STRUCTURAL AND PLASMONIC PROPERTIES OF GOLD NANOCRYSTALS

BY

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DISSERTATION

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ABSTRACT

The design of gold nanoparticles for surface-enhanced Raman scattering (SERS) and plasmonic enhanced fluorescence are more involved than simply maximizing the local field enhancement. The enhancement is a function of the excitation wavelength relative to the plasmon resonance as well as the distance of the reporter molecules from the nanoparticles’ surface. For suspension based measurements, additional considerations must also be made regarding absorption and scattering effects as light propagates through the sample. These effects are in addition to the other more commonly observed effects such as nanocrystal shape. With such a wide number of variables in play, a series of studies breaking down each of these components and their contribution to the observed enhancement is warranted.

In this thesis, a series of experiments were undertaken using a platform based on polyelectrolyte coating of gold nanoparticles by layer-by-layer deposition. The reporter molecules are bound onto the surface of polyelectrolyte coated nanoparticles before trap coating them with an additional oppositely charged polyelectrolyte layer. By etching away the gold nanoparticle using potassium cyanide, we are then able to quantify the number of reporter molecule per nanoparticle using mass spectrometry. With this quantitative approach, we can the directly compare the effects of the aforementioned enhancement mechanisms on the observed signal intensity. This method overcomes some of the disparities in literature between reported values of enhancement due to assumption in the number of reporter molecules contribution to the signal intensity.

Using our group’s expertise, we synthesized gold nanoparticle libraries of nanorods, cubes, trisoctahedra and spheres of different sizes. Each geometric configuration was characterized using a recently developed TEM technique - nano-beam coherent area diffraction.
The as-synthesized were exposed to a coherent electron beam with probe size similar to that of
the nanoparticles. The nanoparticles were then tilted such that were oriented so that the electron
beam was parallel to a major zone axis and the diffraction pattern recorded. We observed streaks
at each Bragg reflection that changed depending on the shape of the nanoparticle. This is in
contrast to the spots for the Bragg reflections observed for normal small area diffraction patterns
of gold nanoparticles. The angles between the streaks were compared using vector analysis to
theoretical simulated three dimensional models and showed good correlation. These studies
indicate such a platform can be used to elucidate the structure of high-index gold nanoparticle
shapes such as trisoctahedra.

The as-synthesized gold nanoparticles had surface plasmon resonances that incrementally
spanned the spectral region of 500-900 nm. The reporter molecules used all have an absorption
maximum far from the excitation wavelength. This ensures that chemical resonant based effects
are minimized and plasmonic electromagnetic effects dominate the observed signal
enhancement. For gold nanorods, the highest SERS signal from six different aspect ratios was
observed with absorption maxima blue-shifted from the laser excitation wavelength. This
finding is in contrast to substrate measurements where the maximum observed signal is red-
shifted from the laser excitation wavelength.

A similar platform was used to compare the effects of changing the nanoparticle shape on
the observed SERS enhancement. We synthesized trisoctahedral, cubic and spherical geometries
with electronic absorption maxima that overlapped within 3 nm. The relative SERS enhancement
with 785 nm excitation was compared to theoretical simulations using finite element analysis.
The observed signal intensities correlated well to the theory, suggesting the electromagnetic
fields focused towards sharp edges and corners dominated the spectral response.
The final chapters of this thesis are tailored towards understanding the distance dependence of plasmonic effects on the two photon absorption (TPA) cross section of an organic chromophore. First, we optimized the protocol to coat as-synthesized gold nanorod with multiple polyelectrolyte layers. By varying the purification and complexation parameters we were able to obtain up to ten layers of wrapping without great losses in nanoparticle concentration. The TPA molecules were then electrostatically attached at different incremental distances to compare the relative enhancement as a function of distance. We compared the TPA enhancement for on-resonant excitation and find a 40 fold enhancement for the molecules closest to the surface of the nanoparticles. For the off-resonant excitation, we observed an interesting trend where the TPA enhancement recovers for higher number of polyelectrolyte layers.
To my mother, father and sister for their dedication and determination throughout my academic endeavors.
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# TABLE OF CONTENTS

CHAPTER 1: AN INTRODUCTION TO GOLD NANOCRYSTALS ................................................1

CHAPTER 2: HIGH-INDEX FACETS IN GOLD NANO PARTICLES ELUCIDATED BY 
COHERENT NANOAREA ELECTRON DIFFRACTION .................................................29

CHAPTER 3: OFF-RESONANCE SERS FROM SUSPENSIONS OF DIFFERENT ASPECT 
RATIO GOLD NANORODS: NOT WHAT WE THOUGHT ...........................................53

CHAPTER 4: SHAPE DEPENDENCE IN SUSPENSION BASED SERS MEASUREMENTS 
OF GOLD NANO PARTICLES ..................................................................................80

CHAPTER 5: PLASMONIC ENHANCEMENT OF THE TWO PHOTON ABSORPTION 
CROSS-SECTION OF AN ORGANIC CHROMOPHORE USING 
POLYELECTROLYTE COATED GOLD NANORODS ..............................................100

CHAPTER 6: OFF-RESONANT TWO- PHOTON ABSORPTION CROSS-SECTION 
ENHANCEMENT OF AN ORGANIC CHROMOPHORE ON GOLD NANORODS.125
CHAPTER 1
AN INTRODUCTION TO GOLD NANOCRYSTALS

1.1 Background

Gold nanoparticles and their unique optical properties have been of interest to scientists and artists for many centuries. History documents their earliest applications to the early 17th century, where their brilliant colors made them ideal for pigments in chinaware and in stained glass. The first documented scientific research on gold sols was initiated by Michael Faraday in 1857, where he reported the formation of a deep red solution of colloidal gold by reduction of aqueous chloroaurate solutions. His experiments for metal sols suggested that gold colloids in particular showed a remarkable correlation between size and color. It was not explained until 1908, when Gustav Mie solved Maxwell’s equations for the absorption and scattering of electromagnetic radiation by spherical noble metal nanoparticles. He showed that when the radius of the noble metal nanoparticles is smaller than the wavelength of light, they exhibit a strong absorption band in the visible region. This phenomenon was attributed to a small particle effect, absent in bulk metals and individual atoms. Incoming electromagnetic radiation induces the formation of a dipole in the nanoparticle, a restoring force is then set up to compensate this, what results is a resonant plasma frequency matching the electron oscillation within the nanoparticle. These oscillation modes are known as surface plasmons and are characterized by a strong field enhancement at the interface between metal and dielectric. For spherical metal nanoparticles, much smaller than the wavelength of the exciting light. Mie theory for the extinction crosssection (absorption + scattering cross section), \( \sigma_{\text{ext}} \) can be written as the following for a nanoparticle of radius \( R \).
\[
\sigma_{\text{ext}}(\omega) = 9 \frac{\omega}{c} \frac{\varepsilon_2 \nu_0}{\varepsilon_1(\omega) + 2\varepsilon_m(\omega)} \frac{\varepsilon_2(\omega)}{[\varepsilon_1(\omega) + 2\varepsilon_m(\omega)]^2 + \varepsilon_2(\omega)^2}
\]  

where \( V_0 = (4\pi/3)R^3 \), \( \omega \) is the angular frequency of the excitation frequency, \( \varepsilon_m \) the dielectric function of the medium surrounding the metallic nanoparticles. \( \varepsilon_1 \) and \( \varepsilon_2 \) correspond to the real and imaginary parts of the dielectric function of the metallic nanoparticles respectively. 

Equation 1 shows that the surface absorption band exists when \( \varepsilon_1(\omega) \approx -2\varepsilon_m(\omega) \) only when it is \( \varepsilon_2 \) is small or weakly dependent on \( \omega \). Typically for metals such as In, Sn and Cd the plasma frequency is in the UV end of the spectrum and hence the strong colors are not observed. The “coinage metals” Au, Ag and Cu are unique in that their conduction band electron transitions push the plasma frequency into the visible part of the spectrum. 

A commonly observed phenomenon is the red shift and broadening of electronic absorption spectra as a function of the aggregation state of the nanoparticles. Here, Mie theory’s assumption of non-interacting nanoparticles, that are well separated breaks down. Instead near-field coupling between particles in close proximity and far-field dipolar coupling interactions dominate the spectral response.

The dipolar fields resulting from the plasmonic oscillation of a single nanoparticle induce plasmonic oscillations in adjacent nanoparticles.

For non-spherical metallic nanoparticles such as gold nanorods, the plasmon resonance splits into a higher and lower energy bands corresponding to the transverse and longitudinal oscillations respectively (Figure 1.1). The following section will discuss the synthesis of these nanoparticles and other shaped nanoparticles. These studies have been able to show the optical response of metal nanoparticles can be tuned by controlling their size, shape, and environment.
providing a starting point for emerging research fields like surface enhanced spectroscopies\textsuperscript{11-13} and biological sensing\textsuperscript{14} based photonics or plasmonics.\textsuperscript{15}
Figure 1.1: (Top) Cartoon of surface plasmon resonance in (left) gold nanospheres and (right) gold nanorods. (Bottom) Corresponding electronic absorption spectra of actual samples in water.
1.2 Wet Chemical Synthesis of Gold Nanocrystals

Our group’s expertise is in the synthesis of gold nanoparticles of different geometries based on wet chemistry. With the aforementioned applications demanding gold nanoparticles of unique shapes capable of the highest electromagnetic fields, researchers have pushed towards synthesizing a wide variety of shaped nanoparticles.\textsuperscript{16} Initial work pioneered by our group looked at creating simple geometries such as rods and cubes,\textsuperscript{17} where the electromagnetic fields could be focused towards the corners with so the called “lightening rod” effect.\textsuperscript{18} Using a specific surfactant molecule (cetyltrimethylammonium) CTA\textsuperscript{+} with different counterions (Br\textsuperscript{-}, Cl\textsuperscript{-} and I\textsuperscript{-}), we showed the ability to control the growth of gold nanoparticles using surfactant micelles and a seed mediated approach.\textsuperscript{19} By varying the amount of silver added to the synthesis, the aspect ratio of the short gold nanorods could be varied between aspect ratio one to five.\textsuperscript{20} Although other approaches have showed the ability to grow gold nanorods, the relative yield of rod shaped particles compared to spheres is typically compromised.\textsuperscript{21} Even slight variations in chain length, head group structure, counterions, and CTAB purity have been found to be critical to the synthesis of gold nanorods.\textsuperscript{22, 23} Additionally it was also observed that any slight variations in the synthetic conditions led to the formation of other shapes such as hexagons and cubes.\textsuperscript{24} With such small changes leading to vastly different shapes, many scientists have leant towards kinetic based mechanisms to explain the growth of gold nanoparticles.\textsuperscript{25} Alternative viable thermodynamic models suggest surfactant molecules bind to certain facets preventing their growth and encouraging the growth of others.\textsuperscript{26} An added complexity is the role of the additive metal ions such as silver that tailor the size of different geometries of gold nanoparticles.\textsuperscript{27} Many different mechanisms have been suggested including; underpotential deposition, halide absorption, surface packing density, and alloy formation among others.\textsuperscript{28}
Confirming these phenomena has proved difficult, however, due to the limitations in characterizing the real time growth of the nanoparticles and monitoring the role of the additive ions on the nanoscale. In order to address this researchers have now begun looking at techniques such atom probe tomography and in-situ, wet cell high resolution transmission electron microscopy (TEM) to characterize such effects.

Wet chemistry synthetic techniques have recently added new classes of gold nanoparticles of different including; higher index polyhedra such as rhombic dodecahedra, tetrahexahedra, and trisoctahedra. These structures consist of higher index facets possessing a high density of low co-ordinated atoms such as steps, edges and kinks which serve as catalytically more active sites. Again driven by surface enhanced spectroscopic applications researchers have identified the potential of such structures to give huge enhancement in signal intensity for surface enhanced Raman spectroscopy (SERS). The higher index facets sites are ideal for the absorption of reporter molecules within the highly focused electromagnetic fields at these facets.
Figure 1.2: Gold nanocrystals of different shapes; (Top) Gold nanorods of varying aspect ratio and the corresponding electronic absorption spectra. (Bottom Left) Gold nanocubes. (Bottom Right) Gold trisoctahedra. All scale bars = 50 nm
1.3 Surface Enhanced Raman Spectroscopy (SERS)

With recent developments in synthesizing noble metal nanoparticles, researchers have moved towards exploring their application in chemical sensing\textsuperscript{38} and biomedical imaging.\textsuperscript{14} Vibrational spectroscopy (Raman and infrared) provides molecular, identity information and offer a powerful tool for these experiments. The techniques overcome limitations in optical spectroscopy techniques such as fluorescence.\textsuperscript{39} In Raman spectroscopy, inelastic scattering of incident radiation with the electron cloud of a functional group is detected. These scattering events are significantly rare compared to elastic (Rayleigh) scattering, resulting in extremely low cross sections (about $10^{-29}$ to $10^{-31}$ cm$^2$/molecule). Surface enhanced Raman spectroscopy (SERS), discovered in the 1970s, uses nanostructured metallic surfaces to greatly enhance the magnitude of Raman scattering.\textsuperscript{40} Typically incoming electromagnetic waves interact with the noble metal nano-surface by exciting localized surface plasmon modes, inducing electromagnetic fields near the surface. The Raman scatterer is placed within the intense electric fields surrounding metal nanoparticles. (Figure 1.3) The culmination of this effect is an increase in the intensity of inelastic scattering by up to 8 orders of magnitude.\textsuperscript{40} Recently researchers have attributed two orders of the observed enhancement to the chemical resonant effects, compared to six orders for electromagnetic based mechanisms.\textsuperscript{40} Assuming that the intensity of both the incident and scattered fields are enhanced, the detected Raman signal will increase significantly.

For the electromagnetic mechanism the enhancement factor, $G$ of each reporter molecule is given approximately by\textsuperscript{41}

\[ G = |E(\omega)|^2 |E(\omega')|^2 \]  

(1.2)
where $E(\omega)$ and $E(\omega')$ correspond to the local electric field enhancement factor at the incident and Stokes-shifted frequency ($\omega'$) respectively. Other more rigorous descriptions of the electromagnetic enhancement mechanism that do not use the product of fields at different field have been shown by Kerker and co-workers.\textsuperscript{41}

However, experiments have shown the actual numerical values of observed enhancement using the more accurate expression of the enhancement are only slightly different.\textsuperscript{41} For typical SERS measurements, the enhancement $G$, is averaged over the surface area of the nanoparticles generating the observed signal intensity. The reported values of $G$, are often complicated by the assumption that $E(\omega)$ and $E(\omega')$ are the same, making $G = |E(\omega)|^4$. The reasoning behind the approximation is that the plasmon width is typically much larger compared to the Stokes shift.
Figure 1.3: Schematic of surface-enhanced Raman scattering (SERS) of methylene blue capped gold nanoparticles with 0.3 uM methylene blue excited at 785 nm (top) and spontaneous Raman scattering of 25 uM methylene blue (bottom).
In their efforts to optimize the design of SERS probes, researchers have focused on electromagnetic enhancement to further the dynamic working range of these materials. Armed with the ability to fabricate highly uniform SERS substrates based on well defined noble metal nanoscale features such as “nanosphere lithography,” researchers have begun to further understand the optimum configurations for the highest electromagnetic enhancement. Most studies to date have looked at the optimal position of the surface plasmon maxima relative to the excitation wavelength. Other variable include; the polarization of the incoming electromagnetic radiation, the aggregation state for colloidal suspensions, the concentration of nanoparticles and the number of Raman reporter molecules undergoing enhancement. For substrate based measurements, the optimal localized surface plasmon resonance (LSPR) configuration and the polarization of the incident radiation have been rigorously studied. SERS measurements on colloidal suspensions however, are inherently more difficult to understand, due to factors such as Brownian motion and unbound free reporter molecules coming into contact with “hot spots” between nanoparticles. Such phenomena are not new to the community, indeed it was only recently identified by Van Duyne and others that the 14 orders of SERS enhancement observed by Kneipp and Nie in 1997 were most likely due to free unbound reporter molecules floating in and out of “hot spots” in colloidal suspensions. These regions exist due to the overlapping electromagnetic fields of noble metal nanoparticles in close proximity (< 2 nm). An artifact of this effect is the “blinking” phenomena where huge spikes are identified by the user in the vibrational spectra of the reporter molecule. The difficulty is the reproducibility of these spectra for the same sample set. For an intrinsically low scattering cross section process the probability of a SERS event typically requires longer acquisition times on the order of five seconds or greater, or a higher incoming incident laser excitation power. Such limitations have
moved researchers towards systems with reporter molecules spatially fixed to the surface of the nanoparticles. A recent example of this being shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS) using a dielectric silica coating to coat the gold nanoparticles. Here the chemically inert shell ensures there is no direct contact between the probed substance and the gold core. The authors also undertook tip-enhanced Raman spectroscopy (TERS) measurements to pinpoint the enhancement at the nanoparticle junction (“hot spots”). They also observed eight orders of enhancement in agreement with Van Duyne and others. The platform serves as a great indicator of future outlook of SERS spectroscopy, with the potential to sprinkle “smart dust” of silica coated nanoparticles over any sample for a fast, non-destructive, flexible and portable characterization tool.
1.4 Plasmonic Enhanced Fluorescence Spectroscopy

