RICE UNIVERSITY

Complexed Multifunctional Metallic and Chalcogenide Nanostructures as Theranostic Agents

by

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ABSTRACT

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Nanostructures have attracted substantial attention due to their distinctive properties and various applications. Nanostructures consisting of multiple morphologies and/or materials have recently become the focus of intense study with particular attention being paid to their optical and magnetic properties and the enhanced role of the interface between materials. Of particular interest are metallic-based plasmonic nanostructures, structures that support surface plasmon resonances that are sensitive to the environment, and ferrimagnetic-based nanostructures, structures that exhibit strong magnetic properties when exposed to an external field. These nanostructures provide theranostic potential in the context of cancer photothermal therapies, diagnostics and imaging. Additionally, chalcogenide based nanostructure complexes are particularly interesting. Metallic chalcogenides offer the ability to combine different types of linear and nonlinear optical properties, enable design of nanostructure complexes with surface plasmon resonance effects in new wavelength ranges, and act as photo-emitting agents for novel theranostic applications.

In this thesis an in depth analysis of plasmonic, magnetic and photo-emitting nanostructures as theranostic agents is presented. We have created several multifunctional nanostructures and the factors contributing to the functional properties of
these nanostructures are explored systematically through experimentation, theory, and simulations. Both in vivo and in vitro testing demonstrates the applicability of these nanostructures as theranostic agents.
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Chapter 1: Introduction

Cancer is a leading cause of death worldwide; in 2007, it accounted for nearly 8 million deaths, and in 2030 this number is projected to increase to 12 million [1]. A critical challenge in dealing with such high incidence of cancer revolves around the separation between diagnosis, treatment and subsequent imaging to confirm therapeutic effect. Thranostics have come forward recently as a way to combine these three phases of medical treatment, thus decreasing time and improving efficacy of treatment. The ideal theranostic approach would include several tunable functions ranging from imaging to treatment with accurate targeting of cancer specific cells. In order to generate a tunable theranostic, metal nanostructures have recently been investigated as potential theranostic agents [2-8]. Metal nanostructures have been the focus of extensive research due to their unique properties at the nanoscale. As metallic materials approach the nanoscale and as the proportion of atoms at the exterior of the material becomes considerable, their properties vastly differ from that of bulk materials [9-10]. The intriguing and sometimes unanticipated properties of nanostructures are consequently largely due to the large surface area of the material, which governs the contributions made by the small bulk of the material [11-12]. These dimensionally dependent properties have attracted substantial attention. In particular, nanostructures derived from gold have generated strong interest, because of their unique and size-dependent electronic, physical, chemical, and optical properties [13-14].
1.1 Nanostructures as theranostic agents

The properties associated with these nanostructures have aided in their use in medical and biological applications, such as immunoassay labeling, biological and chemical sensing, surface enhanced Raman spectroscopy, and medical clinical diagnosis and therapy [13-16]. Gold nanostructures in particular, have also been used as theranostic agents to combat cancer by use of image enhancement scattering mechanisms and plasmonically generated photothermal effects for ablation. Gold nanostructures possess an intense optical absorption attributed to the surface plasmon resonance phenomenon, and serve as thermally stable heat transfer sources that can be utilized for photothermal therapy (PTT) [5-7]. The plasmon derived optical resonance of gold nanostructures is dependent on a variety of factors, such as the relative dimensions of the nanoparticles, interparticle interactions, and their dielectric environment. By simply adjusting the geometry of the gold nanostructures this optical resonance can be positioned over hundreds of nanometers in wavelength across the visible into the near infrared spectrum [17-22]. Commonly used gold nanostructures for PTT include nanospheres, nanoshells, nanotubes, and nanorods [23-24].

Another class of nanoparticles, magnetic nanoparticles, have been widely used as theranostic agents. Among the different types of nanomaterials used in theranostics, iron oxide nanoparticles (IONPs), as magnetic resonance imaging contrast agents, have set an example as effective nanoparticles for medical applications for T2 magnetic resonance imaging for several biological applications including cancer, cardiovascular, and neurological imaging [25]. Furthermore, over the past 10 years multiple articles have been published on various aspects of magnetic nanoparticles ranging from chemical
synthesis, size-dependent magnetization, morphology, surface engineering, in vitro and in vivo applications [26-27].

Chalcogenide based nanostructured complexes are also of particular interest, since they offer the ability to combine linear and nonlinear optical responses or to design surface plasmon resonance effects in nonconventional wavelength ranges. Metal-based chalcogenide nanostructured systems can exhibit strong optical responses ranging from the UV to the shortwave infrared (SWIR) region of the electromagnetic spectrum due to the extinction of the surface plasmons by an alternating electromagnetic field [28-32]. This allows them to serve as light-mediated theranostic agents for cancer detection or PTT treatment. Metal chalcogenides, in the form of quantum nanocrystals, also possess photoluminescent properties due to quantum confinement. Interestingly, these quantum nanocrystals offer photoluminescence in the NIR and SWIR region allowing for biological use where water and oxy- and deoxyhemoglobin absorption is an issue (800 - 1500 nm) [33].

A critical challenge in the design of a theranostic agent revolves around the separation between diagnosis, treatment and subsequent imaging to confirm therapeutic effect. The ideal NP complex would be capable of several tunable functions ranging from imaging to treatment to monitoring with accurate targeting of cancer specific cells. This ideal NP complex would serve as a multifunction theranostic agent. A NP complex capable of combining multiple or all three phases of medical treatment, diagnosis, therapeutics and confirmation would greatly decrease time and improve efficacy of treatment.
The outline of the thesis is as follows: Chapter 2 describes surface plasmon polaritons and discusses the origins of plasmons in metals. Chapter 3 deals with fabrication of AuNPs in the form of solid gold nano-cores and is used as a brief introduction into some of the mechanisms involved in nanoparticle formation and illustrates the intricacies and sensitivity of NP formation to the synthesis environment.

