $R$ and tip angle $\theta$ (as defined in Fig. 4.1) were measured for each of the 250 AuBP found in 25 images using the ImageJ image analysis software package. Table 4.1 shows the results of the measurements performed on typical optimal growths using what we found to be the optimal growth using the experimental growth conditions found in Type A growths. These dimension values are in good agreement with those reported by others for AuBPs to within one standard deviation [12,19,20]. Our particles have a somewhat larger measured value of $W$ than those reported by Liu et al., which is reflected in the short wavelength resonance peaks of our spectra [5].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L$</td>
<td>89.4 ± 5.3 nm</td>
</tr>
<tr>
<td>$W$</td>
<td>28.7 ± 2.5 nm</td>
</tr>
<tr>
<td>$\theta$</td>
<td>27.6 ± 2.3°</td>
</tr>
<tr>
<td>$R$</td>
<td>3.6 ± 0.6 nm</td>
</tr>
</tbody>
</table>

**Table 4.1:** Measurements from a typical AuBP growth

4.5.2 Comparison of Experimental AuBP Morphology and Spectra to Modeled Results

Generally, one wishes the AuBPs to have long sharp tips in order to enhance the plasmon resonance [12,21]. The overall size and the sharpness of the tips affect the wavelength of the LSPR peak as well as its intensity. Thus, we consider how the LSPR peak is affected by variations in physical dimensions – already knowing that we have found the growth process that is optimal for our own application. In Fig. 4.14 we plot the $R/L$ of bipyramidal particles vs. their bulk longitudinal wavelength for 7 different growths. All samples in which the TEM images
contained sufficiently well-formed AuBPs that were clear and distinct enough for accurate measurement and proper calculation of standard deviation are included. We plot R/L because these two dimensions both directly affect the longitudinal LSPR peak. Also, growing particles of completely invariant length or radius is highly impractical, as it would require a great number of growths and additional purification measures in order to obtain a sufficient number with low dimensional variation (significantly less than the observed standard deviation). The data are shown as open squares which were fit with a simple linear regression (the solid line). The error bars represent one standard deviation in the data for each axis.

Plotted along with our experimental values are the calculated resonance peaks as modeled by Liu et al. [3]. Liu used FDTD calculations to determine resonant absorbance spectra for three different AuBPs, all of the same length (L=83.4 nm), in order to determine the effect that tip radius has on the LSPR peak. Absorbance spectra were calculated for 3 radii of curvature values (R= 2.0 nm, 3.0 nm, 4.4 nm). As seen in table 4.1, our L and R values can range between 84.1 nm ≤ L ≤ 94.7 nm and 3.0 nm ≤ R ≤ 4.2 nm within one standard deviation, which is in close agreement with values employed by Liu et al. They found a linear relationship between tip radius and peak resonance for longitudinal LSPR peaks near 800 nm. Thus, their computational study models how changes in R only will affect longitudinal peak resonance wavelength. In our experimental case, both R and L vary, so some variation between points in Fig. 4.14 is not completely surprising.

![Figure 4.14](image)

**Figure 4.14:** Radius / Length measurements vs. Longitudinal LSPR peak wavelength. Open squares are experimental data; closed triangles are theoretical FDTD calculations from Liu et al. [3]

Both FDTD modeling and experiment suggest a linear relationship with a negative slope, indicating that a larger radius of curvature results in a blueshifted longitudinal LSPR peak. Conversely, particles of constant radius of tip curvature will see redshifted longitudinal resonance peaks for longer particle length, as expected. The linear regression fit from Liu’s FDTD calculations gives a slope of -1300 ± 100 (a.u.). Our experimental values also result in a
linear relationship but with a slope of $-2280 \pm 290$ (a.u.). This shows a stronger dependence of LSPR peak on tip radius than is suggested in Liu’s numerical calculations. It is also possible, however, that had Liu et al. calculated resonance peaks for larger radii of curvature, they would have found behavior more closely fitting our own findings. Also note that for particles in our data similar to those used in calculations by Liu et al. (well-formed, long particles with “sharp” ends), we see experimental data and the numerically predicted values are reasonably close given the experimental uncertainty denoted by the error bars. This region is denoted by the shaded region in Fig. 4.14.