Fluorescence spectroscopy techniques in biomedicine, range in applications from DNA sequencing to immunoassays. A major driving force has been the ability of synthetic chemists to engineer molecules to absorb and emit over a wide range of the visible spectrum. Fluorescent molecules are typically aromatic molecules which satisfy the quantum requirements for fluorescence to occur. In the presence of strongly confined electric fields near metallic nanostructures their light-emission properties are greatly altered by three methods: (i) increasing the optical excitation rate, (ii) changing the radiative and (iii) non-radiative decay rates. Typically the fluorescence rate $\gamma_{em}$ below saturation can be expressed as

$$\gamma_{em} = \gamma_{exc} \left[ \frac{\gamma_{rad}}{\gamma_{rad} + \gamma_{non-rad}} \right]$$

where $\gamma_{exc}$ is the excitation rate, and $\gamma_{rad}$ and $\gamma_{non-rad}$ are the radiative and non-radiative decay rates respectively. If one assumes that the structure of the fluorescent molecules remains unchanged during excitation, plasmonic nanoparticles can increase the optical absorption rate $\gamma_{exc}$ as,

$$\gamma_{exc} \propto |p.E|^2$$

where $p$ is the dipole moment and $E$ is the optical electric-field vector, due to the enhanced excitation fields at the molecule’s spatial location, increasing the observed fluorescence intensity. The excited molecules also relax rapidly in the presence of excited localized surface plasmons by near-field coupling. The total decay rate rate ($\gamma_{rad} + \gamma_{non-rad}$) increases considerably due to large density of optical states at the metal nanoparticle’s surface. Recently researchers have observed larger enhancements when the frequency of the plasmon resonance
maxima overlaps that of the molecule emission spectra.\textsuperscript{52} These effects are also magnified when the fields are focused in between two or more adjacent nanoparticles. The mechanism is believed to depend on whether surface plasmons trapped at the metal colloid interface can radiate or not.\textsuperscript{53} The scattering component of extinction is a measure of the extent to which the plasmons can radiate into the far-field.\textsuperscript{54} The electromagnetic enhancement of the fluorescence mechanisms appear to dominate in metal colloidal systems where the extinction coefficient is dominated by scattering, whereas non-radiative decay due to internal damping increases when absorption of incoming photons is dominant.\textsuperscript{53, 55} A representative schematic showing the electromagnetic enhancement of fluorescence as a function of distance from the nanoparticles surface is shown in Figure 1.4.

Recently the validity of these theoretical treatments have been evaluated by rigorous experiments, where parameters such as the nanoparticles’ size and the distance between the chromophores and the gold nanoparticles were compared.\textsuperscript{56} In order to better understand the optimal window where electromagnetic effects dominate over quenching mechanisms researchers have looked at utilizing a variety of spacer molecules. In those studies conjugates of chromophores and nanoparticles in suspension show a pronounced quenching of fluorescence.\textsuperscript{57} Only when separated by larger macromolecules such as proteins was the fluorescence quenched less.\textsuperscript{58} Previous studies have looked at using carbon spacers fixing the distances between 1 to 2 nm.\textsuperscript{59} At these distances the quenching efficiency estimated to be 99.8 \%. The reasons are two-fold; firstly gold nanoparticles cause the non-radiative rate of the molecules to increase due to energy transfer to the particle. Secondly the radiative rate of the molecules is decreased because the molecular dipole and induced dipole on the gold nanoparticle radiates out of phase if the molecules are oriented tangentially to the gold nanoparticle surface.\textsuperscript{60} The problem with this
approach is for distances exceeding 2 nm the carbon chains become increasingly floppy. Alternative strategies include utilizing single strand DNA with one end that binds with chromophore and the other with a thiol moiety that binds to gold nanoparticles.\textsuperscript{61} Such studies have measured radiative decay rates and confirmed quenching effects are present in the distances between 2 – 16 nm from the surface of gold nanoparticles for Cy-5 chromophores.\textsuperscript{57} These experiments correlate well with theoretical models by Gertsen and Nitzan\textsuperscript{62} and shed some light on to how the chromophore’s quantum efficiency varies as a function of distance from the nanoparticle surface.

These studies are of great significance for the application of gold nanoparticles for cellular imaging. Typical fluorophores undergo photobleaching upon prolonged irradiation, due most likely to photon induced chemical damage and permanent covalent modification. Photobleaching becomes very problematic for deep-tissue based experiments where there are already limited numbers of photons exciting the fluorophore molecules. Recently researchers have proposed using fluorophores bound in close proximity to the enhanced electromagnetic field of gold nanoparticles.\textsuperscript{58,63} The benefits are twofold: to increase the fluorescence intensity in the presence of the electromagnetic field and to rapidly decrease the quantum lifetimes such that photobleaching effects are minimized.
Figure 1.4: Schematic of the competing effect between plasmonic enhancement and quenching as function of distance from the gold nanorods (GNR) surface. Purple Line: Decaying plasmonic electromagnetic fields from the surface of noble metal nanoparticles.
Similar plasmonic effects have been used to enhance non-linear optical (NLO) mechanisms such as two photon absorption (TPA). Figure 1.5 shows a schematic of the Jablonski diagram, where two photons are absorbed with a combined energy sufficient to induce a molecular transition to an excited electronic state. As in one-photon processes, excitation occurs when the energy of the absorbed photon matches the energy gap between the ground and excited states. The same transitions are in effect for (TPA) where two, less energetic photons are absorbed simultaneously. With modern day photonic devices moving towards ultrafast signal manipulation, researchers have moved towards understanding the NLO response of plasmonically active noble metal nanoparticles. Somewhat atypically, gold nanoparticles demonstrate both intrinsic enhancement of their TPA, as well the ability to enhance the extrinsic TPA of a chromophore in close proximity. Often however, discrepancies in the reported values of enhancement of the nanoparticles exist for measurements of dried nanoparticle suspensions, on substrates. It is likely, as in substrate based SERS measurements, that the observed signal intensities are complicated by the generation of “hot spots,” between nanoparticles in close proximity. Additionally, the techniques typically, used to probe the TPA cross-section such as Z-scan, do not account for the effect of the nanoparticles’ absorption on the observed enhancement value even for on-resonance excitation.
Figure 1.5 Schematic of Jablonski diagram for one (left) and two-photon (right) absorption, showing one-photon emissions for both (blue arrow)
1.5 Thesis Overview

Gold nanoparticles’ ability to enhance optical and vibrational spectroscopies such as plasmonic enhanced fluorescence and surface enhanced Raman (SERS) has led to many studies on their optimal design parameters. For both these techniques the user’s greatest concern is usually to obtain the highest enhancement of the signal intensity. One such parameter is the position of the localized surface plasmon resonance (LSPR) maxima relative to the laser excitation wavelength. Based on current literature, they should overlap as closely as possible for the highest plasmonic enhancement for both techniques. As mentioned earlier, difficulties arise when considering other factors, such as quenching in the case of fluorescence and the position of the reporter or chromophore molecule within the noble metal nanoparticles’ electromagnetic fields. Additional concerns include how these trends differ for measurements within colloidal suspensions as opposed to substrate oriented measurements.

Based on our group’s expertise we synthesized a library of different gold nanoparticle geometries and sizes. The colloidal stability and shape of these nanoparticle suspensions were characterized by techniques such as Uv-Vis spectroscopy, zeta potential and transmission electron microscopy (TEM). Chapter 2 describes a recently developed TEM technique – “nano-area coherent beam diffraction”, to reveal previously unconfirmed information regarding growth facets in as-synthesized gold nanoparticle geometries such as trisoctahedra. We use polyelectrolyte layer-by-layer deposition to wrap the surfactant-stabilized gold nanoparticle surfaces and electrostatically bind reporter molecules on to the surface of the nanoparticles. Once bound to the surface, we then trapped these reporter molecules onto the surface of the nanoparticles using a further oppositely charged polyelectolyte trap coat layer. Chapter 3-6 describe how this technique was used to study variables including the geometry, distance of the
reporter molecules to the gold nanoparticle surface and the relative position of the LSPR maxima on surface enhanced spectroscopies.

An attribute this platform has over other noble metal nanoparticle based systems is the ability to quantify the number of bound reporter molecules contributing to the signal intensity. By etching away the gold nanorod core and quantifying the number of molecules using electrospray-ionization liquid chromatography mass spectrometry (ESI-LC-MS), we are able to gain valuable insight into the number of molecules contributing to the overall signal intensity. In a similar manner we undertake a prudent approach to confirm the concentration of nanoparticles in all spectroscopic measurements. By confirming the total number of gold atoms in each gold nanoparticle sample using inductively coupled plasma mass spectrometry (ICP-MS) and the size and shape of the nanoparticles using TEM, we calculate an experimental molar extinction coefficient. Combining these measurements, a rigorously quantitative approach to SERS and plasmonic fluorescence measurements can be achieved. Such studies will aid researchers moving towards integrating molecular enhanced spectroscopies for biomedical applications. Examples include using antibody or apatamer conjugated gold nanoparticles to targeting tumour cells with different gene over-expression. Creating libraries which quantitatively compare these differences for a range of tumour cells may lead to significant contributions for cancer treatments and early diagnosis in the future.
1.6 References


(28). Murphy, C. J.; Thompson, L. B.; Chernak, D. J.; Yang, J. A.; Sivapalan, S. T.; Boulos, S. P.; Huang, J.; Alkilany, A. M.; Sisco, P. N. Gold Nanorod Crystal Growth: From


CHAPTER 2

HIGH-INDEX FACETS IN GOLD NANOPARTICLES ELUCIDATED BY
COHERENT NANOAREA ELECTRON DIFFRACTION

2.1 Introduction

Recent developments in synthesizing noble metal nanoparticles of various shapes and sizes using wet chemistry\textsuperscript{1, 2} have spurred interest in different methods to characterize their structure. Gold nanoparticles in particular have found applications in chemical sensing, nanomedicine and catalysis.\textsuperscript{3} The nanoparticle shape has been shown to effect the physiochemical \textsuperscript{4} and thermodynamic properties\textsuperscript{5}; additionally, particles enclosed by high-index facets are thought to have higher catalytic activity.\textsuperscript{3, 6, 7} Currently researchers have an array of tools available including scanning electron microscopy (SEM),\textsuperscript{8} transmission electron microscopy (TEM),\textsuperscript{5} atomic force microscopy (AFM)\textsuperscript{9} and x-ray diffraction (XRD)\textsuperscript{10} characterize structure,\textsuperscript{11} particularly when integrated with detection methods such as electron energy-loss spectroscopy (EELS)\textsuperscript{12} and electron back scattering diffraction (EBSD).\textsuperscript{13}

Motivated by interest in how surface ligands and various biomolecules\textsuperscript{14} bind to the surface of nanoparticles, researchers have delved into confirming their surface reconstructions. For face centered cubic (FCC) metal nanoparticles such as gold, silver and platinum, several unique geometries such as rhombic dodecahedron,\textsuperscript{15} tetrahedra,\textsuperscript{16} and trisoctahedra\textsuperscript{17} have recently been synthesized. Characterization of these nanostructures by TEM using selected area electron diffraction (SAED) suggest the existence of higher index facets not previously observed in metal nanoparticles.\textsuperscript{18} Typically the structures for these nanoparticles are obtained by measuring angles in TEM and SEM images\textsuperscript{19} or atomic level reconstructions using high-resolution transmission electron microscopy (HRTEM). HRTEM is a phase contrast method; the
intensity in a HRTEM image is not always proportional to the sample thickness used to determine the inclined surfaces. In some cases, the contrast will reverse if the sample thickness is larger than a quarter of the extinction distance\textsuperscript{20}. In addition, spherical aberration results in image delocalization and chromatic aberration causes a blurring when imaging thicker specimens.\textsuperscript{21} HRTEM imaging resolution and its associated fast Fourier transform (FFT) depend directly on the precise imaging conditions, alignment, and stability of a TEM \textsuperscript{22-24}. Any instability will degrade the image resolution. Aberration corrected HRTEM can account for some of these effects but is still limited in terms of information regarding the crystallography of metal nanoparticles. Alternatively studies using small area electron diffraction (SAED) with probe sizes on the order of microns can be used to confirm the low indexes Bragg reflection. However, with such large probe sizes many nanoparticles contribute to the observed diffraction pattern making it difficult to directly confirm higher index Bragg reflections.

Coherent nanoarea electron diffraction (NED) is an alternative technique where the direct optical Fourier transform of a single nanoparticle through the objective lens in reciprocal space can be used to study the three dimensional (3-D) morphology of the nanoparticles.\textsuperscript{25} The diffraction pattern is the intersection of the Ewald sphere with reciprocal space. The Ewald sphere has a radius of \(1/\lambda_c\), which has a nearly flat radius of curvature. The reciprocal lattice rods of the specimen can be sliced tangentially by the Ewald sphere when the specimen is tilted onto a zone axis. NED overcomes many limitations of SEM and TEM.\textsuperscript{25} Coherent electron diffraction has been used to determine the shape of nearly-perfect 28 nm MgO cubes\textsuperscript{26} and the atomic resolution structure of sub-10 nm Au\textsuperscript{27} and CdS\textsuperscript{28} nanoparticles. These studies require the particles to be smaller than the coherence length of the electron beam (extinction distance), which is about 35 nm for a 200 kV Schottky field emission microscope.\textsuperscript{27, 28} If the sample is
large compared to the extinction distance, multiple scattering events will wash out 3-D structures in reciprocal space. However, we selected gold nanoparticles that were 2 to 4 times thicker than the extinction distance ($\xi_g = 27$ nm for (111) Au and 30 nm for (200) Au. Using coherent nanoarea electron diffraction, we are able to rapidly determine the facets parallel to the beam direction and measure the angles between high-index facets. The precision of our method is significantly improved over measuring angles between facets by TEM imaging. Our electron diffraction patterns show 3-D information in the Bragg reflections which have never before been directly observed in gold nanoparticles by electron microscopy.
2.2 Results and Introduction

We set up the microscope in parallel nanoarea electron diffraction mode (NED) using small probe forming apertures to limit the beam size between 55 to 120 nm, where the electron dose is much smaller than in HRTEM or convergent beam modes. Figure 2.1 shows a schematic diagram of the microscope setup. The electron beam is accelerated to 197 kV ($\lambda_e = 2.53$ pm). Scattering produces reflections which satisfy the Laue condition in reciprocal space. For small crystals, the Bragg conditions are relaxed and reciprocal lattice points transform into reciprocal lattice rods.

Figure 2.2 displays a diffraction pattern from a 16 nm x 41 nm Au nanorod oriented on the [1 1 0] zone axis. The diffraction pattern shows a well ordered spot pattern indicating the nanorod is single crystalline. The diffuse rings are due to diffraction of the amorphous carbon substrate. Each reflection has a streak pointed in the [0 1 -1] direction, which indicates sharp (0 2 -2) planes and a well ordered facet. Thus these [0 1 -1] streaks come from the reciprocal space shape of the crystal intersected by the Ewald sphere. The absence of discrete satellite reflections indicates the (0 2 -2) planes are exactly 90° normal to the [1 1 0] zone axis direction and the crystal is larger than the coherence length of the microscope. Streaks for (0 0 2) planes in the [0 0 1] direction are weakly observed, but are much shorter than those in the [0 1 -1] direction. This indicates the (0 2 -2) facets are more ordered than (0 0 2) facets. One possibility is that (0 2 -2) planes have a larger flat edge, while (1 -1 1), (-1 1 1), and (0 0 2) planes are not sharp on the edges between planes, leading to two sides of the crystal appearing curved. Figure 2.7 shows a diffraction pattern from a rod oriented on the [0 0 1] zone axis with streaks pointing in the [0 1 0] direction. The two diffraction patterns of the rods show that the (0 2 -2) and (0 2 0) planes are
more ordered than (0 0 2) and other planes. Thus we can obtain 3-D structural information with coherent electron diffraction. Figure 2.8 shows an image of a similar but larger 21 x 50 nm rod on the [1 1 0] zone axis acquired with a chromatic aberration corrected TEM. The FFT of the image shows the same streaks in the [0 1 -1] direction as in Figure 2.2, however, discrete reflections are observed along the streak, as the coherence length is larger in this microscope. We conclude that (0 2 -2) and (0 2 0) planes are thus the lowest energy facets in these nanorods. In the simple case of a rod, this information could be determined from a series of TEM images alone, since the 3-D shape is simple and easy to observe all edges from several orientations. Coherent NED is very powerful for the case where not all the edges and faces can be easily observed.