The relevant publication for the experiments in chapter 3 is:


Chapter 4 discusses the delivery of AuNPs in in vivo environments using T cells. The use of AuNPs and T cells together combines the photothermal therapy and imaging advantages of AuNPs with the immunotherapy and biodistribution advantages of T cells leading to a novel theranostic approach. The relevant publication for the experiments in chapter 4 is:


Chapter 5 discusses the use of chalcogenides as substrates for nanocomplexes. In particular, this chapter discusses the use of spherical metal-based chalcogenides as substrates for Au islet growth to generate near-infrared and short wave infrared optical responses. The work presented in chapter 5 will be published at a later date. Chapter 6 introduces a novel photoluminescent and thermally responsive plasmonic nanoparticle complex that serves as an active triple theranostic agent. The NP complex serves as a triple theranostic agent by affording detection, treatment, and conformational functionality. The work presented in chapter 6 was done in collaboration with Adam Y.
Lin and will be published at a later date. Chapter 7 offers a brief introduction into magnetism and MRI principles. Chapter 8 of this thesis deals with the design and implementation of a multimodal magnetic hollow gold nanoshell complex for enhanced magnetic resonance imaging and photothermal therapy applications. The magnetic properties of iron oxide are combined with the plasmonic properties of gold. The work presented in chapter 8 was done in collaboration with Adam Y. Lin and will be published at a later date. Chapter 9 of this thesis states the conclusion.

1.2 References


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Chapter 2: Surface Plasmon Polaritons

Chapter 2 of this thesis serves as an introduction into plasmonics. In particular, chapter 2
describes the source of the optical properties for the metal nanostructures discussed in
chapter 1. The optical properties for metals are described using Maxwell's equations, the
Drude-Summerfield model, and Mie Theory. At the end of chapter 2, discrete dipole
approximation is discussed as a computational method and serves as an illustration of
how Maxwell equations can be implemented for more complex geometries. The bulk of
the derivations were obtained from references [1-3] listed at the end of this chapter.

Polaritons, quasiparticles resulting from strong coupling of a photon with an
excitation of a material, exist in several forms. Among the several types of polaritons,
surface plasmon polaritons (SPPs) are electromagnetic surface waves confined to the
interface of two materials with different dielectric functions. SPPs can take various
forms, ranging from freely propagating electron density waves along a metal surface, to
localized electron oscillations on metal nanostructures. The interaction of noble metals
with electromagnetic radiation is largely dictated by the free conduction electrons in the
metal. At optical frequencies the metal's free electron gas can sustain surface and volume
charge density oscillations with distinct resonance frequencies. In the case of
subwavelength metal nanostructures, the gas is confined in three dimensions. When the
electrons at the surface of a nanostructure is displaced by an external electromagnetic
field a positively charged lattice remains. The displacement of the electrons with respect
to this positively charged lattice leads to a restoring force that gives rise to a specific
particle plasmon resonance depending on the geometry of the nanostructure. In
nanostructures with suitable geometries, large local charge accumulations can occur that are accompanied by strong enhanced optical fields.

2.1 Optical properties and Maxwell's equations

The optical properties of noble metal nanostructures can be analyzed based on Maxwell's equations. The optical properties of metals can be described by a complex dielectric constant that depends on the frequency of the light. The optical properties of metals are determined mainly by the fact that the conduction electrons can move freely within the bulk of the material and that interband excitations can take place if the energy of the photons exceeds the band gap energy of the respective metal. The presence of an electric field leads to a displacement $r$ of an electron which is associated with a dipole moment $\mu$ according to $\mu = er$. The cumulative effect of all individual dipole moments of all free electrons results in a macroscopic polarization per unit volume $P = n\mu$, where $n$ is the number of electrons per unit volume. The electric displacement $D$ is related to the electron polarization by

$$D(r,t) = \varepsilon_0 E(r,t) + P(r,t). \quad 2.1$$

Where the constitution relation is

$$D = \varepsilon_0 \varepsilon E \quad 2.2$$

Using equations 2.1 and 2.2, assuming an isotropic medium, the dielectric constant can be expressed as

$$\varepsilon = \frac{|P|}{\varepsilon_0 |E|} \quad 2.3$$

The displacement $r$ and therefore the polarization $P$ can be obtained by solving the equation of motion of the electrons under the influence of an external field. The effects of
free electrons can be described by applying the Drude-Sommerfeld model for a free-electron gas.

\[
m_e \frac{\partial^2 \mathbf{r}}{\partial t^2} + m_e \gamma_d \frac{\partial \mathbf{r}}{\partial t} = eE_0 e^{-i\omega t}
\]  \hspace{1cm} (2.4)

where \(\gamma_d\) describes the damping term and \(m_e\) is the effective free electron mass, \(e\) the free electron charge and \(\omega\) and \(E_0\) are the frequency and amplitude of the applied electric field respectively. Equation 2.4 can be solved by the Ansatz \(r(t) = r_0 e^{-i\omega t}\) and using the result in 2.3 yields

\[
\varepsilon_{Drude}(\omega) = \varepsilon_\infty - \frac{\omega_p^2}{\omega^2 + i\gamma_d\omega}, \quad \text{and} \quad \omega_p = \sqrt{\frac{4n_e e^2}{m_e}}
\]  \hspace{1cm} (2.5)

Here \(\omega_p\) is the bulk plasma frequency and \(\varepsilon_\infty\) is a constant that accounts for the higher energy interband transitions in metals. Equation 2.5 is further expanded as follows

\[
\varepsilon_{Drude}(\omega) = 1 - \frac{\omega_p^2}{\omega^2 + i\gamma_d\omega} + i \frac{\gamma_d\omega_p^2}{\omega(\omega^2 + \gamma_d^2)}
\]  \hspace{1cm} (2.6)

The Drude-Sommerfeld model for a free-electron gas gives accurate results for the optical properties of metals in the infrared region yet becomes inaccurate for the visible regime. When metals are bombarded with visible higher-energy photons the bound electrons in the lower-lying shells are promoted to the conduction band. This promotion of bound electrons contribute to the optical response of the metal. The same method used to describe the motion of free-electrons can be used to describe the response of bound electrons. The equation of motion for a bound electron is

\[
m_e \frac{\partial^2 \mathbf{r}}{\partial t^2} + m_e \gamma_d \frac{\partial \mathbf{r}}{\partial t} + \alpha \mathbf{r} = eE_0 e^{-i\omega t}
\]  \hspace{1cm} (2.7)
where \( m_e \) is the effective mass of the bound electrons, which is different from the effective mass of the free electron in a periodic potential. \( \gamma \) is the damping constant describing radiative damping of bound electrons, and \( \alpha \) is the spring constant. The Ansatz is used to solve the equation 2.7. The resultant equation is given below

\[
\varepsilon_{\text{Interband}}(\omega) = 1 + \frac{\tilde{\alpha}^2_p}{(\omega_0^2 - \omega^2) - i\gamma\omega} \quad 2.8
\]