This comparison between experimental data and modeled spectra using the FDTD method is only partially successful due to the difference in the number and the range of AuBP sizes studied. However, both show the same general dependence on length and tip radius to LSPR wavelength whose trend can be explained with our studies reported earlier in Chapter 4. Our work extends the field of AuBP morphology vs. LSPR significantly.

We now have a synthesis protocol that consistently produces well-formed, stable bipyramidal gold nanoparticles with longitudinal spectral peak in the wavelength range required for two-photon absorption in our CdS biosensor device. We have also characterized how changes in this synthesis change the resulting particles, both in their morphology and LSPR behavior. In order to finish preparing these particles for application, they must be functionalized. This functionalization process will result in nanoparticles that have a coating of a “receptor” protein molecule, specifically chosen to capture the desired analyte molecule.
Chapter V
Functionalization of Gold Nanoparticles

5.1. Introduction

Once AuBPs can be synthesized repeatably, to a high quality, and with well-defined characteristics, then the nanoparticles must be functionalized for the semiconductor CdS biosensor. We attempted this functionalization as a final extension of our efforts to prepare gold nanoparticles for use in a device, setting the ground work for future work. As long as the nanoparticles are gold, any molecule with a thiol group at one end can be attached because sulfur bonds strongly to gold. For every molecule that one wishes to sense, or target, a particular capture molecule must be designed and synthesized. In this case, to provide proof of principle of our AuBP plasmon-assisted CdS nanosheet biosensor, two relatively straightforward molecules were chosen – streptavidin and biotin. They are chosen for their availability and extremely high bond strength and specificity. During the functionalization process, either biotin or streptavidin can be made the capture molecule, with the other serving as the target molecule. This is the final step in preparing the Au nanoparticles for application in the biosensing device. The process is accomplished in several steps: first, the CTAB coating the bipyramidal particles is removed and then replaced with a new surfactant. Our receptor molecule is then attached to this new surfactant. Finally, we test the sensitivity of our functionalized particles to the presence of an analyte.

The CTAB must be removed from our particles before functionalization may proceed. This is done because CTAB is toxic and also not suitable for further functionalization. The CTAB must be replaced with a new surfactant that will bond strongly with the gold surface, provide stability for the particles (i.e. prevent aggregation), and it must be possible to chemically attach a receptor functionalization molecule to this surfactant. There are a few choices for molecules that fit these conditions, and we chose to use 3-amino-5-mercapto-1,2,4-triazole (AMTAZ). The molecular structure for AMTAZ is shown in Fig. 5.1.

![AMTAZ molecular structure](image)

Figure 5.1: The molecular structure of AMTAZ. The sulfur bonds strongly with gold. [22]

This molecule was chosen over other possibilities presented in the literature because it is smaller than other molecules presented. A smaller molecule is desirable because we would like the gold surface of the nanoparticle to be as close to the surface of the CdS nanosheet as possible in order to maximize enhancement. The sulfur in AMTAZ has a strong affinity for gold, and the molecule provides a positive charge, thus stabilizing the particles in solution [22].
5.2. Methods

The replacement of CTAB with AMTAZ is performed by closely following the protocol proposed by C Yu et al. [22]. The removal of CTAB and replacement with AMTAZ must be performed simultaneously to ensure the stability of the nanoparticles. First, the nanoparticles are spun down in a centrifuge to remove them from the aqueous solution. The supernatant is removed and the particles are then resuspended in a 1 mM AMTAZ solution of volume matching the original particle solution volume. The solution is then placed in a sonicator equipped with a heating element. Under sonication the temperature is raised from room temperature to 50°C slowly, taking roughly 30 minutes. This elevated temperature drives the CTAB from the nanoparticle surfaces, since CTAB is only weakly physisorbed (a weak dipole interaction) to the gold surface [22]. As CTAB is driven from the surface it will be replaced with AMTAZ from the solution. Sonication is necessary to prevent aggregation during this process because the particles will lose much of their stability until completely coated with AMTAZ. Temperature is then held at 50°C for 30 minutes with constant sonication. At the end of 30 minutes the temperature is returned to room temperature and sonication continues for an additional 2 hours, allowing for AMTAZ to completely bond with the gold nanoparticles. The particles are then spun down to remove them from solution, the supernatant removed, and the particles resuspended in de-ionized water.