Figure 2.3 displays a diffraction pattern from a 44 nm trisoctahedron (TOH) oriented on the [1 1 0] zone axis, with an inset of (00-4) to show the streaks more clearly. Around each of the Bragg reflections, we observe 8 strong steaks. In FCC crystals, the direction is normal to the plane of the same index. Therefore the angle between directions is also the angle between planes. We measure the direction that the streaks were pointing towards by tracing a line from each streak until it intersects a Bragg reflection. In general, we select higher order reflections since the streaks typically point at reflections far from the origin. To determine the direction of these vectors from the origin, the angle between direction vectors is given by Equation 2.1:

\[ \cos(\theta) = \frac{h_1 * h_2 + k_1 * k_2 + l_1 * l_2}{\sqrt{h_1^2 + k_1^2 + l_1^2} \sqrt{h_2^2 + k_2^2 + l_2^2}} \] (2.1)
For example, using (3 -3 -1), two streaks point to [-1 1 9] and [-6 6 -2]; the directions of these streaks are determined to be [-4 4 10] and [-9 9 -1] by a vector difference, and the angle between these directions is 65.0° (see Figure 2.9 for visual traces). Several reflections may be used to index the complete array of streaks. Table 1 displays the measured angles in the [1 1 0] zone axis from the experimental diffraction pattern.

<table>
<thead>
<tr>
<th>Reflection</th>
<th>Points to</th>
<th>Points to</th>
<th>Direction</th>
<th>Direction</th>
<th>θ Between Planes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(3 -3 -1)</td>
<td>(-1 1 9)</td>
<td>(-6 6 -2)</td>
<td>[-4 4 10]</td>
<td>[-9 9 -1]</td>
<td>65.0°</td>
</tr>
<tr>
<td>(3 -3 -1)</td>
<td>(-1 1 9)</td>
<td>(4 -4 2)</td>
<td>[-4 4 10]</td>
<td>[1 -1 3]</td>
<td>54.7°</td>
</tr>
<tr>
<td>(0 0 -4)</td>
<td>(1 -1 -1)</td>
<td>(-6 6 12)</td>
<td>[1 -1 3]</td>
<td>[-6 6 16]</td>
<td>53.2°</td>
</tr>
</tbody>
</table>

The 8 streaks observed in the diffraction pattern are all normal to high index facets. Unlike the nanorod, (0 2 -2) and (0 2 0) are not the lowest energy planes. These angles cannot all be measured from a TEM image, since a TEM image is a projection of many planes and only the outer edges can be observed. Figure 2.4 displays the angles between edge tangentials (white text) and plane normals (black text) normals measured for the image of the TOH particle in black text. In general, the plane tangentials have a precision of 5% since the vertices appear curved in the TEM images. In comparison to the diffraction patterns, the measured angles between plane normals in the image are different and subject to a larger experimental error. The 44 nm TOH geometry is verified through comparison of measured angles (Figure 2.4) to a computer
generated 3-D model with unit edge lengths 1 and $1 + 1/\sqrt{2}$. The corresponding tangential angles are expected to be 117° and the angles between plane normals are predicted at 62.8°.

A larger 101 nm TOH crystal oriented on the [110] zone axis is displayed in Figure 2.5. There are many more streaks visible in the NED pattern of the larger particle than the smaller particle, which indicates additional lowest energy facets. We combine the streak directions from multiple reflections to measure additional angles shown in Table 2. The larger TOH crystal corresponds well to a computer generated 3-D model with unit edge lengths $1 + 1/\sqrt{2}$ and $1 + 1/\sqrt{42}$. Figure 2.6 shows the measurement of angles between normals from the TEM image. We confirm these measurements through comparison to the 3-D model. The outermost tangential angles are ideally 84.8° and the angles between plane normals are expected to be 95.3°. These angles are in close agreement to what we observed with coherent NED but with increased measurement error since the edge sharpness is reduced for larger particles in TEM images. The directions of growing planes cannot be determined from the image alone since the FFT of the image reveals no reflections. Therefore for large particles, only coherent NED provides the direction of plane normal. The precision of measurement of angles between plane normal is increased in coherent NED compared to using TEM images.

<table>
<thead>
<tr>
<th>Reflection</th>
<th>Points to</th>
<th>Points to</th>
<th>Direction</th>
<th>Direction</th>
<th>θ Between Planes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0 0 -2)</td>
<td>(-3 3 -1)</td>
<td>(-5 5 1)</td>
<td>[-3 3 1]</td>
<td>[-5 5 3]</td>
<td>9.7°</td>
</tr>
<tr>
<td>(-2 2 0)</td>
<td>(-5 5 5)</td>
<td>(-3 3 5)</td>
<td>[-3 3 5]</td>
<td>[-1 1 5]</td>
<td>24.5°</td>
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<tr>
<td>(-2 2 0)</td>
<td>(2 -2 2)</td>
<td>(-1 1 5)</td>
<td>[4 -4 2]</td>
<td>[1 -1 5]</td>
<td>54.7°</td>
</tr>
</tbody>
</table>

Table 2.2: Direction and Angles of Diffraction Streaks for Large TOH in Figure 2.4
We have used coherent nanoarea electron diffraction to measure the direction of planes and the angles between planes of complex Au nanocrystals. This technique is especially useful for large nanocrystals, in which lattice fringes are not observed in TEM images due to multiple scattering in thick crystals, directions cannot be determined from FFTs of images, and many facets are invisible due to the transmission geometry. This technique can be used to precisely determine the 3D shape of a nanocrystal without the use of tomography and is accessible in any field emission TEM.
2.3 Experimental Procedure

**Materials.** Hydrogen tetrachloride (HAuCl₄, > 99.999%), sodium borohydride (NaBH₄), and ascorbic acid (C₆H₈O₆, > 99.0%) were obtained from Aldrich and used as received. Cetyltrimethylammonium bromide/chloride (CTAB, > 99%, CTAC > 98%) was obtained from Sigma and used without further purification. All glassware were cleaned using aqua regia and rinsed with Barnstead E-Pure 18 MΩ·cm water.

**Instrumentation**

Transmission electron micrographs (TEM) images were taken on a 197 kV JEOL JEM 2010F Field Emission Microscope with a spherical aberration coefficient (Cₚ) of 1.0 mm. We used parallel probe sizes of 55 - 120 nm to acquire diffraction from individual nanoparticles. It is important to select a probe size slightly larger than the nanocrystal size so that all facets are sampled. In contrast to SAED, in NED, only the part of the specimen illuminated with the electron beam contributes to the observed diffraction pattern, leading to high signal to background diffraction patterns. Gold nanoparticles were tilted onto a low index zone axis such as [1 1 0] or [0 0 1]. Diffraction patterns were recorded onto image plates. The diffraction patterns were calibrated by collecting patterns of polycrystalline aluminum with known d-spacings using the same conditions as the gold nanoparticles for each experiment. We displayed a cropped section of the diffraction pattern for clarity. However for measurement we used the full diffraction pattern as many streaks pointed to high index reflections beyond the 4th Laue zone. For TEM sample preparation, 10 µL of gold nanoparticle suspension was drop cast onto holey and lacey carbon TEM grids (Pacific Grid-Tech).
**Measurement of Diffraction Streaks:** The diffraction streaks are traced until the traced lines intersect a Bragg reflection. An experimental error can arise from the precision in tracing the small streaks. Typically the error can be up to 1° to 2°. The experimental error is lower for smaller particles with fewer facets, as the streaks will appear longer and are easier to trace. Generally the diffraction pattern and image are rotated with respect to each other. Thus for large particles, the FFT of an image will not reveal the orientation.

**Synthesis of seeds for CTAB rods** The nanoparticle seeds were synthesized by modification the method of El-Sayed et al. A CTAB solution (7.5 mL, 0.1 M) was mixed with 0.25 mL of 0.01 M H\textsubscript{Au}Cl\textsubscript{4}. To the stirred solution, 0.6 mL of ice-cold newly made 0.01 M NaBH\textsubscript{4} was quickly added, which resulted in a light brown solution. After stirring the solution vigorously for 2 minutes, the solution was kept for 1 hour in room temperature before use.

**Synthesis of CTAB seeds for trisoctahedra (TOH)** The nanoparticle seeds were synthesized by following the procedure by El-Sayed. et al. CTAB solution (7.0 mL, 75 mM) was mixed with 46 \( \mu \)L of 20 mM H\textsubscript{Au}Cl\textsubscript{4}. To the solution, 0.42 mL of ice-cold newly made 0.01 M NaBH\textsubscript{4} was quickly added under vigorous mixing and to give a pale brown solution. The solution was used within 2-5 h after the preparation.

**Synthesis of CTAB Gold Nanorods.** Gold nanorods of aspect ratio 2.5 were synthesized as reported elsewhere. To 95 mL of 0.1 M CTAB, 5 mL of H\textsubscript{Au}Cl\textsubscript{4}, 0.55 mL of ascorbic acid, and 0.12 mL of seed were added sequentially and left overnight. The gold nanorods were purified twice by centrifugation at 8000 RPM for 20 mins.

**Synthesis of TOH gold nanoparticles.** Trisoctahedra (TOH) nanoparticles were synthesized by a seed mediated method used by Lee et al. A 0.125 mL solution of 20 mM H\textsubscript{Au}Cl\textsubscript{4} was mixed
with 9 mL of 22 mM cetyltrimethylammonium chloride (CTAC). To this mixture, 3.06 mL of 38.8 mM ascorbic acid was added to make the concentration of ascorbic acid 9.5 mM and was thoroughly mixed. Then, seed was diluted 100 fold with nanopure water, and 50 µL of the diluted seed (B) was added to the solution and left overnight. The gold nanoparticles were purified twice by centrifugation at 3381 RCF for 20 mins.
Figure 2.1: (a) Schematic diagram of the TEM microscope in nanoarea electron diffraction mode (NED) a coherent parallel beam of size 50 to 120 nm illuminates a trisoctahedra gold nanocrystal (b) The beam must be larger than the gold nanocrystal in order to include all facets. (c) The resulting diffraction pattern is a slice of the Ewald sphere through the reciprocal lattice, producing a 2D diffraction pattern shown. Features of the reciprocal space which are perpendicular to the beam such as sharp facets will appear as streaks in the diffraction pattern.
Figure 2.2: (a) NED pattern of a gold nanorod oriented on the [110] zone axis. Well-defined streaks point in the [0 1 -1] direction. (b) Magnified view of (0 2 -2) reflection showing streaks pointing in the [0 1 -1] direction. (c) Image of the nanoprobe on the nanorod. (d) A computer generated image of the nanorod.
Figure 2.3: (a) NED pattern of a 44 nm TOH crystal oriented on the [110] zone axis. The streaks are normal to the planes. (b) Magnified view of (0 0 -4) reflection showing streaks measured from this and other reflections. (c) TEM image of the TOH crystal. (d) Computer generated image of the TOH crystal.
Figure 2.4: TEM image of a 44 nm TOH gold nanoparticle. The tangents to the faces are drawn and the angles between tangents are shown in white text. The angles between plane normals are $180^\circ$ minus this angle and are shown in black text. The measurement error arises in drawing lines tangent to the shape edges.
Figure 2.5: NED pattern of a 101 nm TOH crystal oriented on the [110] zone axis. There are many more streaks compared to the crystal in Figure 2.3, which indicates additional facets. (b) Magnified view of \((-2\ 2\ 0)\) reflection showing streaks measured from this and other reflections. (c) TEM image of the TOH crystal under the nanobeam. (d) Computer generated image of the TOH crystal.
Figure 2.6: TEM image of a 101 nm TOH gold nanoparticle. The tangents to the faces are drawn and the angles between tangents are shown in white text. The angles between plane normals are 180° minus this angle and are shown in black text.
Figure 2.7: NED pattern of a rod oriented on the [001] zone axis. Streaks point in the [010] direction.
Figure 2.8: HRTEM image of a rod oriented on the [110] zone axis. The FFT of the image shows streaks pointed in the [0 1 -1] direction.
Figure 2.9: We trace streaks from a reflection (solid circle) until the traces intersect a Bragg reflection (dotted circles). The direction of the streak is the same direction as the facet normal, and is determined by the vector difference from the starting and ending reflection indices yellow text). An example is shown for the diffraction pattern in Figure 2.3.
2.4 References


CHAPTER 3

OFF-RESONANCE SERS FROM SUSPENSIONS OF DIFFERENT ASPECT RATIO GOLD NANORODS: NOT WHAT WE THOUGHT

3.1 Introduction

Surface-enhanced Raman scattering (SERS) is a vibrational technique whose promise for chemical sensing has been debated since the 1970s.¹ A primary design objective in SERS optimization is to tailor the surface plasmon resonance relative to the laser excitation wavelength. This is because the on-resonance field enhancement at the surface of the plasmon-active material can increase the Raman signal intensity of nearby molecules by several orders of magnitude. In the case of SERS on immobilized silver nanostructures, the maximum signal enhancement was observed if the plasmon band position was red-shifted compared to the laser excitation wavelength.² Based on these studies, researchers have fabricated rationally designed nanoparticles for biomedical applications³ such as highly sensitive assays⁴ and multiplexed imaging⁵ The stable signals and multiplexing capabilities of these nanoparticles offer an attractive alternative to fluorescence-based techniques.⁶,⁷ A recent report notes that a SERS-based approach can outperform an enzyme-linked immunosorbent assay (ELISA).⁸ Nanoparticle-based SERS assays could, thus, provide novel sensing capabilities that complement or improve present technologies and lead to next-generation clinical diagnostics. For example, Moskovits et al. have extended such studies to quantitatively confirm the ratio of cancerous to noncancerous cells in samples with two different reporter molecule-antibody combinations.⁹ Using labeled nanoparticles as Raman reporters to achieve contrast in deep-tissue measurements is currently an active area of research.¹⁰,¹¹
Light scattering, absorption, and fluorescence arising from the tissue limit the choice of Raman excitation wavelengths to the near-infrared (NIR) spectral region. In this spectral region (700-1100 nm), gold nanorods and nanoshells can be used as effective SERS-active nanoparticles as they exhibit a tunable plasmon band where tissue has low absorption. Additionally, the presence of the nanoparticles dispersed throughout the tissue adds absorption and scattering effects to the Raman measurement as the light propagates. In this way, nanoparticles that would be injected into tissue behave much like in colloidal suspensions.

For suspensions, as opposed to substrates, accounting for light propagation and attenuation is vital. While the resonant plasmon helps to enhance the Raman signal, attenuation by absorption and scattering complicates experimental design and optimization. This effect is clearly visible in a solution of nanoparticles. For example, Figure 3.1 shows a photograph of a laser beam traversing two cuvettes, illustrating extinction effects in solution. The cuvette on the left in both panels, containing water, displays minimal scattering and absorption, resulting in minor attenuation of the laser beam. The cuvette on the right, containing gold nanorods in suspension, shows that the laser beam is unable to penetrate effectively through the cuvette, due to a combination of absorption and scattering of light by the nanorods. Therefore, when performing SERS experiments on such nanorods in solution, Raman-scattered light would be similarly extinguished. Therefore, it is important to understand that there is an antagonistic interplay between extinction and SERS enhancement in the observed Raman signal collected from colloidal suspensions, and therefore in biological sensing.