The real and imaginary components can be separated into real and imaginary parts

\[
\varepsilon_{\text{Interband}}(\omega) = 1 + \frac{\tilde{\alpha}^2_p(\omega_0^2 - \omega^2)}{(\omega_0^2 - \omega^2)^2 + \gamma^2\omega^2} + i \frac{\gamma\tilde{\alpha}^2_p\omega}{(\omega_0^2 - \omega^2)^2 + \gamma^2\omega^2} \quad 2.9
\]

### 2.2 Plasmon polaritons at dielectric interface

In order to investigate the physical properties of surface plasmon polaritons, Maxwells equations are applied to the flat interface between the conductor and the dielectric. Since we are seeking solutions to Maxwell's equations that are localized at the interface we use the Helmholtz wave equation where \( k = \omega/c \)

\[
\nabla^2 E + k^2 \varepsilon E = 0 \quad 2.10
\]

By following reference [1] one material is a metal with a frequency dependent dielectric \( \varepsilon_1 \) and the other material consist of a real dielectric \( \varepsilon_2 \). The interface between the two materials lies in the \( z = 0 \) plane where \( \varepsilon_g \) exist at \( z < 0 \), and \( \varepsilon_r \) exist at \( z > 0 \). The wave equation now has to be solved separately in each region of constant \( \varepsilon \). Maxwell's equations allow two sets of solutions based on the TM or "p-polarized" and TE or "s-polarized" modes. Here we only consider the p-polarized mode since no solutions exist for the case of s-polarization i.e. s-polarized light does not generate plasmon excitations. P-polarized plane waves in half-space \( j = 1 \) and \( j = 2 \) can be written as
\[ E_j = \begin{pmatrix} E_{xj} \\ 0 \\ E_{zj} \end{pmatrix} e^{ik_x x - i\omega t} e^{ik_{zj} z}, \quad j = 1, 2 \]  

Since the wave vector parallel to the interface is conserved the following \( k \) vector relations hold

\[ k_x^2 + k_{j,x}^2 = \varepsilon_j k^2, \quad j = 1, 2 \]  

Together with Gauss' law, the above equation leads to

\[ k_x E_{j,x} + k_{j,x} E_{j,z} = 0, \quad j = 1, 2 \]  

Since the parallel field component is continuous and the perpendicular component is discontinuous the boundary conditions yield the following relations

\[ E_{x1} - E_{x2} = 0, \]  

\[ \varepsilon_1 E_{x1} - \varepsilon_2 E_{x2} = 0, \]  

To solve equation 2.14 requires that the respective determinants vanish. This happens when

\[ \varepsilon_1 k_{2,x} - \varepsilon_2 k_{1,x} = 0 \]  

In combination with equation 2.12, equation 2.15 leads to a dispersion relation between the wave vector components and the angular frequency \( \omega \)

\[ k_x = \frac{\omega}{c} \left( \frac{\varepsilon_1 \varepsilon_2}{\varepsilon_1 + \varepsilon_2} \right)^{1/2} \]  

\[ k_{zj} = \frac{\omega}{c} \left( \frac{\varepsilon_j^2}{\varepsilon_1 + \varepsilon_2} \right)^{1/2} \]  

We are looking for interface waves that are propagating along the surface, i.e. we require a real \( k_x \). In order to generate a bound interface solution, the normal components of the wave vector must be imaginary in both materials which eventually leads to exponentially
decaying solutions. From the dispersion relation, we determine the conditions for the existence of a bound interface mode which are given by

\[ \varepsilon_1 \varepsilon_2 < 0, \quad \varepsilon_1 + \varepsilon_2 < 0 \]

which results in an electromagnetic wave bound to the interface. The resulting imaginary \( k_{ij} \) corresponds to exponentially decaying waves i.e. evanescent waves. This means that one of the dielectric functions must be negative with an absolute value exceeding the other dielectric. As mentioned previously, metals, especially noble metals such as gold and silver, have a large negative real part along with a small imaginary part of the complex dielectric. Therefore, at the interface between the metal and dielectric, localized modes at the metal-dielectric interface can exist.

In order to excite the surface plasmon polaritons both the energy and momentum conservation has to be satisfied. Excitation of a surface plasmon polariton by light is only possible if the wave vector of the exciting light can be increased over its free-space value. This can be done through the tilt of the light line \( \omega = c \cdot k_x \) to \( c \cdot k_x/n \) using a medium with refractive index greater than \( n > 1 \). This tilting of the light line allows excitation of the surface plasmons by means of evanescent waves created at the interface between the medium with \( n > 1 \).

To accommodate losses associated with electron scattering we have to consider the imaginary part of the metal's dielectric function.

\[ \varepsilon_1 = \varepsilon_1' + i\varepsilon_1'' \]

The imaginary part is related to the energy dissipation of the material. With \( \varepsilon_2 \) assumed to be real we obtain a complex wavenumber \( k_x = k_x' + ik_x'' \). The real part determines the surface plasmon polariton wavelength and the imaginary part accounts for the damping of
the surface plasmon polariton as it propagates along the interface. If a surface plasmon propagates along a smooth surface the propagation length of the intensity is 1/(2k_x) as it approaches 1/e. We have shown that there exist a surface plasmon propagation length along the surface due to an electromagnetic field yet electromagnetic waves incident of a metal surface can only penetrate the solid up to a certain material dependent depth. The decay lengths of the surface plasmon polariton electric fields away from the interface can be obtained from

\[ k_{1,z} = \frac{\omega}{c} \sqrt{\frac{\epsilon'_2}{\epsilon'_1 + \epsilon_2}} \quad \text{for metal} \]

\[ k_{2,z} = \frac{\omega}{c} \sqrt{\frac{\epsilon'_2}{\epsilon'_1 + 2}} \quad \text{for dielectric} \]

We obtain the 1/e decay lengths away from the interface as (1/k_{1,z}, 1/k_{2,z}). This decay length is called the skin depth and is defined as the distance, where the exponentially decreasing evanescent field \( e^{-|k_z||z|} \) falls to 1/e.

2.3 Particle Plasmons

For surface plasmon polaritons propagating on plane interfaces we observed that the electromagnetic field is strongly localized normal to the interface i.e. localized in one dimension. In the case of subwavelength metal nanoparticles, the electromagnetic field is confined in three dimensions. There are distinctive differences between polaritons propagating at the plane interface of a metal and polaritons at the surface of metal nanoparticles. When an electromagnetic wave is incident on the surface of a metal nanoparticle the electron gas becomes polarized and is displaced by the external field.
This electron gas displacement leaves behind a positively charged lattice. The displacement of the electrons with respect to this positively charged lattice leads to a restoring force that gives rise to a plasmonic oscillation with a specific particle plasmon resonance depending on the geometry of the nanoparticle. Figure 2.1 shows the case of a spherical metal nanoparticle exposed to an external electromagnetic field.