The new AMTAZ coating on the particles can be activated and functionalized. This can be accomplished through a well-known mechanism, EDC-NHS coupling. In this process, the nitrogen ring in AMTAZ is “opened,” allowing a functionalization protein with an NHS group to bind to AMTAZ covalently. This covalent bond is extremely strong and stable. For this purpose, we chose to use NHS-ester-Biotin, a form of biotin specifically designed for functionalization purposes. Because we chose to functionalize with biotin, streptavidin will be our analyte target molecule.

In order to facilitate and speed the functionalization process, a functionalization reaction kit was customized and purchased from ArrayIt Corp. This kit contained several buffer solutions: a reaction buffer, a wash buffer, and a rinse buffer. The standard protocol for the use of this kit was then customized to be appropriate for use with suspended gold nanoparticles. First, the particles were removed from aqueous solution and resuspended in an equal volume of the reaction buffer. A solution of biotin dissolved in water was then added. There is no well-defined prescription for how much biotin is required, though it is best to err on the side of more biotin rather than less. Any excess biotin will be removed at the end of the functionalization process. It is recommended to add enough biotin that is it is all just easily dissolved in water, with no solid biotin remaining after thorough mixing. The reaction was allowed to proceed at room temperature under sonication for 30 minutes. A volume of wash buffer equal to the volume of the original solution was added and then sonicated for 1 minute. This solution was spun down in a centrifuge to remove the particles from solution, the supernatant removed, and the particles resuspended in the rinse buffer. This was briefly mixed, spun down once more, and the particles were resuspended in deionized water.
5.3 Results

5.3.1 Replacing CTAB with AMTAZ

The replacement of CTAB with AMTAZ must be confirmed before moving on with the functionalization. Because CTAB is so much larger than AMTAZ (CTAB is approximately 6 nm long, AMTAZ is approximately 1.2 nm long) and cannot itself be functionalized, virtually all CTAB must be stripped from the nanoparticles. This verification can be done by examining Raman spectra as well as VIS-NIR absorbance spectra. The expected Raman spectra for both CTAB and AMTAZ coated nanoparticles are available in the literature and shown below in Fig. 5.2.

![Raman spectra comparison](image)

**Figure 5.2:** The expected Raman shifts for CTAB (top) and AMTAZ (bottom) coated nanoparticles (dotted lines). The Raman spectra for the molecules without nanoparticles are shown in solid lines [22].

The CTAB Raman spectra has distinct peaks at 455, 763, 1061, 1127, 1295, and 1460 cm\(^{-1}\). The AMTAZ spectrum shows Raman bands at 479, 1064, 1425, and 1478 cm\(^{-1}\) [22]. Attaching the molecules gold nanoparticles enhances most of the characteristic Raman response peaks due to their plasmon resonance.

We performed Raman spectra on a CTAB-terminated bipyramidal nanoparticle sample, and a nanoparticle sample in which the CTAB had been removed and replaced with AMTAZ. The experiment was performed at room temperature with a 514 nm laser, a laser power of 2 mW at the sample, and an exposure time of 5 minutes. The results for a wavenumber region in which there are the largest number of expected peaks are shown below in Fig. 5.3. Results for AMTAZ terminated particles are shown in black, CTAB terminated particles in red.
In Fig. 5.3, we see none of the expected characteristic Raman bands of either AMTAZ or CTAB. Therefore, we cannot use these Raman spectra to confirm that we have replaced CTAB with AMTAZ. It is likely that, had a laser been available with a wavelength closer to an LSPR peak of the nanoparticles, we may have seen better results. We also examined the absorbance spectra of both samples. These are shown in Fig. 5.4 and Fig. 5.5 for the CTAB and AMTAZ samples, respectively.