Here we explore the competition between SERS enhancement and extinction on propagation through the sample. We investigate the dependence on plasmon resonance frequency by using gold nanorods of six different aspect ratios which provide longitudinal surface plasmon
resonances at wavelengths spanning 600-800 nm. The Raman reporter, methylene blue, was trap-coated with a polyelectrolyte layer near the surface of each nanorod. SERS spectra were acquired using a 785 nm excitation wavelength in transmission mode. In order to compare signals across batches of nanorods, the average number of reporter molecules per nanorod was quantified using electrospray ionization liquid chromatography mass spectrometry (ESI-LC-MS). We report the Raman signal per nanorod as a function of aspect ratio, correcting for the attenuation due to extinction in suspension using methanol as an internal standard.
3.2 Results and Discussion

SERS measurements are typically based on Raman reporter molecules attached directly to the surface of the nanoparticles either by covalent or electrostatic interactions.\textsuperscript{17} Other reports have examined the use of SERS using reporter molecules separated at fixed distances from the surface of the nanoparticles by employing a dielectric silica shell.\textsuperscript{18} Here, we utilize a polyelectrolyte dielectric layer to wrap gold nanorods of a variety of aspect ratios.\textsuperscript{19} A schematic of this technique is illustrated in Figure 3.2a. First, positively-charged CTAB capped gold nanorods were wrapped with negatively charged polyacrylic acid (PAA). We then attached methylene blue reporter molecules by electrostatic interactions. The reporter molecules were then trap-coated by an additional polyallylamine hydrochloride (PAH) polyelectrolyte layer.\textsuperscript{20} Layer wrapping was confirmed at each step by zeta potential measurements (Figure 3.2b) and electronic absorption spectra as previously described.\textsuperscript{21} Shifts in the longitudinal plasmon peak of 5 nm or less are observed as the surface functionalization proceeds (Figure 3.8). The polyelectrolyte coating also stabilizes the as-synthesized CTAB capped gold nanorods from aggregating in polar protic solvents.\textsuperscript{22} As a rough guide, assuming a 2.5 nm thick CTAB bilayer and 1.5 nm thicknesses for the polyelectrolyte layers,\textsuperscript{19,21} the Raman reporter dyes should be about 4 nm from the metal surface. There is no apparent aggregation in solution, as suggested by the lack of plasmon band-broadening. In the case that two nanorods are in contact, the spacer layers guarantee that the reporter molecule is approximately 4 nm away from the proximal metal surface and about 12 nm away from the distal metal surface. Although we cannot rigorously prove that there are zero aggregates in solution, these relative distances suggest that the reporter molecules are not expected to lie in hot spots.
The number of reporter molecules per nanorod was quantified for each aspect ratio by ESI-LC-MS. We found the average number of reporters per gold nanorod was 100-300 reporter molecules (Figure 3.2c). These ESI-LC-MS measurements were carried out in triplicate for three independent batches, for each aspect ratio of nanorods. The means of 9 measurements per aspect ratio, with attendant error bars of one standard deviation from the means, are shown in Figure 3.2c with further details in Figure 3.9. The values of 100-300 reporters per nanorod are far fewer than monolayer coverage. For example, methylene blue adsorbs to charged surfaces from water with a footprint of 0.66 nm$^2$. A full monolayer of methylene blue, hence, would imply approximately 3000 molecules per nanorod. Therefore, experimental loadings are less than 10% of monolayer coverage. Using these values we compare the experimentally observed SERS signal intensity from each gold nanorod suspension and relate them to theory.

To characterize the observed spectral signatures, we present three reference spectra in Figure 3.3. The electronic absorption spectrum of methylene blue in methanol is shown in Figure 3.3a. An absorption maximum can be seen around 670 nm with a shoulder at 650 nm, both of which are blue-shifted from the Raman laser excitation wavelength of 785 nm. This should minimize any chemical resonance effects such as those observed in surface enhanced resonant Raman spectroscopy (SERRS).

For Raman measurements, we collected signal from a sample of methylene blue polyelectrolyte coated gold nanorods resuspended in methanol. This approach was first introduced by Kneipp and co-workers and has the benefit that the methanol Raman band at 1030 cm$^{-1}$ can serve as an internal standard. For reference, the Raman spectra of methylene blue in methanol and of neat methanol are shown in Figure 3.3b and Figure 3.3c.
We synthesized gold nanorods with aspect ratios from 2 to 4.5, resulting in a systematic variation of the longitudinal plasmon resonance band. Figure 3.4a shows the electronic absorption spectra of each of the gold nanorod suspensions after the final PAH polyelectrolyte trap-coating. On the same spectral axes, we also depict the laser excitation wavelength with a red dotted line and the resulting Raman spectrum (black curve) for reference. Traditionally, Raman spectra are reported in terms of Raman shift from an excitation wavelength, as shown in Figure 3.4b where the spectra are normalized for concentration and the number of reporter molecules per rod and then offset for clarity. From the shaded region in Figure 3.4a, it is obvious that the extinction profile of higher aspect ratio nanorods in suspension overlaps both the spectral profile of the Raman excitation laser and the wavelengths of Raman scattered photons. The largest Raman signal is observed for nanorods that have a plasmon band blue shifted from the excitation frequency. To quantify the recorded signal, we examined both the reporter Raman signal as well as that of the suspending medium (methanol).

Two spectral features to characterize our suspensions are the Raman band originating from methanol at 1030 cm\(^{-1}\) shift and the Raman band originating from methylene blue at 1616 cm\(^{-1}\) shift (Figure 3.5).\(^{26}\) The signal intensity at 1030 cm\(^{-1}\) shift should only decrease from extinction of the Raman excitation wavelength since we assume methanol is not enhanced by the gold nanorods.\(^{24}\) However, the Raman signal from the reporter at 1616 cm\(^{-1}\) shift will be affected by the location of the longitudinal surface plasmon resonance determined by the aspect ratio of the gold nanorod suspensions. By examining these two bands as a function of aspect ratio, we illustrate the effects of the competing physical processes. In addition, we can select an aspect ratio that would provide the largest Raman signal in suspension.
Extinction measurements (Figure 3.4a) and Raman measurements of the methanol band (Figure 3.5) provided two estimates of the extinction due to nanorods. This extinction is quantified in Figure 3.6a. The competing process of SERS electromagnetic enhancement when extinction effects are considered to be negligible (i.e. substrate measurements) is presented in Figure 3.6b. A prolate-spheroidal approximation for the rods was used to estimate absorption-free electromagnetic enhancement. It is clear from Figure 3.6 that maximum extinction of the Raman excitation occurs near the maximum of electromagnetic enhancement. The collected signal seen in Figure 3.5 illustrates this competition. reporter molecule quantification.

As the SERS signal will vary as a function of the nanoparticle concentration and the number of reporter molecules per nanoparticle, it is important to account for these variations in order to understand the spectral data. Using theory we account for these experimental variations between samples. The observed Raman spectra are quantified using the reporter signal at 1616 cm\(^{-1}\) shift for each aspect ratio. These results are shown in Figure 3.7. The normalized Raman signal in transmission mode from a suspension of gold nanorods can be shown to be equal to:

\[
R(\omega, \omega_0, h) = AR(0)\left(G_N(\omega, \omega_0)\right)e^{\frac{mC_{ext}(\omega_0)h}{mC_{ext}(\omega)}}e^{\frac{mC_{ext}(\omega)h}{mC_{ext}(\omega_0)}}
\]

where the frequencies \(\omega_0\) and \(\omega\) correspond to the incident light and the Stokes’ shifted frequencies, respectively, \(A\) is the effective cross-sectional area of the illuminating and collecting beams, \(R(0)\) is the raman signal from a single reporter molecule absent the nanorod, \(\rho\) is the concentration of the nanorods in the solution, \(N\) indicates the number of bound Raman reporter molecules, \(h\) is the interaction path length, \(C_{ext}\) is the extinction cross-section of the individual
nanorods, $m$ is the refractive index of the solution, and $\langle G_N(\omega, \omega_0) \rangle$ is the ensemble-averaged extinction modified enhancement factor. The ratio in Eq. 1 models the propagation of incident light and Raman scattered light through the suspension and is a form of Beer’s Law. This expression can be simplified if the number of reporter molecules is small, in which case the ensemble-averaged, extinction-modified enhancement factor $\langle G_N(\omega, \omega_0) \rangle$ can be linearized, i.e.,

$$\langle G_N(\omega, \omega_0) \rangle = \langle G(\omega, \omega_0) N \rangle = \langle G(\omega, \omega_0) \rangle \langle N \rangle.$$

This allows us to obtain the averaged per particle extinction modified enhancement factor.

$$\langle G(\omega, \omega_0) \rangle = \frac{R(\omega, \omega_0)}{R(0)} \frac{mC_{\text{ext}}(\omega)}{e^{-mC_{\text{ext}}(\omega) h}} e^{-mC_{\text{ext}}(\omega_0) h}.$$

(3.2)

The single-particle, extinction-modified enhancement factor was computed using Eq. (2) and is shown in Figure 3.7. A maximum per-particle signal enhancement is found at an aspect ratio of 2.75, with 2.5 also within error bounds. This implies that maximum observed signal occurs with gold nanorods blue-shifted from the laser excitation wavelength. This differs from the field enhancement maximum expected at an aspect ratio of 4.

### 3.3 Conclusion

In contrast to SERS experiments of immobilized nanoparticle substrates, where absorption effects are minimal, a significant extinction contribution is realized for SERS particles in suspension. In analyzing the signal generation and recording rigorously, we have demonstrated that Raman-active molecules do not provide monolayer coverage of our polyelectrolyte bearing nanorods as usually assumed. In our experiments, loadings are less than 10% of the expected maximum for monolayer coverage. The calculated ensemble-average
signal intensity based on experimentally determined molecular coverage suggests the maximum Raman scattered signal is obtained from that plasmon resonance that is blue-shifted from excitation. This implies the use of nanorods of lower aspect ratios for optimal sensing. Extinction is an important consideration in combination with maximal SERS enhancement when designing tagged Raman probes for suspension applications such as collecting Raman reporter signal through tissue. Efforts toward refining our model by accurately spatially localizing reporter binding sites on the gold nanorods is currently underway in our laboratories.
3.4 Experimental Section

Materials

Hydrogen tetrachloroaurate(III) hydrate (HAuCl$_4$·3H$_2$O, > 99.999%), sodium borohydride (NaBH$_4$, 99.99%) and silver nitrate (AgNO$_3$, > 99.0%) were obtained from Aldrich and used as received. Methylene blue (>82%) with the remainder in organic salts, cetyltrimethylammonium bromide (CTAB, > 99 %) and ascorbic acid (C$_6$H$_8$O$_6$, > 99.0%) were obtained from Sigma Chemical and used as received. The polyelectrolytes polyacrylic acid, sodium salt, $M_w$~15,000 g/mol, (35 wt % solution in H$_2$O) (PAA) and polyallylamine hydrochloride, $M_w$~15,000 g/mol, (PAH) were obtained from Aldrich and used without further purification. Sodium chloride (NaCl, > 99.0%) was obtained and used as received from Fischer Chemicals. All solutions were prepared using Barnstead E-Pure 18 MΩ-cm water. All glassware used was cleaned with aqua regia and finally rinsed with 18 MΩ-cm water.

Gold Nanorod Synthesis

CTAB-coated gold nanorods of aspect ratio (AR) 2, 2.5, 2.75, 3, 3.5 and 4.5 corresponding to dimensions (35 ± 2 nm x 17 ± 1 nm, 39 ± 6 nm x 16 ± 4 nm, 40 ± 5 nm x 14 ± 2 nm, 43 ± 4 nm x 15 ± 1 nm, 45 ± 3 nm x 12 ± 1 nm, 48 ± 3 x 11 ± 1 nm) were synthesized as previously described. The gold nanorods were purified twice by centrifugation (8000 RPM, 2 hours).

Polyelectrolyte Layer By Layer (LBL) Coating

We coated the gold nanorods with PAA and PAH using an adapted procedure to maintain the nanoparticle concentration throughout each step. For each polyelectrolyte layer we
prepared stock aqueous solutions of PAA (-) or PAH (+) at concentrations of 10 mg/ml prepared in 1 mM NaCl and a separate aqueous solution of 10 mM NaCl. To 30 mL aliquots of twice centrifuged CTAB gold nanorods (0.15 nM in particles) we added 6 mL of PAA or PAH (+) solution followed by 3 ml of 10 mM NaCl. The solutions were left to complex overnight (12 – 16 hours) before purification using centrifugation (5000 RPM, 2 hours). We then centrifuged the supernatant and concentrated the two pellets to minimize losses in gold nanorod concentration. Zeta potential measurements and UV-Vis absorption measurements were made between each layering step to confirm successful coating without aggregation of the gold nanorods.

Zeta potential was measured on a Brookhaven ZetaPals instrument. Absorption spectra were recorder on a Cary 500 UV-Vis-NIR spectrometer and transmission electron microscope images were taken on a JEOL 2100 Cryo TEM microscope at a 200 kV accelerating voltage. All TEM grids were prepared by drop-casting 10 µL of purified gold nanorods on a holey Carbon TEM grid (Pacific Grid-Tech). A Thermo Scientific Sorvall Legend X1 centrifuge in a “swinging bucket” orientation was used for purification as detailed below.

*Methylene Blue Gold Nanorod Complexation*

We used the same initial concentration of gold nanorods of 0.15 nM prior to addition of 10 uL stock 1 mM methylene blue solution to 0.99 mL of gold nanorods, to give a final methylene blue concentration of 1 µM during complexation. The mixture was left for an hour before removing excess reporter molecules using centrifugation (2350 RCF, 15 min). The supernatant was also centrifuged and both pellets concentrated to maintain gold nanorod concentration. The concentrated pellet was then re-suspended to 1 mL with DI water before adding 0.2 mL of PAH (10 mg/mL) in 1 mM NaCl and 0.1 mL of 10 mM aqueous NaCl.
solutions. This mixture was left overnight before purification by centrifugation (2350 RCF, 15 min); again the supernatant was also centrifuged (2350 RCF, 15 min) and the pellets concentrated. For final purification we dialyzed the aqueous solutions in Spectrum Labs 100k MWCO G2 membranes against 4L of Barnstead E-Pure (18 MΩ-cm) water for 48 hours. Any remaining unbound methylene blue molecules and excess polyelectrolyte were removed via centrifugation followed by dialysis in a 100 kDa cutoff membrane. By using a membrane pore size 5 times larger than the molecular weight of the polyelectrolyte used, any multi-layer polyelectrolyte bundles that may have formed during synthesis should be removed.

**ESI-LC-MS Quantification of Methylene Blue**

For electrospray ionization liquid chromatography mass spectrometry (ESI-LC-MS) quantification of the number of methylene blue molecules, we centrifuged the methylene blue complexed polyelectrolyte gold nanorods (2350 RCF, 15 min) and the supernatant again (2350 RCF, 15 min) before concentrating the pellets. We re-suspended the pellet in 50 uL of methanol and the gold nanorod cores were then etched by adding 0.010 ml of 1 M KCN and waiting 1 – 2 hours. It was observed that etching had completed once the solution turned colorless. KCN itself does not disturb the mass spectral analysis of methylene blue (see Supporting Information). All data in Figure 3.2c are the result of triplicate measurements for each of three independent batches of nanorods for each aspect ratio.

The ESI-LC-MS analysis was performed in Metabolomics Center at UIUC with a 5500 QTRAP mass spectrometer (AB Sciex, Foster City, CA) which is equipped with a 1200 Agilent LC. Analyst (version 1.5.1, Applied Biosytems) was used for data acquisition and processing. An Agilent Zorbax SB-Aq column (5u, 50 x 4.6mm) was used for the separation.
The HPLC flow rate was set at 0.3 mL/min. HPLC mobile phases consisted of A (0.1% formic acid in H₂O) and B (0.1% formic acid in acetonitrile). The gradient was: 0–1 min, 98% A; 6–10 min, 2% A; 10.5–17 min, 98% A. The autosampler was kept at 5°C. The injection volume was 1µL. The mass spectrometer was operated with positive electrospray ionization. The electrospray voltage was set to 2500 V, the heater was set at 400 °C, the curtain gas was 35, and GS1 and GS2 were 50, 55, respectively. Quantitative analysis was performed via multiple reaction monitoring (MRM) where m/z 284.2 to m/z 240.1 for methylene blue was monitored. Calibration curves were run on methylene blue standards from 0.01 µM to 0.1 µM concentrations; our data found 0.029-0.108 µM concentrations. (See Figure 3.8 for further details).