![Figure 2.1. Diagram showing the collective oscillation of conduction electrons of a spherical metal particle when exposed to an external field.](image)

According to the Drude model, the electron gas oscillates 180 degrees out of phase relative to the driving electric field. For the remainder of this work, relating to plasmonics, subwavelength nanostructures will be discussed in their various forms. Therefore, it is useful to theoretically analyze the electromagnetic properties associated with these nanostructures. As an initial introduction, we will analyze the theoretical properties of particle surface plasmons based on a metallic nanoparticle with spherical geometry. In order to keep the initial analysis simple we limit the discussion to the quasi-static approximation. In the limit of small particles compared to the wavelength of light we can neglect retardation effects. In the quasi-static approximation the Helmholtz equation for potentials

\[ \nabla^2 \Phi + k^2 \varepsilon \Phi = -4\pi \rho, \]

\[ \nabla^2 A + k^2 \varepsilon A = -\frac{4\pi}{c} j, \]
reduces to the much simplified Laplace equation. In the quasi-static limit the electric field
can be represented by a potential as \( E = -\nabla \Phi \). If there are no external charges present,
this potential has to satisfy the Laplace equation
\[
\nabla^2 \Phi = 0
\]

We analyze the solutions for the Laplace equation for a small metal nanoparticle by
expressing the Laplace equation in spherical coordinates as
\[
\frac{1}{r^2 \sin \theta} \left[ \sin \theta \frac{\partial}{\partial r} \left( r^2 \frac{\partial \Phi}{\partial r} \right) + \frac{\partial}{\partial \theta} \left( \sin \theta \frac{\partial \Phi}{\partial \theta} \right) + \frac{1}{\sin \theta} \frac{\partial^2 \Phi}{\partial \varphi^2} \right] \Phi(r, \theta, \varphi) = 0
\]

The solutions are of the form
\[
\Phi(r, \theta, \varphi) = \sum_{l,m} b_{l,m} \cdot \Phi_{l,m}(r, \theta, \varphi).
\]

Here, the \( b_{l,m} \) are constant coefficients and the \( \Phi_{l,m} \) are of the form
\[
\Phi_{l,m} = \left\{ \begin{array}{l}
\frac{r^l}{r^{l-1}} \left\{ P^m_l(\cos \theta) \right\} \left\{ e^{im\varphi} \right\} \\
\end{array} \right.
\]

where the \( P^m_l(\cos \theta) \) are the associated Legendre functions and the \( Q^m_l(\cos \theta) \) are the
Legendre functions of the second kind. The continuity of the tangential electric fields and
the normal components of the electric displacements at the surface of the sphere imply
that
\[
\frac{\partial \Phi_1}{\partial \theta} \bigg|_{r=a} = \frac{\partial \Phi_1}{\partial \theta} \bigg|_{r=a}
\]
\[
\varepsilon_1 \frac{\partial \Phi_1}{\partial r} \bigg|_{r=a} = \varepsilon_2 \frac{\partial \Phi_1}{\partial r} \bigg|_{r=a}
\]

Here, \( \Phi_1 \) is the potential inside the sphere and \( \Phi_2 = \Phi_{\text{scat}} + \Phi_0 \) is the potential outside
the sphere consisting again of the potentials of the incoming and the scattered fields. For
the incoming electric field we assume the it is homogeneous and directed along the x-
direction. Consequently, \( \Phi_0 = -E_0 x = -E_0 r P_1^0(\cos \theta) \) and evaluation of the boundary conditions leads to

\[
\Phi_1 = -E_0 \frac{3\varepsilon_2}{\varepsilon_1 + 2\varepsilon_2} r \cos \theta
\]

2.27

\[
\Phi_2 = -E_0 r \cos \theta + E_0 \frac{\varepsilon_1 - \varepsilon_2}{\varepsilon_1 + 2\varepsilon_2} a^3 \cos \theta \frac{\cos \theta}{r^2}
\]

The electric field can be calculated using the above equation and \( E = -\nabla \Phi \).

\[
E_1 = E_0 \frac{3\varepsilon_2}{\varepsilon_1 + 2\varepsilon_2} (\cos \theta e_r - \sin \theta e_\theta) = E_0 \frac{3\varepsilon_2}{\varepsilon_1 + 2\varepsilon_2} e_x
\]

2.28

\[
E_2 = E_0 (\cos \theta e_r - \sin \theta e_\theta) + \frac{\varepsilon_1 - \varepsilon_2}{\varepsilon_1 + 2\varepsilon_2} \frac{a^3}{r^3} E_0 (2 \cos \theta e_r + \sin \theta e_\theta)
\]

The field inside the nanoparticle is homogenous. Consequently, the quasi-static approximation is only valid for particles that are smaller in size than the skin depth \( d \) of the metal \( (d = \lambda / [4\pi \sqrt{\varepsilon}] \)). The dipole is induced by the external field \( E_0 \) and has a polarizability defined by \( P = \varepsilon_0 \varepsilon_2 \alpha E_0 \) which then becomes

\[
\alpha(\omega) = 4\pi \varepsilon_0 a^3 \frac{\varepsilon_1(\omega) - \varepsilon_2}{\varepsilon_1(\omega) + 2\varepsilon_2}
\]

2.29

The scattering cross section of the sphere is then obtained by dividing the total radiated power of the dipole \( P = \omega^4 / (12\pi \varepsilon_0 \varepsilon_d c^3) |P|^2 \) by the intensity of the exciting wave \( (I = (1/2) c \varepsilon_0 \varepsilon_d E_0^2) \):

\[
\sigma_{scat} = \frac{k^4}{6\pi \varepsilon_0^2} |\alpha(\omega)|^2 = \frac{8\pi}{3} k^4 a^6 \left| \frac{\varepsilon_1 - \varepsilon_2}{\varepsilon_1 + 2\varepsilon_2} \right|^2
\]

2.30

The power removed from the incident light by the particle is not due to only scattering but also due to absorption. Using Poynting’s theorem the dissipated power by a point dipole is determined as \( P_{abs} = (\omega/2) \text{Im}(\mu \cdot E_0^*) \) where \( \mu = \varepsilon_2 \alpha E_0 \), the cross section then becomes
\[ \sigma_{abs} = \frac{k}{\varepsilon_0} Im(\alpha(\omega)) = 4\pi k a^3 Im\left(\frac{\varepsilon_1 - \varepsilon_2}{\varepsilon_1 + 2\varepsilon_2}\right) \]  

The extinction cross section is the combination of the scattering and absorption components \( \sigma_{ext} = \sigma_{scat} + \sigma_{abs} \). We can see that scattering scales with \( a^6 \) and absorption scales with \( a^3 \).