Figure 5.4: Absorbance spectrum for CTAB terminated AuBP. There are two LSPR peaks, at 580 nm and 840 nm. The large peak at 260 nm is from CTAB on the particles and in solution.
Figure 5.5: The absorbance spectrum of the AMTAZ terminated AuBP sample. The left shoulder of the 580 nm LSPR peak is present, with a very broad absorbance at longer wavelengths. The 260 nm CTAB peak has disappeared, indicating that CTAB has been removed successfully.

In Fig. 5.4 we see clear LSPR peaks at 580 nm and 840 nm. This indicates the presence of well-formed gold bipyramidal nanoparticles. The large absorbance peak near 260 nm is due to CTAB in solution and coating the nanoparticles. Figure 5.5 shows that this CTAB absorbance peak has disappeared, indicating the successful removal of CTAB from the solution and the surface of the nanoparticles. However, we have also lost the two clear LSPR absorbance peaks. The left shoulder of the 580 nm LSPR peak is visible, and there is a single broad absorbance at longer wavelengths. Previous work has shown that this type of spectrum is indicative of a large number of badly formed and aggregated nanoparticles with no clearly identifiable shape, and thus no well-defined, narrow LSPR resonant behavior. Repeated attempts at replacing CTAB with AMTAZ produced similar results. This suggests that, while it was successful for other groups using gold nanorods [22], our protocol is not suitable for application with bipyramidal particles. This could be because bipyramidal particles have been seen to be less stable and much more reactive than nanorods. Another protocol will need to be identified and tested if bipyramidal nanoparticles are to be used in our biosensor.

5.3.2. Sensitivity Measurement with Gold Nanorods

Because our attempts to functionalize bipyramidal particles were unsuccessful, we used gold nanorods with similar LSPR absorbance spectra that had already been functionalized to test the possible sensitivity of our biosensor. These nanorods are 42 nm long and have an axial diameter of 10 nm. For these tests, we used gold nanorods that have been functionalized with Neutravidin, which is identical to streptavidin in chemical behavior but has the advantage that Neutravidin is a slightly shorter molecule. The absorbance spectrum for these functionalized nanorods is shown in Fig. 5.6. The peak was fit using a dual Gaussian curve. Because of the low resolution of this spectrum, the uncertainty in that peak is approximately 1 nm. Note that the longitudinal LSPR peak is many times larger than the short wavelength peak. This is because
the sample is extremely pure, comprised almost entirely of nanorods. Because of their geometry, nanorods are also expected to have a slightly weaker short wavelength LSPR peak than a bipyramidal particle [9].

Figure 5.6: The absorbance spectrum for Neutravidin functionalized gold nanorods with longitudinal LSPR peak at 820 nm.

These Neutravidin functionalized nanorods are designed to capture biotin. So, to test their sensitivity, varying quantities of biotin are added to a solution of nanorods and the absorption spectra are then examined, looking for shifts in the longitudinal resonance peak. The nanorods solution was first diluted because the stock solution is very highly concentrated. We took 0.1 mL of nanorod solution and added 5.0 mL of deionized water. This was then split into samples of 1 mL each. It is known that there are $3.1 \times 10^{13}$ nanorods in 1 mL of stock solution. Therefore, each of the 1 mL diluted nanorod samples contains approximately $6 \times 10^{11}$ nanorods. Approximating the nanorods as cylinders with a length of 42 nm and diameter of 10 nm, the surface area is approximately 1480 nm$^2$. Approximately 3700 Streptavidin molecules can fit in this area. With 8 biotin binding sites, there are a total of approximately 30,000 available sites for biotin binding on each nanorod.