Raman Spectroscopy

For Raman acquisition the dialyzed gold nanorod samples were centrifuged to concentrate the pellets (2350 RCF, 15 min) and the supernatant poured off. The samples were then re-suspended in 2 mL of methanol (HPLC Fischer Scientific > 99.9% purity) and placed in a quartz cuvette. Triplicate samples were synthesized, with seven spectral acquisitions of each sample were collected to minimize the signal-to-noise ratio.

Raman spectra were acquired on liquid samples in transmission mode (LabRAM, Horiba). The excitation wavelength for all measurements was 785 nm with a 30 second acquisition time. The Raman shift from 400-1800 cm⁻¹ was collected at ~9 cm⁻¹ spectral resolution. Laser light was focused through a 1cm path length cuvette with a 40 mm focal length lens and collected with a 125 mm focal length lens to collimate the transmitted light and direct it to the spectrograph. Laser power at the sample was 25mW. Between measurements the cuvette was rinsed with aqua regia (3:1 HCl:HNO₃) followed by multiple rinses with Barnstead E-Pure.
(18 MΩ-cm) water and methanol. For Raman measurements the gold nanorod concentrations were 0.36 nM, 0.36 nM, 0.31 nM, 0.33 nM, 0.33 nM, and 0.29 nM for aspect ratios 2, 2.5, 2.75, 3, 3.5, and 4.5 respectively, necessitating a small correction to the recorded data which did not change the relative trend observed in the Raman spectra between aspect ratios. Electronic absorption spectra were confirmed before and after Raman measurements.
Figure 3.1. Illustrative image demonstrating extinction effects in solution. Upon laser illumination, minimal extinction (scattering + absorption) is observed in water (left cuvette). In contrast, suspensions of gold nanorods in water exhibit extinction under illumination (right cuvette). a) Laser illumination under ambient lighting b) Laser illumination without ambient lighting.
Figure 3.2.  a) Schematic of a gold nanorod with a (red) polyacrylic acid coating, followed by methylene blue reporter molecules (blue) and a polyallylamine hydrochloride (green) trap coat. b) Corresponding zeta potential for aspect ratio 3 gold nanorods as a function of layer coating corresponding to the stages in (a).  c) Quantification of the number of methylene blue reporter molecules per gold nanorod as a function of aspect ratio.
Figure 3.3. Reference spectra. a) UV/Vis of 15 µM methylene blue in methanol b) Raman spectrum of 500 µM methylene blue in methanol c) Raman spectrum of neat methanol with 785 nm excitation
Figure 3.4.  a) Extinction spectra for gold nanorods (AR 2 - pink, AR 2.5 - purple, AR 2.75 - blue, AR 3 - green, AR 3.5 - brown, and AR 4.5 - orange), normalized to concentration plotted on the same axes as the position of the Raman excitation wavelength (red dots) and the resulting Raman spectrum (black). The spectral region shown in Figure 3.5b is highlighted by shadow in Figure 3.5a b) Surface enhanced Raman spectrum of methylene blue attached to six different aspect ratios of gold nanorods bearing PAA polyelectrolyte layer (offset for clarity) normalized for gold nanorods and reporter molecule concentration
Figure 3.5. Comparison of Raman spectra acquired from gold nanorod suspensions bearing polyelectrolyte layers plus methylene blue reporter in methanol. The variation in Raman intensity for the methanol bands is illustrated by the peak at 1030 cm\(^{-1}\) which varies as a function of aspect ratio. Gold nanorod suspensions are normalized for concentration and the number of reporter molecules per gold nanorod.
Figure 3.6. a) Blue dots: Experimentally observed extinction of Raman excitation at 785 nm. Red triangles: Difference in Raman band at 1030 cm\(^{-1}\) shift with neat methanol and each aspect ratio of gold nanorods suspended in methanol. b) Predicted electromagnetic enhancement from varying aspect ratios of spheroids in the quasi-static limit. Mean-free electron path and depolarization/radiative damping corrections were applied.
Figure 3.7. Determined averaged per particle extinction modified enhancement factor $\langle G(\omega, \omega_0) \rangle$ for the used aspect ratios (some error bars are smaller than the data points).
Figure 3.8: Uv-vis absorption spectra of aspect ratio 2 CTAB capped gold nanorods (black) and sodium polyacrylate (PAA) methylene blue (MB) polyallylamine hydrochloride (PAH) gold nanorods (red).
Figure 3.9: ESI-LC-MS elution time peak area calibration curve of Methylene Blue with equivalent additions of KCN
3.5 References


76


CHAPTER 4

SHAPE DEPENDENCE IN SUSPENSION BASED SERS MEASUREMENTS OF GOLD NANOPARTICLES

4.1 Introduction

Plasmonic metal nanoparticles show promise for biological applications ranging from diagnostic imaging to in-vivo therapies.\textsuperscript{1-4} Intense electromagnetic fields near the metal surface have been employed to cultivate highly sensitive surface enhanced Raman spectroscopy (SERS) substrate-based assays as well as nanoparticle probes approaching single molecule detection limits.\textsuperscript{5} SERS has a dynamic range surpassing that of fluorescence spectroscopy\textsuperscript{6} and the ability to multiplex vibrational fingerprints of distinct reporter molecules.\textsuperscript{4} The design considerations for such probes focus on maximizing the electromagnetic enhancement defined by their shape, size, and polarization of the incident light.\textsuperscript{7} These parameters have been aided by computer simulations based on the discrete dipole approximation (DDA), finite-difference time-domain (FDTD) method, and the finite element method (FEM), among others.\textsuperscript{8-10}

Recent studies have indicated SERS signal intensities to be the highest for noble metal nanoparticles where the nanostructures concentrate the electromagnetic fields to sharp corners or edges.\textsuperscript{11, 12} Previously, we have demonstrated that gold nanocubes dried on substrates have a higher SERS enhancement when compared to gold nanorods.\textsuperscript{13} The nanoparticles were electrostatically bound to a self-assembled monolayer of 4- mercaptobenzoic acid (4-MBA) onto gold coated substrates. The interpretation of these results is complicated by the presence of “hot spots” from overlapping electromagnetic fields in adjacent nanoparticles. Theoretical treatments have given some indication as to the relative expected magnitude for scattered intensities from reporter molecules residing in “hot spots”. For example Van Duyne and co-workers, observed
that electric fields may actually be focused in nanoscale crevices on the surface of the noble metal nanoparticles.\textsuperscript{14} We approach this subject by undertaking theoretical simulations followed by SERS measurements on suspensions of different shaped polyelectrolyte coated gold nanoparticles. The polyelectrolyte coating ensures the nanoparticles are separated by at least 5 nm even when in direct contact, minimizing hot spot effects.

In this study, nanoparticles were then synthesized and stabilized with a PAA polyelectrolyte layer before electrostatically binding methylene blue reporter molecules. An oppositely charged PAH polyelectrolyte layer was used to trap the bound molecules on to the surface of the nanoparticles. After Raman spectroscopic analysis we measured the number of reporter molecules per nanoparticle using electrospray ionization liquid chromatography mass spectrometry (ESI-LC-MS). Additionally we quantified the amount of gold atoms per particle by inductively coupled plasma mass spectrometry (ICP-MS). These numbers allowed us to confirm the molar extinction coefficient of each shape to calculate the number of reporter molecules per particle. Quantification of the SERS enhancement is then reported relative to the spontaneous Raman signal obtained from a dilution series of methylene blue. Experimental results were compared to finite element method based theoretical simulations for nanoparticles with a spherical, trisoctahedral, and cubic shape to understand edge effects observed on the surface of these structures.
4.2 Results and Discussion

In non-resonant Raman spectroscopy, the chemical enhancement effect generally contributes no more than three orders of magnitude to the overall Raman scattering cross section. Conversely, electromagnetic enhancement may contribute up to eleven orders of magnitude. To date a variety of computational studies have described the importance of edge effects. Nanostructures with high radii of curvature concentrate electric fields through the so-called “lightning rod” effects of SERS.

Three nanoparticle geometries (spheres, cubes, trisoctahedra) are schematically represented in Figure 4.1 along with the zeta potential observed in-between the synthetic steps. Synthesis was carried out using well-established techniques (supplementary material). A Raman reporter, methylene blue, was electrostatically attached to the surface of anionic polyacrylic acid (PAA) polyelectrolyte bearing gold nanoparticles. A final trap-coat layer of cationic polyallylamine hydrochloride (PAH) polyelectrolyte traps the reporter within the vicinity of the nanoparticle surface. Zeta potential analysis after each synthetic step verified successful binding as depicted in Figure 4.2.

In Figure 4.2, the experimental absorption spectra of the nanoparticles are confirmed by UV-Vis spectroscopy with each nanoparticle showing the following localized surface plasmon resonances ($\lambda_{\text{LSPR}}$) at 537 nm, 543 nm, and 529 nm for spheres, cubes, and trisoctahedra, respectively. The LSPR maxima of each colloidal suspension are similar and blue-shifted from the laser excitation (785 nm) such that in-suspension absorption effects are expected to be similar across each measurement (ACS nano). The similarity in electronic absorption spectra for all three nanostructures arises from the inherent far-field nature of electronic absorption
measurements. Characterization of our colloidal suspensions, through UV-Vis and transmission electron microscopy (TEM), indicates minimal aggregation (dimers, trimers, etc.) and well-defined edges for cubes and trisoctahedra. Hot-spots are additionally minimized by wrapping polyelectrolyte layers around the nanoparticle surface to prevent aggregation and inhibit nanoparticle interaction. Studies have indicated that PAA/PAH trap coating provides a buffering layer of at roughly 3 nm between colloidal nanoparticles.\(^{17}\)

To date few reports experimentally quantify the number of reporter molecules electrostatically bound to SERS-active nanoparticles. While it is typical to assume a monolayer of molecules both experimentally\(^ {18}\) and theoretically\(^ {19}\), our quantification results (Figure 4.3(d)) indicate less than monolayer coverage in all three shapes. We quantify the average number of bound reporter molecules (methylene blue) per nanoparticle shape through ESI-LC-MS against known concentrations of reporter standards. As shown in figure 4, SERS spectra of all nanoparticle shapes in colloidal suspension were acquired in triplicates from four batches. Spectra were baselined, and normalized to the gold nanoparticle concentrations (as determined by ICP-MS) as well as the average number of reporter molecules. Cubes are shown to have the highest signal intensity followed by trisoctahedra and spheres. We performed FEM electromagnetic computations to visualize edge effects on nonspherical geometries. In contrast to other popular computational techniques such as FDTD and DDA, FEM utilizes adaptive meshes rather than cubic grids subsequently improving near-field accuracy as well as significantly decreasing computation time.\(^ {20}\) All calculations were performed using a commercial software package (COMSOL Multiphysics 4.2) on an 8-core 2.93 GHz Intel Xeon workstation with 32 GB of RAM and an NVIDIA Quadro 6000 graphics processor. Solutions including post-processing were obtained after 1-2 hours of computation per geometry.
The computational domain consists of a three-dimensional scatterer embedded within a dielectric media (water, $n = 1.33$) surrounded by a swept perfectly matched layer (PML) and a spherical scattering boundary condition. All meshes consist of tetrahedral elements with varying degrees of spacing (typically 1–10 nm) depending upon geometric considerations. Adjustments to mesh density were applied until convergent solutions were obtained. All simulations were performed on one-quarter of the entire symmetric domain to reduce CPU time. Bulk gold optical constants were obtained from Johnson and Christy.\textsuperscript{21}

In Figure 4.5 we compute the electromagnetic enhancement and extinction cross section of all three shapes. The validity of the FEM scattering domain is verified through comparison to Mie theory, a generalized theory of spherical scattering.\textsuperscript{22} Using ideal geometries for the three different shapes, trisoctahedra show the highest electric field enhancement followed by cubes and spheres. This trend is different from what we observe for in our SERS measurements. On closer inspection of the different geometries using TEM Figure 4.5 we observe a variation in the radius of curvature in the trisoctahedra vertices from between 1 nm and 5 nm. By comparison the cube’s radius of curvature remains close to 3 nm for the majority of nanoparticles characterized using TEM. This is in agreement with recent studies of trisoctahedra gold nanoparticles showing higher-index facets which we expect to be less sharp.\textsuperscript{23}

Using COMSOL simulations, we compare the electromagnetic enhancement of trisoctahedra gold nanoparticles with radii of curvature of between 1 - 5 nm. (Figure 4.6). We find that the electromagnetic enhancement increases for increasing radius of curvature, as is expected from the lightning rod effect. This is in agreement with Schatz and co-workers who have shown the significance of edge length on the observed electromagnetic enhancement of SERS on silver nanoparticles.\textsuperscript{9} It is therefore likely that the higher SERS signal intensity
observed for cubic nanoparticles is due to the sharper corners and edges compared to similar sized trisoctahedra nanoparticles.

Another important consideration is the role of the polyelectrolytes persistence length and how they conform around the gold nanoparticles. With higher order index facets such as those found in trisoctahedra gold nanoparticles, it is likely that the polyelectrolytes will preferentially bind to certain facets. Crystal growth of high-index facets in the presence of the Cl\(^-\) counterion contributes to the formation of high-index facets such as (221), (331), and (441).\(^{24}\) High-index facets have been shown to be more chemically reactive and therefore are more likely to contain a higher density of CTA\(^+\) micelles, PAA/PAH polyelectrolyte and electrostatically trapped reporter molecules. As a result we expect the distribution of reporter molecules attached to the polyelectrolytes to be different for trisoctahedral and cubic nanoparticles. This theme is supported by our ESI-LC-MS quantification (Figure 4.2d) showing a lower number of reporter molecules for trisoctahedra nanoparticles versus cubes. This is counter-intuitive to the larger surface area offered by the trisoctahedra nanoparticles relative to cubes. We therefore envision that the reporter molecules preferentially bind to certain corners or facets of polyelectrolyte coated trisoctahedra gold nanoparticles during complexation. It remains a challenge however to ascertain the exact location of the molecules on the surface of the nanoparticles relative to the focused electric fields at sharp corners and edges.

Here, we have demonstrated the electromagnetic enhancement of three shapes (cubes, spheres, trisoctahedra) using FEM and quantified the amount of reporter molecules electrostatically bound to surface of each shape. We find that experimentally gold nanocubes show the highest SERS enhancement followed by trisoctahedra and spheres respectively. This is contrary to our initial idealized simulations suggesting trisoctahedra with the higher number of
sharp vertices should give the highest enhancement. Based on the higher index facets present in triscoctahedral nanoparticles, the radius of curvature is likely to be lower and hence the observed electromagnetic enhancement.

4.3 Experimental Section

Materials. Hydrogen tetrachloride (HAuCl$_4$, > 99.999%), sodium borohydride (NaBH$_4$), and ascorbic acid (C$_6$H$_8$O$_6$, > 99.0%) were obtained from Aldrich and used as received. Cetyltrimethylammonium bromide (CTAB, > 99%) was obtained from Sigma and used without further purification. All glassware were cleaned using aqua regia and rinsed with Barnstead E-Pure 18 MΩ-cm water.

Instrumentation

SERS spectra were obtained on a high-resolution confocal Horiba LabRAM Raman microscope configured with a 785 nm excitation laser line. Triplicate measurements with integration times of 30 seconds each were performed in solution each were performed in suspension. Laser power at the sample was measured to be 12.5 watts. Zeta potential measurements were performed on a Brookhaven Zeta Pals instrument. Absorption spectra were confirmed by a Cary 500 scan UV-Vis-NIR spectrophotometer. Transmission electron micrographs (TEM) images were taken on a JEOL 2010F Field Emission Microscope at 200 kV accelerating voltage. For grid preparation 10 µL of gold nanoparticle suspension was drop cast onto holey carbon TEM grids (Pacific Grid-Tech). Size distributions were verified by analyzing at least 300 representative particles per shape. We use a Thermo Scientific Sorvall Legend X1 Centrifuge for purification as detailed in the synthesis below.
Synthesis of seeds for CTAB cubes (A) The nanoparticle seeds were synthesized by modification the method of El-Sayed et al. \(^{25}\)CTAB solution (7.5 mL, 0.1 M) was mixed with 0.25 mL of 0.01 M H\(_{2}\)AuCl\(_4\). To the stirred solution, 0.6 mL of ice-cold newly made 0.01 M NaBH\(_4\) was quickly added, which resulted in a light brown solution. After stirring the solution vigorously for 2 minutes, the solution was kept for 1 hour in room temperature before use.