### 2.4 Mie Theory

Optical properties of isolated metal nanoparticles and their dependence on particle size effect have been intensively investigated through Mie scattering theory. In particular, Mie theory is a mathematical physical description of the scattering of electromagnetic radiation by spherical particles immersed in a continuous medium. A thorough derivation of Mie's formal theory can be found in [1]. For the purpose of this dissertation a more general presentation of Mie's theory will be described. The differential scattering cross section for unpolarized incident light is given by the following relation

\[ \sigma_{scat} = \frac{\lambda^2}{8\pi^2} (i_1 + i_2) \]

In this formulation, the intensity functions are calculated from the infinite series given by

\[ i_1 = \left| \sum_{n=1}^{\infty} \frac{2n - 1}{n(n + 1)} \left[ a_n \pi_n(\cos \theta) + b_n \tau_n(\cos \theta) \right] \right|^2 \]

\[ i_2 = \left| \sum_{n=1}^{\infty} \frac{2n + 1}{n(n + 1)} \left[ a_n \tau_n(\cos \theta) + b_n \pi_n(\cos \theta) \right] \right|^2 \]

In the above equation, the angular dependent functions \( \pi_n \) and \( \tau_n \) are expressed in terms of Legendre polynomials by
\[ \pi_n(\cos \theta) = \frac{P_n^{(1)}(\cos \theta)}{\sin \theta}, \]  
\[ \tau_n(\cos \theta) = \frac{dP_n^{(1)}(\cos \theta)}{d\theta}, \]
where parameters \( a_n \) and \( b_n \) are defined as
\[ a_n = \frac{\Psi_n(\alpha)\Psi'_n(m\alpha) - m\Psi_n(m\alpha)\Psi'_n(\alpha)}{\xi(\alpha)\Psi'_n(m\alpha) - m\Psi_n(m\alpha)\xi'_n(\alpha)}, \]
\[ b_n = \frac{m\Psi_n(\alpha)\Psi'_n(m\alpha) - \Psi_n(m\alpha)\Psi'_n(\alpha)}{m\xi(\alpha)\Psi'_n(m\alpha) - \Psi_n(m\alpha)\xi'_n(\alpha)}. \]

The size parameter \( \alpha \) is defined as \( \alpha = \frac{2\pi rn_m}{\omega} \) where \( r \) is the radius of the particle, \( n_m \) is the refractive index of the medium, and \( \omega \) is the wavelength of the incident light in vacuum. The Ricatti-Bessel functions \( \psi \) and \( \xi \) are defined in terms of half-integer-order Bessel functions of the first kind \( J_{n+1/2}(z) \), where
\[ \Psi_n(z) = \left( \frac{nz}{2} \right)^{\frac{1}{2}} J_{n+\frac{1}{2}}(z), \]
and \( H_{n+1/2}(z) \) is the half-integer-order Hankel function of the second kind, where
\[ \xi_n(z) = \left( \frac{nz}{2} \right)^{\frac{1}{2}} H_{n+\frac{1}{2}}(z) = \Psi_n(z) + X_n(z), \]
and parameter \( X_n \) is defined in terms of the half-integer-order Bessel function of the second kind, \( Y_{n+1/2}(z) \). Once the Mie coefficients are determined, the extinction and scattering cross sections can be calculated. The Mie total extinction and scattering cross sections are expressed as
\[ \sigma_{\text{ext}} = \frac{2}{\alpha^2} \sum_{n=1}^{\infty} (2n + 1) \text{Re}\{a_n + b_n\} \]

\[ \sigma_{\text{scat}} = \frac{2}{\alpha^2} \sum_{n=1}^{\infty} (2n + 1)(|a_n|^2 + |b_n|^2) \]

2.5 Complex geometries and discrete dipole approximation

Exact solutions to Maxwell's equations are known only for special geometries such as spheres, spheroids, or infinite cylinders, so approximate methods are in general required. The discrete-dipole approximation (DDA) is one such method. The DDA for computing scattering and absorption by particles is a general method developed by Purcell and Pennypacker as a flexible and general technique for calculating the optical properties of particles of arbitrary shapes. The DDA replaces the solid particle by an array of N point dipoles, with the spacing between the dipoles small compared to the wavelength of light incident on the particle. Each dipole has an oscillating polarization in response to both an incident plane wave and the electric fields due to all of the other dipoles in the array. Draine et. al. expounded on the work by Purcell by incorporating corrections for radiative reactions [6]. Draine and Flatau further tested the applicability and accuracy of the DDA method for periodic targets with excellent results [7].

Conceptually, the DDA consists of approximating the target of interest by an array of polarizable points. Once the polarizations are specified, Maxwell's equations can be solved accurately for the dipole array. For a monochromatic incident plane wave

\[ E_{\text{inc},j}(\mathbf{r}, t) = E_0 e^{i(k \mathbf{r} - \omega t)} \]
the polarizations $P_j$ of the dipoles in the target oscillate coherently and is given by $P_j = \alpha_j E_{ext,j}$. Each dipole $j$ is affected by the incident wave plus the electric field at location $j$ due to all the other point dipoles where

$$E_{ext,j} = E_{inc,j} - \sum_{k \neq 1} A_{jk} P_k$$

and $-A_{jk} P_k$ is the contribution to the electric field at position $j$ due to the dipole at position $k$:

$$A_{jk} P_k = \frac{e^{ikr_{jk}}}{r_{jk}^3} \left\{ k^2 r_{jk} \times (r_{jk} \times P_k) + \frac{(1 - ikr_{jk})}{r_{jk}^2} \times \left[ r_{jk}^2 P_k - 3r_{jk}(r_{jk} \cdot P_k) \right] \right\} \quad (j \neq k),$$

where $r_{jk} \equiv r_j - r_k$. By defining the matrix elements for $j=k$ as $A_{jj} = \alpha_j^{-1}$, The scattering problem can be formulated as a set of $N$ inhomogeneous linear complex vector equations:

$$E_{inc,j} = \sum_{k=1} A_{jk} P_k \quad (j = 1, \ldots, N)$$

The $3 \times 3$ matrices $A_{jk}$ are symmetric: $(A_{jk})_{lm} = (A_{jk})_{ml}$. It is further convenient to define two $3N$-dimentional vectors $\vec{P} = P_1, P_2, \ldots, P_N$ and $\vec{E}_{inc} = E_{inc,1}, E_{inc,2}, \ldots, E_{inc,N}$ and a $3N \times 3N$ symmetric matrix $A$ such that $\vec{A}_{3j-3,3k-m} = (A_{jk})_{3j-3,3k-m}$, so that the problem is reduced to a single matrix equation:

$$\vec{A} \vec{P} = \vec{E}_{inc}.$$ 

Many different methods for solving for $P$ are available. Draine and Flatau published a free FORTRAN software package (DDSCAT) that uses an iterative method [7]. Once the polarizations $P_j$ are known, the extinction cross section for array is computed from the forward-scattering amplitude using the optical theorem:
\[ C_{ext} = \frac{4\pi k}{|E_{inc}|^2} \sum_{j=1}^{N} \text{Im}(E_{inc,j} \cdot P_j) \]  