A 1.0 mM biotin solution was then prepared by dissolving 1.2 mg of biotin in 5.0 mL water. Varying the volume of this 1.0 mM biotin solution added varied the quantity of biotin introduced to the nanorod solutions. We used volumes of 4 μL, 8 μL, 20 μL, and 500 μL. A 1.0 mM solution means there are 0.001 moles of biotin in every Liter of solution. This translates to $6.02 \times 10^{14}$ molecules in every μL of solution. Therefore, when 4 μL of biotin are added to 1 mL of diluted nanorods, there are 4000 biotin molecules for every nanorod, and so on for the larger biotin volumes. This means that the smallest volume of biotin will fill just 13% of the available sites if all of the biotin molecules bind to streptavidin, and the largest volume will saturate the nanorods.

Absorption spectra were obtained for each of the samples with biotin introduced. The longitudinal LSPR peaks were then fit with dual Gaussians, allowing for different curve widths
on either side of the peak. These higher resolution spectra have an uncertainty in their peak position of approximately 0.3 nm. These Gaussian fits of the longitudinal LSPR peaks are shown in Fig. 5.7, 5.8, 5.9, and 5.10 for 4 μL, 8 μL, 20 μL, and 500 μL of introduced biotin, respectively.

Figure 5.7: Fitting 4 μL of added biotin with a dual Gaussian. Fit LSPR peak at 821 nm.

Figure 5.8: Fitting 8 μL of added biotin with dual Gaussian. Fit LSPR peak at 821 nm.
We see a 1 nm shift with the first 2 additions of biotin, a 2 nm shift with the third, and a 3 nm shift with the final, largest addition of biotin. The results are summarized below in Table 5.1.

<table>
<thead>
<tr>
<th>Volume of Biotin µL</th>
<th>Molecules / Particle a.u.</th>
<th>% Binding Sites</th>
<th>LSPR Peak Shift from 820 nm nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4000</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>8000</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>20</td>
<td>20000</td>
<td>67</td>
<td>2</td>
</tr>
<tr>
<td>500</td>
<td>5000000</td>
<td>100</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 5.1: Summarizing the results of the biotin sensitivity experiment for gold nanorods.
We can see from the results summarized in Table 5.1 that the addition of even a relatively small number of biotin molecules (the 4 µL sample contains 1.6 x 10^-18 grams of biotin) will cause a shift in longitudinal LSPR peak, though because of the uncertainty in initial peak position we can only say with 100% certainty that there is a shift once we reach 67% binding site coverage. This shift is not immediately obvious by looking at the spectra against each other, but becomes clear when the peaks are carefully fit. Doubling the number of biotin molecules, however, does not double this shift. We do not see a measurably larger shift until we reach 5 times the initial volume of biotin. Adding a much larger volume (up to 500 µL) of biotin results in a 3 nm shift, just 1 nm more than the shift for 1/25 that volume. So, while there may not be enough data here to confirm a precise sensitivity function, the data suggest that there is a diminishing return on adding additional biotin.

We have seen in this chapter that our proposed protocol for functionalizing bipyramidal particles must be either adjusted or replaced entirely. The current protocol results in the destruction of the bipyramidal particles. We also saw that functionalized gold nanoparticles are able to successfully capture biotin. In doing so, their longitudinal LSPR peaks shift 1-3 nm in the presence of a very small quantity of biotin. While this work has laid the groundwork for these tests, it will be necessary to perform a more extensive sensitivity test when preparing to use these or similar nanorods or other nanoparticles in a semiconductor biosensor device.
Chapter VI
Conclusion

In this work, our goal was to design, fabricate, and test a plasmon-assisted CdS semiconductor nanosheet biosensor. This required several experiments, starting with the characterization of the spectral behavior of gold nanospheres. We then synthesized bipyramidal gold nanoparticles, which required the synthesis of gold seed particles, synthesizing gold bipyramids, conducting a silver nitrate concentration study, and finally characterizing the morphology and spectral behavior of the bipyramidal gold nanoparticles. This series of experiments resulted in the consistent synthesis of high quality, stable, well-formed bipyramidal gold nanoparticles with sharp tips. Finally, we attempted to functionalize our nanoparticles. This first required the removal of CTAB to be replaced by AMTAZ. We then performed a sensitivity test on functionalized gold nanorods.