Synthesis of CTAB seeds for trisoctahedra (TOH) gold nanocrystals (B) The nanoparticle seeds were synthesized by following the procedure by El-Sayed et al. \(^{25}\)CTAB solution (7.0 mL, 75mM) was mixed with 46 µL of 20 mM H\(_{2}\)AuCl\(_4\). To the solution, 0.42 mL of ice-cold newly made 0.01 M NaBH\(_4\) was quickly added under vigorous mixing and gave a light brown solution. After NaBH\(_4\) was added, the solution was gently stirred to decompose the excess NaBH\(_4\). The solution was used within 2-5 h after the preparation.

Synthesis of CTAB spheres and cubes Spheres and cubes were synthesized in 40 mL batches to maximize reproducibility. In 32 mL of nanopure water, 6.4 mL of 0.1 M CTAB and 0.8 mL of 0.01M H\(_{2}\)AuCl\(_4\) was added, respectively. This resulted in a yellow-brown solution. 3.8 mL of 0.1 M ascorbic acid was added as the reducing agent which made the solution clear. From here depending on the seed concentration, spheres or cubes will form. To obtain spheres, a 0.02 mL of 5 times diluted seed (A) is needed. To obtain cubes, 0.03 mL of 10 times diluted seed (A) is needed.

Synthesis of TOH gold nanocrystals. Trisoctahedra (TOH) nanocrystals were synthesized by a seed mediated method used by Lee et al. \(^{26}\) A 0.125 mL solution of 20 mM H\(_{2}\)AuCl\(_4\) was mixed with 9 mL of 22 mM cetyltrimethylammonium chloride (CTAC). To this mixture, 3.06 mL of 38.8 mM ascorbic acid was added to make the concentration of ascorbic acid 9.5 mM and was
thoroughly mixed. Then, seed (B) was diluted 100 fold with nanopure water, and 50 \( \mu \)L of the diluted seed (B) was added to the solution. This resulted in a red-pinkish solution.

**Complexation of Methylene Blue to polyelectrolyte coated gold nanorods**

Before quantification all samples were dialyzed in 10,000 g/mol dialysis cassettes (Fischer Scientific) for 48 hours in 4 L of water. For quantification of the number of methylene blue molecules by liquid chromatography mass spectrometry with tandem mass spectrometry (LC-MS/MS) we centrifuged the methylene blue complexed polyelectrolyte gold nanorods (2350 RCF, 15 min) and the supernatant again (2350 RCF, 15 min) before concentrating the pellets. After resuspension of the pellet in 50 \( \mu \)L of methanol and the gold nanorods were then etched by adding 0.010 ml of 1 M KCN.

**ESI-LC-MS/MS Quantification of Methylene Blue**

The LC-MS analysis was performed in Metabolomics Center at UIUC with a 5500 QTRAP mass spectrometer (AB Sciex, Foster City, CA) which is equipped with a 1200 Agilent LC. Analyst (version 1.5.1, Applied Biosytems) was used for data acquisition and processing. An Agilent Zorbax SB-Aq column (5u, 50 x 4.6 mm) was used for the separation. The HPLC flow rate was set at 0.3 mL/min. HPLC mobile phases consisted of A (0.1% formic acid in \( \text{H}_2\text{O} \)) and B (0.1% formic acid in acetonitrile). The gradient was: 0-1 min, 98% A; 6-10 min, 2% A; 10.5-17 min, 98% A. The autosampler was kept at 5ºC. The injection volume was 1 \( \mu \)L. The mass spectrometer was operated with positive electrospray ionization. The electrospray voltage was set to 2500 V, the heater was set at 400 ºC, the curtain gas was 35, and GS1 and GS2
were 50, 55, respectively. Quantitative analysis was performed via multiple reaction monitoring (MRM) where m/z 284.2 to m/z 240.1 was monitored.
Figure 4.1 Schematic of polyacrylic acid (PAA) polyelectrolyte (purple) bearing gold nanoparticles (a) cubes (b) spheres (c) trisoctahedra with methylene blue (MB) reporter molecules (orange) and polyallylamine hydrochloride trap coat (dark red) with corresponding zeta potential of gold nanoparticles (a) CTAB capped cubes, (b) CTAB capped spheres and (c) CTAC capped trisoctahedra, CTAB/CTAC + PAA gold nanoparticles and CTAC/CTAB + PAA+ MB + PAH gold nanoparticles.
Figure 4.2. Normalized UV-Vis spectra of synthesized colloidal suspensions of (a) cubes, (b) spheres, and (c) trisoctahedra. The average number of methylene blue molecules per shape is shown in (d) indicating sub-monolayer coverage for all three shapes. Scale bar 10 nm.
Figure 4.3. Surface enhanced Raman spectra of all gold nanoparticle shapes suspensions; cubes (green), spheres (red), trisoctahedra (blue) normalized to concentration and the average number of reporter molecules as quantified by ESI-LC-MS/MS. 785 nm excitation, 30 seconds acquisition time, 12.5 mW power
Figure 4.4. Electromagnetic enhancement ($|E|^4$) evaluated at $\lambda = 785$ nm (left) and simulated extinction cross section curves for varying nanoparticle diameters (right). Water ($n = 1.33$) is assumed to be the surrounding media. (a) cube (b) sphere with comparison to Mie theory (c) trisoctahedra. Scale bar 20 nm.
Figure 4.5: TEM image of trisoctahedra (top) and cubes (bottom). All scale bars 50 nm
Figure 4.6. Electromagnetic enhancement ($|E|^4$) evaluated at $\lambda = 785$ nm (left) and simulated extinction cross section curves for varying trisoctahedra nanoparticle radius of curvature. a) 0 nm b) 1nm c) 3 nm d) 5 nm. Water ($n = 1.33$) is assumed to be the surrounding media. Scale bar 30 nm.
4.4 References


CHAPTER 5

PLASMONIC ENHANCEMENT OF THE TWO-PHOTON ABSORPTION CROSS-SECTION OF AN ORGANIC CHROMOPHORE USING POLYELECTROLYTE COATED GOLD NANORODS

5.1 Introduction

Plasmonic noble metal nanoparticles offer a variety of “enhanced” spectroscopies in which a chromophore’s optical output is increased due to the presence of the nanoscale metal. The most common example is surface-enhanced Raman spectroscopy (SERS) \(^1^5\) where electromagnetic fields associated with surface plasmons increase the intensity of molecular inelastic scattering events by 5-7 orders of magnitude.\(^6^8\) SERS systems of chromophore-metal nanoparticles can be used in multiplexed imaging,\(^9\) for the case of immobilized chromophores serving as labels, and in ultrasensitive assays to detect sub-picomolar amounts of free analyte.\(^9\) Similar mechanisms are believed to be in effect with metal enhanced fluorescence spectroscopy, with the associated changes in fluorescence intensity and reduction in decay times being observed as a function of distance from the plasmonic surface.\(^1^0,1^1\)

Although less examined, upon excitation, large nonlinear optical (NLO) responses either in the plasmonic material or in nearby molecules may be observed.\(^1^2\) Driven by applications such as three-dimensional fluorescence microscopy,\(^1^3\) optical data storage,\(^1^4\) microfabrication,\(^1^5\) and nonlinear optical materials,\(^1^6\) researchers have examined the ability of noble metal nanoparticles to produce NLO effects. Early examples by Descher and co-workers used composite systems of metallic nanoparticles embedded in a dielectric polymer matrix showing NLO using degenerate four wave mixing.\(^1^7\) More direct measurements include observing the slightly higher nonlinear scattering of silver nanoprisms relative to a known nonlinear material such as carbon nanotubes.
Only with the recent advent of Z-scan measurements have researchers been able to directly compare the surface enhancement of NLO chromophores. These studies have now been extended to measure the two-photon absorption (TPA) cross section unique to specifically designed chromophore molecules. Perry and co-workers combined TPA macromolecules with silver fractal clusters giving up to 5 orders of magnitude enhancement in the TPA of the macromolecules. Their studies suggested that such effects were due to aggregates of clusters in the films using two-photon fluorescence microscopy measurements. Complementary work by Hernandez et al. using salt-aggregated spherical gold nanoparticles complexed with organic chromophores showed a 157-fold enhancement of TPA of the chromophores using Z-scan measurements. The discrepancies in the magnitude of the reported enhancements between these studies raise questions to whether such effects are limited to aggregates where electromagnetic “hot spots” exist. To the best of our knowledge, studies on the effects on colloidal suspensions of unaggregated nanoparticles on TPA cross sections have been limited, although gold nanorods alone exhibit two-photon luminescence relative to gold nanospheres. In this paper we use our group’s expertise in synthesizing gold nanorods of a particular aspect ratio and couple an organic chromophore (“AF-348-3A”) with known TPA properties. Using electrostatic interactions we bind these molecules to oppositely charged polyelectrolyte coated gold nanorods. By varying the number of polyelectrolyte layers in even increments of 2, 4, 6 and 8, we examine the role of the position of the molecules within the enhanced electromagnetic field the gold nanorod on the molecule’s effective TPA cross-section.
5.2 Results and Discussion

Gold nanorods are of great interest to many communities due to their optical properties.\textsuperscript{33} Gold nanorods alone have shown to exhibit two photon luminescence.\textsuperscript{23} We examine how these enhancements effect two-photon absorption (TPA) in specifically designed organic chromophores in close proximity to the gold nanorods. Our hypothesis is that TPA should depend on the distance between the molecular and the plasmonic surface. We used the molecule AF-348-3A synthesized, by the dialkylation of the bisphenolate anion of AF366-OH with 1,3-propane-sultone in THF (Scheme 1).

The product solid was easily resuspended in water for complexation with polyelectrolyte coated gold nanorods. We used simple electrostatic binding of the negatively charged chromophore on to positively charged PAH polyelectrolyte coated rods (Figure 5.1). We added approximately 10,000 dye molecules per gold nanorod based on previous data\textsuperscript{34} for electrostatic binding to the polyelectrolyte layer. For comparable number of chromophore molecules for each layered construct the initial concentration of nanorods was kept constant at 0.1 nM.

For the chromophore we confirmed the stability of the polyelectrolyte coated gold nanorods using UV-VIS spectroscopy (Figure 5.2) and the final positive charge using zeta potential measurements (Figure 5.3). The UV-VIS absorption spectra of the 2, 4, 6, and 8 polyelectrolyte layer coated gold nanorod exhibited a transverse plasmon band at ~ 517 nm and a longitudinal plasmon band at ~ 770 nm for the 2 layer sample. Both peaks were observed to slightly blue shift as subsequent layers were added, indicating a change in the dielectric constant of the gold nanorod’s surroundings; however, this blue shift could also be ascribed to the minor
disaggregation of the 2 layer gold nanorods, based on the larger full-width at half maximum of
the longitudinal mode compared to the other spectra (Figure 5.2). As an interesting observation,
the AF-348-3A molecules are electrostatically associated with the gold nanorod-polyelectrolyte
layer assembly, and as discussed later, typically contain 200-1200 dye molecules. However, the
absorption of the dye is noticeably absent from the UV-VIS spectra.

The zeta potentials of the gold nanorod samples switch from positive to negative,
indicating the successive depositions of oppositely charged polyelectrolyte layers. AF-348-3A is
a water-soluble, anionic dye, which is able to electrostatically bind to the positively charged,
outer layer of the gold nanorod-polyelectrolyte assembly. This indicates it is indeed possible to
build multilayer polyelectrolyte assemblies on gold nanorods.

To determine the number of AF348-3A molecules on each gold nanorod-polyelectrolyte
layer sample, the gold was dissolved using KCN, and LC-MS (ESI) data acquired. Figure 5.4
indicates that there are typically around 1000-1400 dye molecules per nanorod, except for the 4
layer sample, which is anomalously low with about 200 dye molecules per particle.

The ability of the gold nanorods to increase the effective two photon absorption cross-
section, $\sigma^{(2)}$, of AF-348-3A was determined by calculating the ratio of $\sigma^{(2)}$ for AF348-3A with
gold nanorods to $\sigma^{(2)}$ of AF-348-3A without gold nanorods. Only this ratio will be reported, and
it will be referenced as the two photon absorption cross-section enhancement. The $\sigma^{(2)}$ was
determined using the method of Hermann and Ducuing, which compares the one photon
fluorescence resulting from a known absorption cross-section to the two photon fluorescence
arising from an unknown absorption-cross section. Unlike methods of determining $\sigma^{(2)}$ such as Z-scan, absorption from the gold nanorod does not affect the calculation of $\sigma^{(2)}$; also, because the two photon fluorescence intensity is referenced to the one photon fluorescence intensity, optical scattering from the gold nanorods is minimized, as are other unwanted optical mechanisms that would otherwise yield an artificially high $\sigma^{(2)}$.

The measured $\sigma^{(2)}$ enhancement of AF-348-3A versus the number of polyelectrolyte layers separating it from the gold nanorods can be found in the left axis of Figure 5.4. At a separation of two polyelectrolyte layers (approximately 3 nm beyond the initial ~ 2.5 nm CTAB bilayer coating) from the gold nanorod, the $\sigma^{(2)}$ of AF348-3A is increased by a factor of 40 ± 12 relative to a neat solution of the dye in water. This factor exponentially decreases to 19 ± 3, 14 ± 5, and 8 ± 1 for separations of 4, 6 and 8 polyelectrolyte layers (6 nm, 9 nm, and 12 nm), respectively. A fit of this data following the equation, $y = b + Ae^{mx}$ yields a slope of -0.4 ± 0.2 layer$^{-1}$ ($R^2 = 0.9$).

To explain the variation in the $\sigma^{(2)}$ enhancement with the distance from the gold nanorod, finite element method simulations, using COMSOL Multiphysics, of the localized surface plasmon polariton electric field were performed. A cylindrical gold nanorod of radius 7.5 nm and length 50 nm was modeled, corresponding to the experimentally obtained nanorods. These dimensions experimentally afforded gold nanorods longitudinal surface plasmon polariton resonance (LSPR) at 800 nm, which was confirmed by the simulations. The complex index of refraction for gold was taken from Johnson and Christy.32
The gold nanorod was modeled as being surrounded by a layer of isotropic, aliphatic polymer with refractive index $1.50-0i$, typical of polymers such as PAH in the visible-near-infrared region. The thickness of this layer, measured from the surface of the gold nanorods, was taken to be the experimentally determined film thickness of the PAA|PAH multilayers, 3, 6, 9, and 12 nm, respectively, for the 2, 4, 6, and 8 polyelectrolyte layered samples.

The aliphatic polymer was modeled as being surrounded by a jacket of insulating water, with refractive index $1.33-0i$. The experimentally measured samples were in an aqueous solution; since the outermost layer of AF-348-3A is a water-soluble dye, it is surrounded, and likely heavily swollen, by water. Light incident on the dye would be attenuated by the water’s refractive index, and since the complex refractive index of AF-348-3A is unknown, the outermost layer of the simulation was designated as water. The thickness of the outer dye layer is also unknown, so the thickness of the water jacket was assumed to be 1 nm, a value close to monolayer or near-monolayer thicknesses.

Figure 5.5 depicts the LSPR of the gold nanorods electric field upon excitation at 800 nm for 6 aliphatic polymer layers. The electric field is concentrated at the tips of the gold nanorod, with a minimum forming in the center. Most of the LSPR electric field is confined within the polymeric spacer layer, with only a small amount reaching the outer water layer. It is therefore expected that only those dye molecules near the tips of the gold nanorod will have their $\sigma^2$ enhanced.

The electric field concentrated in the outer water layer was evaluated as a surface integral at the polymer spacer layer-water boundary, and divided by the boundary surface area. Two photon absorption is proportional to the fourth power of the electric field present; as a point of
comparison, the electric field to the fourth power with the gold nanorods was divided by the electric field to the fourth power with gold nanorods for the 8 layer sample (Figure 5.4). Fitting the data to the equation (1), \( y = b + A e^{mx} \) yields a slope of \(-0.477 \pm 0.020 \text{ V-m}^{-1}\text{layer}^{-1} \) \((R^2 = 0.998)\). However, this fit is valid only when small distances (less than 3 nm) are neglected. When distances approaching 0 nm from the gold nanorod are included in the fit, a single exponential does not fit the data. This deviation is likely due to quantum effects of the electric field, which COMSOL does not include in its calculation.