2.44

For a more thorough description of the relationship between the extinction cross section and the forward scattering amplitudes see [6]. The absorption cross section is obtained by summing over the rate of energy dissipation by each of the dipoles. The absorption cross section for the entire array is

\[ C_{abs} = \frac{4\pi k}{|E_{inc}|^2} \sum_{j=1}^{N} \left\{ \text{Im}(P \cdot (\alpha^{-1})^* P_j^*) - \frac{2}{3} k^3 P \cdot P_j^* \right\}. \]  

2.45

The scattering cross section can be obtained from the difference of the extinction and the absorption cross sections. It may be difficult to compute the scattering cross section this way if the absorption component is dominant. This is due to the requirement that the absorption and extinction cross sections be computed to high accuracy. It is also possible to compute the scattering cross section directly by computing the power radiated by the array of oscillating dipoles:

\[ C_{sca} = \frac{k^4}{|E_{inc}|^2} \int d\Omega \left| \sum_{j=1}^{N} \left( P_j - \hat{n}(\hat{n} \cdot P_j) \right) e^{-ik\hat{n} \cdot \mathbf{r}_j} \right|^2. \]  

2.46

where \( \hat{n} \) is a unit vector in the direction of scattering, and \( d\Omega \) is the element of solid angle.

### 2.6 Summary

In this chapter we introduced surface plasmon polaritons and discussed the basic properties. We examined the optical properties of metals using Maxwell's equations. Particle generated plasmonics was discussed and scattered electromagnetic radiation from
a spheroid was described using Mie theory. DDA was introduced as a potential computational method to described the electromagnetic scattering from targets with complex geometries.

2.7 References


Chapter 3: Synthesis of Au Nanostructures

Chapter 3 of this thesis deals with fabrication of AuNPs in the form of solid gold nano-cores. Before proceeding I would like to mention that several novel nanoparticle structures are discussed later in this thesis, however, chapter 3 of this thesis is used as a brief introduction into some of the mechanisms involved in nanoparticle formation and illustrates the intricacies and sensitivity of NP formation to the synthesis environment.

As previously mentioned in the introductory chapter, AuNPs are useful as building blocks that pave the way for fabricating biological labels, biological sensors, environmental detection of biological reagents, and clinical diagnostic techniques [1-4]. Since the plasmon-derived optical resonance of AuNPs is strongly related to the dimensions and morphology of the nanoparticles, the ability to synthesize monodispersed AuNPs is essential. In this chapter, an in depth analysis of AuNP synthesis utilizing carbon monoxide gas as a reducing agent is presented. After synthesis, AuNP mono- and polydispersity was examined. The size and monodispersity of the AuNPs were tunable by controlling variables such as H\textsubscript{3}AuCl\textsubscript{4} concentration and gas flow during synthesis. The CO reduction method offered excellent tunability over a broad range of sizes while maintaining a high level of monodispersity. Ensemble extinction spectra and TEM images provide clear evidence that CO reduction offers a viable alternative to other synthesis methods.

3.1 Established alternatives for AuNP synthesis

The most popular and reliable method of producing AuNPs is an aqueous phase synthesis, which relies on the reduction of tetrachloroauric acid in the presence of a
reducing agent to form colloid [5-10]. A number of different reducing agents can be used for the tetrachloroauric acid reduction. These agents have a significant influence on the morphology of the final product, and most of them lead to polydispersed nanoparticle solutions. To date, only a few methods have been established to synthesize AuNPs from about one nanometer to several hundred nanometers in diameter. A widely used method is based on the reduction of tetrachloroaurate ions in water using sodium citrate as a reductant to obtain AuNPs with diameters ranging from 16 to 147nm [11-13]. While this method has demonstrated good quality control over particle size, a high level of monodispersity is limited to the synthesis of larger particles typically in the range of 22 to 120nm. Another disadvantage to this synthesis method is that excess citrate remains in the solution. The residual citrate that acts as a passivation layer on the surface of the nanoparticles, can reduce the effectiveness of surface functionalization with other biological markers [14].

Smaller-sized AuNPs, 1 to 5nm, are usually prepared by borohydride reduction in the presence of thiol capping agents [15]. Disadvantages of this method include the use of toxic organic solvents and the potential presence of impurities introduced by using capping agents, which can also hinder the surface modification and functionality of particles for particular applications [16]. Also, AuNPs have been synthesized using formaldehyde as a reducing agent. One disadvantage is that formaldehyde is toxic, and the excess formaldehyde in the solution leads to solution instability and eventual particle aggregation [8].

Non-chemical based reduction methods, to synthesize AuNPs, have also been employed. Size selected AuNPs have been synthesized by use of laser irradiation in a
surfactant based aqueous environment [17]. Yet this method limits AuNPs sizes to sub 10nm diameters. Meuier et al. were able to synthesize gold nanoparticles from 3nm to ~80nm via a femtosecond laser-assisted method [18]. An involved multi-step process, including a seeding step, was necessary to produce the larger particles. This process requires a complicated femtosecond laser setup and nanoparticle synthesis was also dependent on polymer utilization. Dispersed AuNPs were also synthesized using glow discharge plasma [19-22]. Researchers showed that this method can produce particles in less than 5 minutes yet these particles were limited to ~4nm diameters [22]. Takai et al. used discharge plasma to produce larger AuNPs of irregular shapes [20]. Polydisperse spherical AuNPs ~20nm in diameter were only produced after exposure times greater than 45 minutes.