Chapter 3 details the experiments performed to characterize the spectral behavior of gold nanospheres. After obtaining absorption spectra for bare, functionalized, and particles that have captured biotin, we note that as we add more molecules to the coating of the nanoparticle there is a corresponding redshift. We first redshift 3 nm going from bare to functionalized nanoparticles, and shift another 7 nm once these nanoparticles have captured biotin.

We also observed the scattering spectra for all three nanoparticle types under white light excitation. There was a large redshift of 124 nm moving from bare to functionalized nanoparticles. Once the particles have captured biotin, however, we observe a blueshift of 22 nm. This is possibly because “roughness” on the surface of the nanoparticle is smoothed out. These experiments confirm that the capture of a target molecule will result in a measurable redshift in the absorption LSPR spectral peak of gold nanospheres.

Chapter 4 examines the synthesis and characterization of bipyramidal gold nanoparticles (AuBP). We first synthesized two types of gold seeds: CTAB-terminated and citrate-terminated spherical nanoparticles. We found that they had nearly identical spectral behavior. However, the CTAB seed resulted in more stable final particles with a much longer lifetime, so the CTAB seed solution was chosen for all further AuBP synthesis.

During the AuBP synthesis process, it was found that a synthesis may result in a Type A, Type B, or Type C growth, of which only Type A consists of well-formed, stable AuBP suitable for further study and application in a biosensor device. These Type A growths have a vivid purple color in bulk solution and have two LSPR absorbance peaks. The longitudinal peak can be anywhere from 800 nm and 840 nm, depending on growth conditions. Type A growths are achieved by carefully maintaining a temperature of 30.0 ± 0.2° C and using reagents that are all freshly prepared no more than an hour before use in a growth process. Allowing the temperature to vary more than 0.2° C or using reagents that are not fresh will almost always result in a Type B or C growth. These Type A growths consist of ~50% AuBP and 50% other particles with varying shapes and sizes. It is possible to purify our samples by spinning the samples down in a centrifuge, causing the heavier bipyramids to fall out of solution while leaving the lighter, smaller nanoparticles suspended in solution. This supernatant consisting of water and undesirable nanoparticles, as well as any remaining synthesis reagents can then be removed and the bipyramids resuspended in de-ionized water. It was found that the best purification results were obtained with a spin down force of 3200 g.

All three types of AuBP growth processes were monitored in real time in a temperature controlled spectrometer. We found that Type A growths have an immediate period of rapid
growth followed by sudden saturation, at which time the growth halts. The growth is complete
(meaning the wavelength and absorbance have both stopped changing) at approximately 70
minutes of growth time. The wavelength of the longitudinal LSPR peak is typically saturated at
50 minutes of growth. Both Type B and C growths have an initial growth phase that is slower
than the initial phase of a Type A growth. At approximately 40 minutes of growth we then see a
degredation of the growth, at which time Type B growths exhibit a marked blueshift in
longitudinal LSPR peak and Type B growths saturate in wavelength and exhibit a loss of
absorbance value, falling into the background.

Time-resolved TEM images of Type A and B growths reveal possible physical
explanations for their spectral differences. Type A growths start as small, sometimes
asymmetrical AuBP and simply grow steadily into larger, symmetrical, well-formed AuBP.
Type B growths also contain many AuBP at 50 minutes of growth. After that time, however, the
sharp tips become much larger and more rounded and the particles become thicker at the waist.
These changes correspond directly in time with the marked blueshift in their LSPR spectra.