It is important to note that a refractive index of 1.5 was assumed for the polymeric spacer layer. The Re(n) of most organic polymers can range from 1.3-1.7; using these values as extrema for the spacer layer refractive index, and neglecting distances less than 3 nm, yields electric field- polyelectrolyte layer slopes of \(-0.512 \pm 0.020 \text{ layer}^{-1}\) to \(-0.438 \pm 0.020 \text{ layer}^{-1}\), respectively. Accounting for the experimental error in the measured slope \((-0.434 \pm 0.18 \text{ layer}^{-1})\), the two sets of slopes agree with each other. This suggests that the variation in the \(\sigma^2\) enhancement follows the change in the LSPR electric field of the gold nanorod with distance, and that the effect can be ascribed to the concentrated localized surface plasmon polariton electric field of the gold nanorod.

5.3 Conclusion

It was successfully demonstrated that a charged, water soluble dye, AF348-3A can be electrostatically attached to a gold nanorod via an intermediary, electrostatic bilayer of aliphatic polymers. There were typically 200-1200 dye molecules per bound nanorod. The enhancement of the two photon absorption of the dye was measured by a technique that isolates the nonlinear
absorption of the dye. Experiments indicate, that relative to the dye in solution, its two-photon absorption cross section is enhanced by a maximum of 40-fold for a 3 nm dye-gold nanorod separation; this factor decreases to a minimum of 8.37 for a 12 nm separation. Finite element method simulations suggest that this change is due to the exponentially decaying, longitudinal surface plasmon polariton electric field of the gold nanorod.
5.4 Experimental Section

**Materials.** Hydrogen tetrachloroaurate (III) hydrate (HAuCl$_4$·3H$_2$O, 99.999%), sodium borohydride (NaBH$_4$, 99.99 %) and silver nitrate (AgNO$_3$, > 99.0 %) were obtained from Aldrich and used as received. Cetyltrimethylammonium bromide (CTAB, > 99 %) and ascorbic acid (C$_6$H$_8$O$_6$, >99.0%) were obtained from Sigma Chemical and used as received. The polyelectrolytes (polyacrylic acid sodium salt) $M_w$ ~ 15,000 (35 wt % solution in H$_2$O) (PAA) and polyallyamine hydrochloride, $M_w$~ 15,000, (PAH) were obtained from Aldrich and used without further purification. Sodium chloride (> 99.0%) was obtained and used as received from Fischer Chemicals. All solutions were prepared using Barnstead E-Pure 18 MΩ water. All glassware used was cleaned with aqua regia and finally rinsed with 18 MΩ water.

**Instrumentation.** $^1$H- NMR spectra were obtained using a Bruker Advance 400MHz spectrometer, and chemical shifts were referenced to the solvent residual peak. Elemental analyses and mass spectral analyses were performed at Systems Support Branch, Materials & Manufacturing Directorate, Air Force Research Lab, Dayton, Ohio. Melting points were obtained on either a Buchi-B545 melting point apparatus or MelTemp apparatus. Zeta potential measurements were performed on a Brookhaven Zeta Pals instrument. Absorption spectra were taken on a Cary 500 scan UV-VIS-NIR spectrophotometer. Transmission electron microscope images were taken on a JEOL 2100 Cryo TEM microscope at 200 kV accelerating voltage. All TEM grids were prepared by drop-casting 10 µL of purified gold nanorods on a holey Carbon TEM grid (Pacific Grid-Tech). A Thermo Scientific Sorvall Legend X1centrifuge in a “swinging bucket” orientation were used for purification as detailed in the synthesis below. Two photon absorption cross-sections were calculated using the method of Hermann and Ducuing.$^{25}$ Only the ratio of the two photon absorption cross-section of the AF348-3A-gold nanorod
samples to the cross-section of AF-348-3A in water will be reported. This ratio will be referred to as the two photon absorption cross-section enhancement. Two photon excitation of the samples was performed using the 800 nm output of a 150 fs, 1 mJ diode-pumped, Ti:Al₂O₃ regenerative amplifier (Spectra-Physics Hurricane) with a 1001 Hz repetition rate. For the purposes of two photon absorption cross-sections, single photon excitation of the samples was achieved by frequency doubling the 800 nm output of the laser to 400 nm. The sample’s one- and two-photon fluorescence spectra were obtained using a Jobin-Yvon SPEX 270M imaging monochromator, equipped with Princeton Instruments PIXIS 400B back-thinned CCD camera.

The LC/MS/MS quantification of chromophore AF-348-3A was performed at the Metabolomics Center at UIUC with a 5500 QTRAP mass spectrometer (AB Sciex, Foster City, CA) which is equipped with a 1200 Agilent HPLC. Analyst (version 1.5.1, Applied Biosystems) was used for data acquisition and processing. A Phenomenex (Torrance, CA) column (Gemini 3u C6-Pheyl 11A, 100 x 4.6mm) was used for the separation. The HPLC flow rate was set at 0.25 mL/min. HPLC mobile phases consisted of A (25 mM ammonia acetate in H₂O) and B (Acetonitrile). The gradient was: 0-1min, 95% A; 8-13min, 5% A; 13.5-21.5min, 95% A. The autosampler was kept at 10ºC. The injection volume was 1μl. The mass spectrometer was operated with negative electrospray ionization. The electrospray voltage was set to -4500 V, the heater was set at 600 ºC, the curtain gas was 32, and GS1 and GS2 were 60, 50, respectively. Quantitative analysis was performed via MRM where m/z 797.2--> m/z 675.1 was monitored.

**Chromophore AF-348-3A synthesis.** All chemicals were reagent grade, purchased from Aldrich, and used as received unless otherwise noted. Reactions were performed under a dry nitrogen atmosphere unless otherwise noted. 7-(Benzothiazol-2-yl)-9,9-diethyl-2-
bromofluorene,\textsuperscript{26} the precursor to N,N-di(3-methoxyphenyl)-7-(benzothiazol-2-yl)-9,9-diethyl-fluoren-2-amine\textsuperscript{27} (AF\textsuperscript{336}), was prepared in a 4-step synthesis from commercially available 2,7-dibromofluorene, according to previously reported procedure. Water-soluble, two-photon chromophore AF\textsuperscript{348}-3A was synthesized from dialkylation of the bisphenolate anion of AF\textsuperscript{366}-OH with 1,3-propane-sultone in THF.

N,N-Di(3-methoxyphenyl)-7-(benzothiazol-2-yl)-9,9-diethyl-fluoren-2-amine (AF\textsuperscript{336}). A mixture of 7-(benzothiazol-2-yl)-9,9-diethyl-2-bromofluorene (10.85 g, 25 mmol), 3,3’-dimethoxydiphenylamine (6.87 g, 30 mmol) and toluene (100 mL) was azeotroped dry under nitrogen and cooled. Bis(dibenzylidene acetone)palladium (0) (0.28 g, 0.49 mmol), bis(diphenylphosphino)ferrocene (0.25 g, 0.45 mmol) and sodium-t-butoxide (3.5 g, 36.4 mmol) were then added and the mixture was heated to 100 °C. After 24 hours at 100 °C, the mixture was cooled, diluted with toluene and filtered. The filtrate was washed with water, dried and concentrated on a rotary evaporator. The residue was chromatographed over silica gel. Elution with toluene-heptane (3:1) mixture gave the product, which was recrystallized from a mixture of toluene-heptane, m.p. 178-179.5°C, 11.13 g (76% yield). \textsuperscript{1}H NMR (CDCl\textsubscript{3}) δ ppm: 0.35-0.41 (t, 6H), 1.91-2.14 (m, 4H), 3.69 (s, 6H), 6.54-6.74, 7.05-7.68, 7.84-8.10 (m, 18H). \textsuperscript{13}C NMR (CDCl\textsubscript{3}) δ ppm: 8.61, 32.66, 55.18, 56.44, 108.62, 109.77, 116.66, 119.16, 119.42, 121.00, 121.44, 121.52, 122.94, 123.77, 124.95, 126.28, 127.28, 129.82, 131.55, 134.91, 135.61, 144.48, 147.84, 148.94, 150.67, 151.99, 154.24, 160.46, 168.81. Anal. Calcd for C\textsubscript{38}H\textsubscript{34}N\textsubscript{2}O\textsubscript{2}S: C, 78.33%; H, 5.88%; N, 4.81%; S, 5.49%. Found: C, 78.26%; H, 5.96%; N, 4.68%; S 5.47%.

N,N-Di(3-hydroxyphenyl)-7-(benzothiazol-2-yl)-9,9-diethyl-fluoren-2-amine, (AF\textsuperscript{336}-OH).\textsuperscript{27} A mixture of N,N-di(3-methoxyphenyl)-7-(benzothiazol-2-yl)-9,9-diethyl-fluoren-2-
amine (1 g), and pyridine hydrochloride (10 g) was heated at 200°C under nitrogen atmosphere in an oil bath for 10 hours. The reaction mixture was allowed to cool to room temperature, slurried in water, and the red solids were collected on a fritted filter funnel. The crude (protonated) red product was subsequently slurried in dilute ammonium hydroxide to obtain the greenish yellow solid product, 1.13 g, m.p. 314-316°C. EIMS: m/z 554 (M⁺). Analytically calculated for C₃₆H₃₀N₂O₂S: C, 77.95%; H, 5.45%; N, 5.05%; S, 5.78%. Found: C, 77.74%; H, 5.39%; N, 4.83%; S, 5.78%.

**Sodium3,3’-(3,3’-(7-(benzo[d]thiazol-2-yl)-9,9-diethyl-9H-fluoren-2-ylazanediyl)bis(3,1-phenylene))bis(oxy)dipropylene-1-sulfonate (AF-348-3A).** To a magnetically stirred solution of AF366-OH (3.00 g, 5.41 mmol) in 100 mL dry THF was added NaH (60% in mineral oil; 0.46 g, 11.4 mmol) in one portion. The suspension was stirred for 1 hr at room temperature to give a solution and propane sultone (1.35 g, 11.1 mmol) was added. After stirring for 17 hrs, the viscous mixture was heated to 65°C for 4 hr and isopropanol (150 mL) was added. The precipitate that formed on cooling was filtered and dissolved in 100 mL of hot water. This solution was hot-filtered and concentrated to ~50 mL. Isopropanol (500 mL) was added followed by acetone to precipitate an off-white solid which was dried at 90°C/0.5 torr for 24 hr to give 2.7g (60% yield), m.p. dec > 300°C. 

1H NMR (400 MHz, DMSO) δ 8.14 (d, J = 7.7 Hz, 1H), 8.11 (d, J = 1.3 Hz, 1H), 8.07 (d, J = 7.9 Hz, 1H), 8.04 (dd, J = 8.0, 1.6 Hz, 1H), 7.90 (d, J = 7.9 Hz, 1H), 7.83 (d, J = 8.2 Hz, 1H), 7.59 – 7.50 (m, 1H), 7.50 – 7.41 (m, 1H), 7.21 (t, J = 8.1 Hz, 2H), 7.17 (d, J = 1.9 Hz, 1H), 7.00 (dd, J = 8.2, 2.0 Hz, 1H), 6.67 – 6.58 (m, 4H), 6.55 (t, J = 2.2 Hz, 2H), 3.95 (t, J = 6.4 Hz, 4H), 2.54 (t, J = 7.5 Hz, 4H), 2.07 (dq, J = 14.2, 7.1 Hz, 2H), 1.96 (dt, J = 13.4, 6.4 Hz, 6H), 0.30 (t, J = 7.3 Hz, 6H). 13C NMR (101 MHz, DMSO) δ 167.77,
159.54, 153.66, 151.55, 150.38, 148.31, 147.42, 144.05, 135.05, 134.38, 130.97, 130.16, 127.14, 126.61, 125.31, 123.57, 122.24, 121.73, 120.92, 120.03, 118.90, 115.98, 109.93, 109.09, 66.61, 55.94, 47.85, 31.69, 25.25, 8.45. MALDI MS (m/z): 842 M$^+$, 820 (M+H-Na)$^+$, 798 (M+2H-2Na)$^+$. Anal. Calcd for C$_{42}$H$_{40}$N$_2$O$_8$S$_3$$\cdot$H$_2$O: C, 59.59%; H, 4.92%; N, 3.25%; Na, 5.34%; Found: C, 59.21%; H, 5.01%; N, 3.44%; Na, 5.51%.

**Gold nanorod synthesis.** Gold nanorods of aspect ratio 3 (AR=3) (length 47±4 nm, width 15±2 nm) were synthesized as previously described.$^{28}$ First, gold seed particles were made by the addition of 600 µL of freshly made 10.0 mM NaBH$_4$ (ice cold) to a 2.5 x 10$^{-4}$ M HAuCl$_4$ solution prepared in 0.1 M CTAB. $^{29}$ The resulting pale brown solution was stirred vigorously for a further 10 min and used 30 mins after synthesis. To form gold nanorods we added the following reagent solutions in the assigned order to a 500 ml conical flask: 190 ml 0.1 M CTAB, 2 ml of 10 mM silver nitrate, 10 ml 10 mM chloroauric acid. To this solution ascorbic acid was added with gentle agitation until the solution turned colorless. Finally 0.24 ml of seed solution was added and the solution left overnight (12-16 hours). To remove excess CTAB we used two centrifugation cycles (5000 RPM for 2 hours).

**Gold nanorod functionalization by polyelectrolyte layer by layer (LBL) coating.** We coated the gold nanorods with PAA and PAH using an adapted procedure$^{30}$ to maintain the nanoparticle concentration throughout each step. For each polyelectrolyte layer we prepared stock solutions of PAA (-) or PAH (+) at concentrations of 10 mg/ml prepared in 1 mM NaCl and a separate solution of 10 mM NaCl. To 30 ml aliquots of twice centrifuged CTAB gold nanorods we added 6 ml of PAA or PAH (+) solution followed by 3 mL of NaCl. The solutions were left to complex overnight (12-16 hours) before purification using centrifugation (5000 RPM, 2 hours). We then centrifuged the supernatant and concentrated the two pellets to
minimize losses in gold nanorod concentration. Zeta potential measurements and uv-vis absorption measurements were made between each layering step to confirm successful coating without aggregation of the gold nanorods. Using this method, we prepared gold nanorods coated with 2, 4, 6 and 8 polyelectrolyte layers.

**AF-348-3A chromophore gold nanorod complexation.** To electrostatically bind the chromophore to the polyelectrolyte coated gold nanorods we added 10 µL of 1 mM chromophore stock solution to 0.9 mL of 0.1 nM suspensions of 2, 4, 6 and 8 polyelectrolyte layer coated gold nanorods. The mixture was left to complex for an hour before removing excess reporter molecules using centrifugation (2350 RCF, 15 mins); the supernatant was also centrifuged and both pellets concentrated to maintain gold nanorod concentration. The concentrated pellet was then resuspended to 1 mL with DI water before adding 0.2 ml of PAA (10mg/ml) in 1 mM NaCl and 0.1 ml of 10 mM NaCl solutions. This mixture was left overnight before purification using centrifugation (2350 RCF, 15 mins), again the supernatant was also centrifuged (2350 RCF, 15 mins) and the pellets concentrated. For final purification we dialyzed the solutions in Spectrum Labs 100,000 MWCO G2 membranes against 4L of Barnstead E-Pure (18 MΩ) water for 48 hours.

**ESI LC-MS quantification of AF-348-3A chromophore.** For LC/MS/MS quantification of the number of chromophore molecules we centrifuged the AF-348-3A polyelectrolyte gold nanorods (2350 RCF, 15 mins) and the supernatant again (2350 RCF, 15 mins) before concentrating the pellets. We resuspended the pellet in 300 uL of DI water and kept 300 ul of final supernatant (after two centrifugation cycles). The gold nanorod’s metallic cores were then
etched by adding 0.010 ml of 1M KCN; as a control, this was repeated for the supernatant to quantify any unbound chromophore molecules.

Fluorescence quantum yields for AF-348-3A in both water and DMSO were determined using an actinometry method. Quinine sulfate was used as an actinometer with a known fluorescence quantum yield of 0.55 in 1.0 N H_2SO_4. All samples were excited at 355 nm with a matched optical density of 0.1. For AF-348-3A in water the fluorescence quantum yield is 0.014 +/- 0.001 and in DMSO is 0.401 +/- 0.006.