As compared to the current synthesis methods, CO has an advantage in that no excess reducing agent remains in solution. This eliminates the need for purification. The reduction of HAuCl₄ with CO can also take place at room temperature, which is unlike other methods such as citrate, which require boiling of solutions. The time necessary to produce AuNPs using CO is less than 2 minutes compared to 20 minutes for comparable particle sizes using citrate reduction and 45 minutes for discharge plasma synthesis. CO reduction offers a cheap and flexible alternative to femtosecond laser-based AuNP synthesis processes while eliminating the need for surfactants and polymers to tune the nanoparticle sizes.
3.2 Synthesis of AuNPs Using CO

AuNPs, synthesized by CO reduction, with average diameter ranging from 4 to 52nm, were prepared as described below. A set of solutions consisting of HAuCl₄ concentrations ranging from 0.01mM up to 0.09mM was used. Each HAuCl₄ concentration was duplicated to ensure reproducibility. For each HAuCl₄ concentration, five 40mL samples were prepared. Each sample was aerated at different flow rates controlled by a control valve. The five solutions were exposed to CO gas at flow rates of 16.9, 25.45, 31.59, 37.0 and 42.9 mL/min respectively. The effect of stirring speed was examined, and it was found that the number of revolutions per minute (rpm), by which the solution was stirred, played a role in particle size and morphology. The optimal stir speed, for producing the most monodispersed particles, was found to be 500 rpm. For the following discussion, each solution was constantly stirred at a rate of 500 rpm during synthesis unless noted otherwise. Additionally, the effect of gas-injection flow rates and diffuser pore size on nanoparticle monodispersity and reaction completion times were investigated. It was found that a 60um average diffuser pore size was sufficient for producing monodispersed particles. The solution temperature, prior to aeration, was maintained between 20 and 22⁰C.

The Au³⁺ reduction, by CO, to Au⁰ takes place via a number of redox reactions. When the CO gas is introduced into the aqueous HAuCl₄ solution, electrons are donated to the [AuCl₄]⁻ ions. For [AuCl₄]⁻ ions to be reduced to gold atoms, a series of redox reactions take place. This includes the liberations of Cl⁻ ions and is described by equation 3.1 and equation 3.2.
\[ \text{AuCl}_4^- + 2e^- \rightarrow \text{AuCl}_2^- + 2Cl^- \quad 3.1 \]
\[ \text{AuCl}_2^- + e^- \rightarrow \text{Au}^0 + 2Cl^- \quad 3.2 \]

The electrons are contributed from the reaction of carbon monoxide and dihydrogen monoxide and the reducing half reactions are given in equation 3.3 and equation 3.4.

\[ \text{CO}(g) + H_2O \rightarrow \text{CO}_2(aq) + 2e^- + 2H^+ \quad 3.3 \]
\[ \text{CO}(g) + 2H_2O \rightarrow \text{HCO}_3^- + 2e^- + 3H^+ \quad 3.4 \]

To illustrate the effects of CO gas flow injection rates on nanoparticle synthesis, nanoparticles were synthesized from an aqueous solution of HAuCl\(_4\) acid at a concentration of 0.01mM. Even at this lower concentration, which is normally not used for the synthesis of AuNPs, the extinction spectra is clearly visible and well formed as evident in Figure 3.1. A smoother, more pronounced spectrum was generated at the minimum flow rate of 16.9 mL/min when compared to the other injection flow rates. As the flow rate was increased from 16.9 to 42.9 mL/min the change in spectral symmetry was clearly visible. TEM micrographs of the corresponding nanoparticles are displayed in Figure 3.1.
Figure 3.1. UV-visible extinction spectra of nanoparticles synthesized from a chloroauric acid concentration of 0.01mM aerated at flow rates of 16.9, 25.5, 37.0, and 42.9 mL/min corresponding to A, B, C, and D, respectively, with accompanying TEM micrographs. A smoother, more pronounced spectrum was generated at the minimum flow rate of 16.9 mL/min when compared to the other injection flow rates. As the flow rate was increased from 16.9 to 42.9 mL/min the change in spectral symmetry was clearly visible.

The gas injection flow rate of 16.9 mL/min produced individual nanoparticles compared to the other injection rates. The nanoparticles produced by the 16.9 mL/min flow rate ranged in size from 5 to 11nm in diameter. A flow rate of 25.45 mL/min, Figure 1B, produced nanoparticle aggregates and irregularly shaped particulate matter. Nanoparticles
synthesized at a flow rate of 31.59 mL/min consisted of aggregated particle chains. A CO
flow rate of 37 mL/min (Figure 3.1C) resulted in aggregated particle chains similar to
that of nanoparticles produced at a flow rate of 25.45 mL/min. The particle aggregation in
Figure 3.1B and 3.1D was evident by the broad spectral band. As the flow rate increased
to 42.9 mL/min, the nanoparticles became elliptical in shape and very polydispersed. The
nanoparticle sizes, when aerated at 42.9 mL/min, ranged from 5 to 40nm in diameter with
some aggregated particles; this size distribution is reflected in the broad spectral band.

Increasing the chloroauric acid concentration reduced the polydispersity of the
nanoparticles, yet the gas injection flow rate continued to influence the AuNP size
distribution profiles. Figure 3.2 shows the UV-visible spectra of AuNPs synthesized from
a chloroauric acid concentration of 0.03mM at flow rates of 16.9, 25.5 and 37.0 mL/min
(Figure 3.2A, 3.2B, and 3.2C).
Figure 3.2. UV-visible extinction spectra of nanoparticles synthesized from a chloroauric acid concentration of 0.03mM aerated at flow rates of 16.9, 25.5, and 37.0 mL/min corresponding to A, B, and C, respectively, with accompanying TEM micrographs and histograms.

The polydispersity of the AuNPs aerated at 16.9 mL/min (3.2A) is represented by a broad particle distribution curve. The particle sizes for 3.2A ranged from 2.5 to 17nm in diameter. Increasing the CO flow reduced the width of the particle distribution curve where an optimum inlet gas flow was obtained at 25.5 mL/min (3.2B). The standard deviation for 3.2B was 7%, well below the 13 to 15% normally obtained for comparable sizes via citrate reduction [23]. These particles remained stable in excess of 12 months when stored at 4°C.
To confirm the formation of Au atoms from HAuCl₄, the valence state of Au was identified by x-ray photoelectron spectroscopy (XPS). Figure 3.3 shows an XPS spectrum of AuNPs synthesized via CO gas reduction. The Au 4f₇/₂ peak appeared at a binding energy of 83.98 eV and the Au 4f₅/₂ peak appeared at a binding energy of 87.71 eV. This indicates the formation of metallic gold [24-25].

![XPS spectrum of AuNPs synthesized via CO gas reduction.](image)

**Figure 3.3.** XPS spectrum of AuNPs synthesized via CO gas reduction. The Au 4f₇/₂ peak appeared at a binding energy of 83.98 eV and the Au 4f₅/₂ peak appeared at a binding energy of 87.71 eV. This indicates the formation of metallic gold [17-18].