We then performed studies to determine the effect of silver nitrate concentration in the
synthesis solution on the growth process and the resulting spectral behavior of AuBP. It was
found that at very low concentrations (22 μM), there is insufficient silver nitrate to produce
AuBP. We also found that the growth rate increases with increasing AgNO₃ concentration. It
was observed that increasing silver nitrate concentration monotonically results in a longer
longitudinal LSPR absorbance peak wavelength, ranging from approximately 800 nm for the
lowest concentration that resulted in AuBP to 845 nm for the highest concentration. This study
will allow for the fine-tuning and selection of any longitudinal LSPR peak between 800 nm and
840 nm which may be valuable when constructing a biosensor device.

Though it is possible to obtain considerable variation in dimensions and spectral behavior
between AuBP growths, any one growth is fairly homogenous. Single growths typically vary in
Length by only 6% and in tip radius of curvature by 13% (which is just a 0.6 nm variation). Data
was compiled for several growths with varying mean dimensions and corresponding varying
longitudinal LSPR peaks. This data was then analyzed in an attempt to find some correlation
between radius / length and longitudinal spectral peak position. It was found that there is a
negative, linear correlation between Radius / Length vs Longitudinal Peak position. Numerical
calculations performed by Liu et al. also predict a negative linear relationship, though our
experimental slope suggests a stronger dependence of LSPR peak position on particle
dimensions.

Once we have consistently well-formed and stable AuBP samples with the desired
spectral peaks, we move on to the final steps in preparing our nanoparticles for application in the
CdS biosensor as a brief extension to the primary focus of this work. Chapter 5 details the
experimental attempts to functionalize nanoparticles as well as sensitivity tests for capturing
target molecules. Attempts to replace the CTAB nanoparticle coating with AMTAZ seemed to
destroy the desirable AuBP population, resulting in spectra with no discernable longitudinal
LSPR peak, and were thus unsuccessful. Future attempts must be made to revise or completely
replace the protocol used to perform this surfactant replacement.

We then performed sensitivity tests on gold nanorods that had already been
functionalized with Neutravidin. These functionalized nanorods have a longitudinal LSPR
spectral peak of 820 nm and each has approximately 30,000 available binding sites for biotin.
Quantities of biotin ranging from 4000 molecules / nanorod to 500,000 molecules / nanorod (in
an attempt to achieve saturation) were added to nanorod samples. The resulting longitudinal
LSPR spectral peaks were then fit with dual Gaussian curves to accurately identify the absorbance peaks. It was found that small quantities of biotin (4000 molecules / nanorod, or 13% binding site coverage) result in a 1 nm spectral redshift while near saturation we see a 3 nm shift. This proves that there is a measurable spectral shift when the nanorods capture biotin and that this shift is sensitive to the number of molecules captured.

With this, we conclude this study of bipyramidal gold nanoparticles and their application in CdS nanosheet biosensor devices. The particular geometry and plasmon behavior of gold bipyramids makes them an excellent candidate for application in our CdS biosensor. We are now poised to disperse gold bipyramidal nanoparticles onto a CdS nanosheet and test the device for plasmon-enhanced two-photon absorption and then perform the corresponding sensitivity tests for target molecule capture.
Works Cited


[16] Material Safety Data Sheet for Silver Nitrate 2011 Version 5.0 Sigma Aldrich, St Louis, MI


Appendix A
Details of Solution Preparation for Nanoparticle Synthesis

Provided here are details for preparing the individual solutions for seed and bipyramidal gold nanoparticle synthesis to the necessary concentrations. In all cases where water is required, it is necessary to use 18 MΩ-cm deionized water.

Seed Particle Synthesis Solutions:

0.2 M CTAB: 182.2 mg of powder CTAB dissolved in 5.0 mL H₂O. This should then be sonicated until this turns from milky white to completely clear. This typically takes approximately 15-20 minutes.