The simulations of the localized surface plasmon electric field of the gold nanorods were performed using COMSOL Multiphysics, a commercial finite element package. The simulated gold nanorod was given the dimensions of the synthesized gold nanorods with a radius of 7.5 nm and a length of 50 nm Further details can be found in the following section. The complex index of refraction for gold was taken from Johnson and Christy.
Scheme 5.1: Synthesis of Sodium\(3,3'-(3,3'-(7\text{-}(\text{benzo[d]thiazol-2-yl})-9,9\text{-diethyl-9H-fluoren-2-ylazanediyl})\text{bis}(3,1\text{-phenylene})\text{bis(oxy)dipropene-1-sulfonate (AF-348-3A)\)}}
Figure 5.1: Experimental schematic of polyelectrolyte coated gold nanorods with the AF-348-3A chromophore. Gold Bar: gold nanorods. Red lines: layers of polyelectrolytes. Green Stars: chromophore. Blue line: a final polyelectrolyte layer that serves to trap coat.
Figure 5.2: Electronic absorption spectra of gold nanorods, aspect ratio 3 bearing 2 (black), 4 (red), 6 (green) and 8 (blue) polyelectrolyte layers. Inset: Transmission electron micrograph of gold nanorods bearing 2 polyelectrolyte layers, scale bar = 20 nm.
Figure 5.3. Zeta potential measurements of aspect ratio 3 gold nanorods as a function of polyelectrolyte layer number.
Figure 5.4. Two photon absorption cross-section enhancement (left axis) and simulated surface plasmon polariton electric field (right axis) versus distance from the gold nanorod. The red, dashed line is an exponential fit including the 2 layer data while the red, solid line does not include 2 layer data.
Figure 5.5: The calculated longitudinal surface plasmon polariton resonance of the gold nanorod.
5.5 References


CHAPTER 6

OFF-RESONANT TWO-PHOTON ABSORPTION CROSS-SECTION ENHANCEMENT OF AN ORGANIC CHROMOPHORE ON GOLD NANORODS

6.1 Introduction

Plasmonic electromagnetic effects define the linear and non-linear optical (NLO) properties of confined metallic structures.\(^1\)\(^2\) For noble metals these electric fields are induced by localized surface plasmon resonance (LSPR) frequencies in the visible part of the spectrum.\(^3\) Their amplitude and spectral response are a function of their dimensions, local environment and state of aggregation.\(^4\) Additionally the electric fields set-up by the accumulation of charges at sharp edges and corners, termed the “lightening rod effect” also contribute to the signal enhancement.\(^5\) In anisotropic gold nanorods, both these phenomena are in effect, making them ideal for applications to maximize their NLO enhancement. These include novel devices for optical data storage\(^6\)\(^7\) and actively controlling the propagation of light for nano-photonics.\(^8\) A recent elegant demonstration using gold nanorods by Zijlstra and co-workers showed optical storage up to 7.2 terabits was possible.\(^9\) With the premise of ultra-fast manipulation of data in the future, researchers have looked into understanding their NLO behavior using femto-second spectroscopy.\(^10\) Depending on the laser power and fluence, electron and lattice non-equilibrium effects contribute to the plasmonic optical non-linearity.\(^11\) Previously, various groups have demonstrated that for on-resonant excitation of the plasmon resonance frequencies, the non-linearities that exist are a function of the coherent oscillations themselves.\(^12\) Often overlooked is how such plasmonic non-linearities differ away from the surface of the nanoparticles but within the near-field regime of their induced electromagnetic fields. By describing the surface plasmons resonance as damped harmonic oscillations, Ausnegg and co-workers showed constructive and
destructive interference effects for off-resonant two-photon excitations of lithographically patterned gold nano-structures.\textsuperscript{13}

Based on these findings, the effect of an off-resonant gold nanorod’s localized surface plasmon polariton resonance on the two-photon absorption (TPA) cross-section enhancement factor of an organic dye, AF348-3A, is examined. In a previous study, the TPA spectrum of AF348-3A overlapped the longitudinal localized surface plasmon resonance of a gold nanorod to which it was bound. The TPA cross-section enhancement factor of the AF348-3A gold nanorod system was found to be dependent on the dye-gold nanorod separation, with a maximum enhancement factor of 40 being obtained closest to the surface.\textsuperscript{14} In this study, AF348-3A is placed at variable distances from gold nanorods whose localized surface plasmon polariton resonance does not overlap the dye’s TPA spectrum. The AF348-3A dye features a two photon absorption resonance at 800 nm,\textsuperscript{14} a wavelength at which the gold nanorod to which it is bound does not feature a localized surface plasmon polariton resonance. Using a method described by Hermann and Ducuing,\textsuperscript{15} the dye’s TPA cross-section at 800 nm is examined as a function of its separation from the gold nanorod. This method has been previously demonstrated to give accurate two photon absorption cross-section enhancement factors that are free of parasitic optical and photophysical effects that would yield an artificially high TPA enhancement factor.\textsuperscript{14,16}
6.2 Results and Discussion

Aspect ratio two gold nanorods were synthesized with a plasmon resonance at approximately 650 nm using a previously reported procedure.\textsuperscript{17} The as-synthesized nanorods are stabilized by a positively charged CTAB bilayer,\textsuperscript{18} onto which polyelectrolytes can be wrapped using layer by layer deposition.\textsuperscript{19} Previously, we have successfully demonstrated that it is possible to bind at variable distances, a TPA chromophore, AF348-3A, to a gold nanorod by using sodium polyacrylate (PAA) and polyallylamine hydrochloride (PAH) as dielectric polyelectrolyte spacer layers.\textsuperscript{14} Figure 6.1 shows the experimental schematic of the gold nanorods with the attached chromophore molecules on 2, 4, 6 and 8 polyelectrolyte layer-bearing gold nanorods; this corresponds to spatial distances of 3, 6, 9 and 12 nm respectively. These distances are estimations based on previous data of stained TEM images of polyelectrolyte coated gold nanorods.\textsuperscript{19}

Electronic absorption spectra of the AF348-3A-bearing, 2, 4, 6, and 8 polyelectrolyte layer-coated gold nanorods are shown in Figure 6.2. Slight blue and red shifts in the absorption maxima are observed and are associated with the variable refractive index of the space surrounding the gold nanorods. The blue-shifts are somewhat counter-intuitive as they suggest that the polyelectrolytes’ refractive indicies are smaller than that of water. A possible explanation is the varying amounts of hydration of these polyelectrolyte layers have a bearing on the direction of their shifts. The absorption spectra also show an increase in the width of the absorption spectra with each polyelectrolyte layering step. We attribute this to a minor amount of aggregation between each layering and centrifugation step that cannot be completely eliminated. For each sequentially polyelectrolyte assembly, zeta-potential measurements indicate a change in the surface potential (Figure 6.5) of the gold nanorods, suggesting that electrostatic, layer-by-
layer self assembly is occurring. The absorption spectrum of AF348-3A is not observed in Figure 6.2 because the molar absorptivity of the longitudinal plasmon resonance of the gold nanorod is several orders of magnitude higher than that of AF348-3A.\textsuperscript{14}

All samples were measured in aqueous solutions (Millipore) with an absorbance of approximately 0.25 at the global absorption maximum. Measurements of the two photon absorption cross-section enhancement were obtained using an instrument and technique previously described.\textsuperscript{14} Briefly, the 800 nm, 150 fs, 1000 Hz output of a Ti:Al\textsubscript{2}O\textsubscript{3} regenerative amplifier (Spectra-Physics Hurricane) excited an aqueous solution of the neat dye or dye complexed to gold nanorods for measurement of the dye’s two photon fluorescence. The 800 nm output of the laser was also doubled by way of a frequency doubling crystal to a frequency-equivalent wavelength of 400 nm. The sample was excited again and its one photon fluorescence spectrum obtained. The two photon absorption cross-section of the neat dye or the dye complexed to a gold nanorod was obtained using a method described by Hermann and Ducuing.\textsuperscript{15} The ratio of the two photon absorption cross-section of the gold nanorod-bound dye to that of the neat dye solution is defined as the two photon absorption cross-section enhancement. Only this ratio will be reported; it is not necessary to obtain the absolute two photon absorption cross-section of the dye because of the relative enhancement method utilized.

The calculation of the AF348-3A two photon absorption cross-section, $\sigma^{(2)}$, is described by (1), where $\sigma^{(1)}$ represents the linear, one photon, absorption cross-section, $\phi_{2\omega}$ is the one photon excitation power, $\phi_\omega$ represents the two photon excitation power, and $I(2\text{PL})$ and $I(1\text{PL})$ are the two photon- and one photon-fluorescence intensities, respectively.\textsuperscript{15}
\[
\sigma^{(2)} = \sigma^{(1)} \frac{\phi_2 I(2PL)}{\phi_0 I(1PL)}
\]  

(6.1)

By taking the ratio of \( \sigma^{(2)} \) of AF348-3A on gold nanorods to \( \sigma^{(2)} \) of AF348-3A in a neat solution, several factors that would give inaccurate \( \sigma^{(2)} \) enhancement values are corrected. Gold nanorods can act as light scatterers; in a solution with an absorbing dye, this light scattering would increase the observed fluorescence intensity by increasing the effective pathlength of photons through the sample. In the \( \sigma^{(2)} \) calculation, the ratio of the power-corrected fluorescence intensities is obtained and the scattering effect is greatly minimized. Gold nanorods also exhibit linear- and nonlinear-absorption at the same wavelengths used to excite AF348-3A in this study. Using a technique such as Z-scan to measure the AF348-3A \( \sigma^{(2)} \) would require a separation of the gold nanorod’s absorption from that of the dye. Using the fluorescence technique, the absorption of the gold nanorods is not measured and not needed.

The \( \sigma^{(2)} \) measurement technique described in equation 6.1 works with only one pair of linear- and two photon-fluorescence intensities; however, a much more robust approach involves several sets of excitation and fluorescence intensities. Using the latter method has two main benefits. The first is that with several sets of intensities, a statistically meaningful set of data can be obtained. Secondly, the correct power law relationship for the fluorescence can be confirmed for the \( \sigma^{(2)} \) measurement; for a plot of the one photon fluorescence intensity versus excitation energy density, the relationship should be linear with a slope of 1.0. For two photon
fluorescence, the plot should be quadratic. Figures 6.6-6.13 confirm that the correct excitation power laws are followed for the neat dye and dye-bound gold nanorod samples.

The $\sigma^{(2)}$ enhancement factors (Figure 6.3) for AF348-3A separated from a gold nanorod by two, four, six and eight layer samples are $8.08 \pm 1.27$, $2.32 \pm 0.38$, $3.26 \pm 0.28$, and $9.28 \pm 1.44$, respectively. This is an unusual, unexpected pattern: the $\sigma^{(2)}$ enhancement decreases by a factor of almost four then increases by the same amount. The AR2 nanorods have a longitudinal localized surface plasmon resonance (LSPR) at 653 nm and a transverse resonance at 521 nm. The two photon absorption cross-section of the dye was measured using an 800 nm laser. The result is off-resonant two photon excitation: AF348-3A exhibits two photon absorption at a wavelength where the gold nanorod does not have a localized surface plasmon polariton resonance.

Aussenegg and co-workers examined a similar situation using a third order autocorrelation function (THG-ACF). Two THG-ACFs were obtained: one using gold nanorods whose LSP resonance was at the wavelength of the laser and a second when the LSPR was shifted away from the laser’s wavelength. When compared to an ACF obtained by substituting a beta-borium borate (BBO) crystal for the gold nanorod sample, it was found that the THG-ACF of the off-resonant gold nanorods featured beating. Beating was not observed for the on-resonant gold nanorod case. The beating was attributed to a mixture of constructive and destructive-interference. The gold nanorod samples will partially reflect and scatter the incident laser pulse; a surface plasmon polariton generated by this laser pulse will have an electric field that extends into the space surrounding it. Depending on the distance from the gold nanorod, the electric field of the back-scattered or back-reflected laser pulse will show either constructive- or destructive-interference with the localized surface plasmon polariton’s electric field. In Figure
6.3, if the distance of the AF348-3A molecule from the gold nanorod lies in a region of constructive interference, a higher \( \sigma^{(2)} \) enhancement factor will be observed. If the dye molecule is in a region of destructive interference, the \( \sigma^{(2)} \) enhancement factor will decrease.

Finite element method simulations using COMOSOL Multiphysics were used to verify the measured \( \sigma^{(2)} \) enhancement pattern. The rate of excitation, \( R \), for a molecule can be described using equation 6.1, where \( N \) is the number density of the absorbing species and \( I \) is the light intensity of the exciting wavelength, \( \lambda \).

\[
R = N \sigma^{(1)} f^2(\lambda_i) I(\lambda_i) + N \sigma^{(2)} f^4(\lambda_i) I^2(\lambda_i) \quad (6.2)
\]

The local field factor, \( f \), represents the sensitivity of the absorption mechanism to an increase in the electric field experienced by a molecule. For a two photon absorption process, \( \sigma^{(2)} \) increases proportionally to the fourth power of the electric field. Using the electric field calculated in COMSOL, the electric-field ratio can be calculated using (equation 6.3). Details of the simulation can be found elsewhere.

\[
Ratio = \frac{(Electric\ field\ at\ x\ layers)^4}{(Electric\ field\ at\ 8\ layers)^4} \quad (6.3)
\]

The calculated electric field to the fourth power for the two-, four-, six-, and eight-layer samples is divided by the electric field to the fourth power for the eight layer sample. A plot of the electric field ratio versus the number of layers can be found in Figure 6.4. The calculated electric field ratio follows the same pattern found in the measured \( \sigma^{(2)} \) enhancement factors (see also the red line in Figure 6.3). As better comparison, the measured \( \sigma^{(2)} \) enhancement factors
were normalized to the $\sigma^{(2)}$ enhancement factor at eight layers (Table 6.1). Within the standard deviation of the measured $\sigma^{(2)}$ enhancement factors, their ratios exhibit quantitative agreement with the calculated ratios, indicating that the interference model described by Aussenegg et al.$^{13}$ is a valid description for the measured $\sigma^{(2)}$ enhancement factors.

<table>
<thead>
<tr>
<th>Number Layers</th>
<th>Calculated Ratio*</th>
<th>Measured Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.60</td>
<td>1.06 ± 0.85</td>
</tr>
<tr>
<td>4</td>
<td>0.47</td>
<td>0.34± 0.26</td>
</tr>
<tr>
<td>6</td>
<td>0.63</td>
<td>0.48± 0.19</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*for $n = 1.5$

Our studies indicate that the plasmonic induced interference effects of gold nanorods can be observed using TPA of an organic chromophore attached to polyelectrolyte gold nanorods. By accounting for scattering and absorption effects in our enhancement of TPA using Herman and Ducing method, we observe such effects are pronounced at distances of approximately 12 nm for the surface of the nanoparticles. We envision understanding these effects will be of great value to fabricating plasmonic NLO devices of the future.
Figure 6.1. Experimental schematic of polyelectrolyte coated gold nanorods with the AF-348-3A chromophore. Gold bar: gold nanorods. Blue lines: Different numbers of polyelectrolyte spacer layers with distance, d. Green star signs: AF-348-3A chromophore (Inset). Red lines: PAA Polyelectrolyte trap coat layer.
Figure 6.2. Electronic absorption spectra of gold nanorods of aspect ratio two in water, each bearing two (black), four (red), six (blue), eight (pink) polyelectrolyte layers with AF-348-3A. The blue, vertical dash represents the two photon laser excitation wavelength at 800 nm.
**Figure 6.3.** Measured two photon absorption cross-section enhancement (left axis, black points) and the calculated electric field ratio (right axis, red points) for aspect ratio two polyelectrolyte-bearing gold nanorods.
Figure 6.4. Electric field ratio of aspect ratio two gold nanorods, calculated using COMSOL Multiphysics: ■, n = 1.3; ●, n = 1.4; ▲, n = 1.5; ▼, n = 1.6; ♦, n = 1.7.
Figure 6.5. Zeta potential measurements of aspect ratio two gold nanorods as a function of polyelectrolyte layer number.
Figure 6.6. Linear fluorescence power scan for two layers AF348-3A-AuNR in water.
Figure 6.7. Linear fluorescence power scan of four layers AF348-3A-AuNR in water.
Figure 6.8. Linear fluorescence power scan for six layers AF348-3A-AuNR in water.
Figure 6.9. Linear fluorescence power scan for eight layers AF348-3A-AuNR in water.
Figure 6.10. Nonlinear fluorescence power scan for two layers AF348-3A-AuNR in water.
Figure 6.11. Nonlinear fluorescence power scan of four layers AF348-3A-AuNR in water.
Figure 6.12. Nonlinear fluorescence power scan for six layers AF348-3A-AuNR in water.
Figure 6.13. Nonlinear fluorescence power scan for eight layers AF348-3A-AuNR in water.
6.3 References