0.5 mM HAuCl₄: Dissolve in H₂O at a rate of 0.197 mg/mL. 5 mL is required for synthesis, making at least 6 mL is recommended. This means at least 0.98 mg is required, though preparing a larger volume (approximately 10 mL) is recommended. This chemical comes in powder form and is highly hydroscopic, meaning that when exposed to air it will rapidly pull water out of the atmosphere. If this continues too long, the salt-like powder turns to a yellow sludge. Even before that occurs, however, pulling water from the atmosphere makes any mass measurements of the powder much less accurate. To avoid this, one must measure by mass difference rather than measure the mass directly. First take the mass of the vial of salt. Then (once it has come to room temperature) open the vial, quickly take a small amount on the end of a glass pipette and dissolve this immediately. Then quickly close the vial and measure its change in mass, which is the mass of salt removed. This method means the vial is open for only a few seconds for any measurement, and allowing it to come to room temperature prevents condensation on the interior of the vial. This vial of powder must be refrigerated for long-term storage, as it will slowly decompose at room temperature. Should dissolve easily and quickly, forming a light yellow solution.

0.01 M NaBH₄: This substance is highly volatile and should be handled with care. Any use of it should be done in a fume hood. Read MSDS data sheets carefully, and note that this chemical will react violently with water (even moisture in the air) when at room temperature. Therefore, it must be kept refrigerated before use and must be ice cooled when diluted in water or used in synthesis. This reducing agent comes stock as a 0.5 M liquid solution, which must be drawn out of the container with a syringe. Pull nitrogen gas into the syringe (approximately the same volume you intend to draw from the container), and inject this into the solution container. Then invert it and draw the desired volume into the syringe, ejection it into a clean, dry test tube. Then measure your precise desired volume from this stock test tube. It is recommended to dissolve 80 μL NaBH₄ (ice cooled) in 3.92 mL water (also ice cooled). This provides you more than is necessary for synthesis but allows for easy and accurate preparation of the necessary concentration. This final solution should be kept ice cooled right up until it is used in synthesis.
**Bipyramid Synthesis Solutions:**

**0.4 M CTAB:** 619.6 mg CTAB powder dissolved in 4.25 mL H₂O. This should then be sonicated until this turns from milky white to completely clear. At this concentration this typically takes approximately 30-40 minutes. Once dissolved, should be placed in temperature bath to be allowed enough time to come to temperature (30°C).

**0.1 M Ascorbic Acid:** Powder form dissolved in water, with 17.61 mg/mL. For 10 mL of AA solution, this requires 176.1 mg in 10 mL of water. Will dissolve easily, requiring just one or two inversions of a closed test tube to dissolve, forming a clear solution. Once dissolved, should be placed in temperature bath to be allowed enough time to come to temperature (30°C).

**0.01 M AgNO₃:** Powder dissolved in water, with 1.70 mg/mL. Prepare a large enough volume that the mass of the powder is easily accurately measured (20-30 mL most likely sufficient). Will dissolve easily, requiring just one or two inversions of a closed test tube to dissolve, forming a clear solution. Once dissolved, should be placed in temperature bath to be allowed enough time to come to temperature (30°C).

**0.01 M HAuCl₄:** Dissolve in water at 3.94 mg/mL. It should dissolve easily in water and will form a vivid yellow (though not cloudy) solution. This chemical comes in powder form and is highly hydroscopic, meaning that when exposed to air it will rapidly pull water out of the atmosphere. If this continues too long, the salt-like powder turns to a yellow sludge. Even before that occurs, however, pulling water from the atmosphere makes any mass measurements of the powder much less accurate. To avoid this, one must measure by mass difference rather than measure the mass directly. First (once it has come to room temperature) take the mass of the vial of salt. Then open the vial, quickly take a small amount in the end of a glass pipette and dissolve this immediately by placing the end of the pipette in some known volume of water. Then quickly close the vial and measure its change in mass, which is the mass of salt removed. This method means the vial is open for only a few seconds for any measurement, and allowing it to come to room temperature prevents condensation on the interior of the vial. This vial of powder must be refrigerated for long-term storage, as it will slowly decompose at room temperature.
Appendix B
Additional TEM Images Used in Analysis

TEM Used for Morphology Measurements – 800 kx magnification:
Wide-field TEM images used for yield statistics and morphology measurements (100 kx – 250 kx magnification):