A better understanding of the effect of the gas flow rates and chloroauric acid concentrations on nanoparticle synthesis can be obtained by considering the mechanisms involved in nanoparticle nucleation and growth. When aerating the aqueous HAuCl₄ solution with CO gas, the precursor concentration increases continuously with increasing time. As the concentration reaches supersaturation, nucleation takes place and leads to a decrease in concentration. The continued decrease of the concentration is due to the growth of the particles. During the growth process, two growth mechanisms could take
place or a combination of the two. The first growth mechanism is due to the formation of particles from coalescence of the nuclei only. The second growth mechanism is due to the coalescence of nuclei into simple and multiple twins with further growth from monomer attachment of Au atoms on the surface [12].

To produce monodispersed AuNPs with CO gas, the rate of nucleation must be high enough so that the precursor concentration does not continue to climb. Instead a large amount of nuclei are formed in a short period. Turkevich et. al found that the nucleation process consists of a polymerization step [26]. When the aqueous HAuCl₄ solution is neutral or acidic, the nucleus is formed by gold organic polymer. While the aqueous HAuCl₄ solution is alkaline, a polymerization of gold hydroxide takes place [12, 27]. The rate of growth of these nuclei should be fast enough to decrease the concentration below the nucleation concentration rapidly. This results in the creation of a limited number of seed particles. The rate of growth must be slow enough that the growth period is long compared with the nucleation period. This produces AuNPs with narrowed size distributions which are the result of the limited nucleation period.

### 3.3 Factors Affecting AuNP Synthesis

Since the morphology is found to depend strongly on injection flow rates and HAuCl₄ concentrations, a relationship between the HAuCl₄ concentration and gas-injection flow rates on particle monodispersity can be found. Solution stir speeds during synthesis were examined and it was found that stir speeds had an effect on synthesis and played a role in nanoparticle size disparities. Slow solution stir speeds had the biggest affect on solutions aerated at a flow rate of 16.9mL/min or below. Increasing the stir speed of the solution
aided in the solubility and dispersal of the CO gas molecules during synthesis. It was found that adjusting the gas injection flow rate compensated for a reduction or increase in solution stir speed. The gas diffuser pore size affected the synthesis process considerably when the solution was at a standstill or stirred at a relatively slow speed below 75rpm. Once the solution stir speed approached and/or crossed the 75 rpm threshold, injection-hole size produced only small variances. Once the stir speed reached 500 rpm, there was no difference between samples produced with different diffuser pore sizes, and only the Au concentration or gas injection flow rates affected particle sizes. Therefore, the solution stirring speed was maintained at 500 rpm to isolate the gas injection flow rate and Au concentration effect on nanoparticle synthesis.

A chloroauric concentration of 0.03mM and an inlet gas flow of 16.9 mL/min stirred at 500 rpm resulted in coalescence and growth of nanoparticles before the nucleation reached equilibrium. In essence, the induction period was initiated with a slow autocatalytic rise in the number of nuclei due to the lack of sufficient reducing agent in the solution. Because of this slow nucleus formation, new nuclei were being formed while existing nuclei had already undergone coalescence resulting in polydispersity. Increasing the flow rate to 25.5 mL/min increased the autocatalytic rise in the number of nuclei. Particle growth took place after cessation of the nucleation process resulting in monodispersity. This is illustrated by the fact that the particle distribution curve for Figure 3.2B consisted of particle sizes in the range of 4 to 6nm as opposed to the range of 2 to 17nm (Figure 3.2A). By increasing the flow rate further (Figure 3.2C), rapid coalescence of the nuclei takes place. The resulting polydispersity of the sol at increased gas injection flow rates is still marginal compared to the lower flow rate of 16.9 mL/min.
When comparing the spectra of 3.2A, 3.2B, and 3.2C, the more polydispersed sample possesses a broadened spectrum.

### 3.4 Increasing HAuCl$_4$ Concentration

When the chloroauric acid concentration approached 0.2mM, the gas injection flow rate had a less pronounced effect on the spectra symmetry yet the flow rate continued to dictate the monodispersity of the particles. When particles were synthesized from a chloroauric acid concentration of 0.3mM, the most monodispersed sample was produced at a flow rate of 25.5 mL/min. The mean diameter for this sample was 9nm with a standard deviation of 11%.

As the concentration increased to 0.5mM, 20 to 25 nm particles were produced. Continual increase of the chloroauric acid concentration beyond 0.5mM to 0.6mM only produced small changes in nanoparticle size with increased absorbance. The standard deviation for the AuNPs produced at 0.6mM was 8% indicating monodispersity. As the concentration was increased to 1mM, nanoparticles approaching 30nm in diameter were produced but the standard deviation approached 20%. Further doubling the concentration to 2mM had no uniform effect on particle growth, with the majority of the particles in the 30nm size regime and some of the particles in the 40 to 55nm size regime with a standard deviation approaching 35%. The UV-visible spectra of the sol prepared at different concentrations (Figure 3.4), increasing from 0.02 to 1mM, shows an increase in absorbance which correlates to an increase in particle concentration and volume.
Figure 3.4. UV-visible spectra of gold nanoparticles with increasing chloroauric acid concentrations from 0.02 to 0.05mM in 0.01mM increments, from 0.1 to 0.5mM in .1mM increments, and at 1mM. The inset is the absorbance spectra of gold nanoparticles produced from concentrations of 0.02mM to 0.1mM.

Figure 3.5 shows the pronounced red shifting of the plasmon, which is associated with increased nanoparticle size. This shifting effect is in line with the prediction described by Mie theory. The statistical analysis of the particles synthesized from aqueous solutions of HAuCl$_4$ ranging from 0.02 to 0.6mM revealed an average standard deviation of ~11%.
Figure 3.5. Normalized UV-visible spectra of gold nanoparticles with increasing chloroauric acid concentrations from 0.02 to 0.05 mM in 0.01 mM increments, from 0.1 to 0.5 mM in .1 mM increments, and at 1 mM. A red-shifting of the plasmon is observed as the chloroauric acid concentration is increased.

3.5 Influence of pH on AuNP Synthesis

It is known that pH is a factor influencing the nucleation and growth of AuNPs [8, 12, 27]. Since the synthesis process takes place in an acidic environment, the particle is formed from gold polymer with a small contribution from gold hydroxide polymer reduction. As the concentration of chloroauric acid increases, the pH of the solution decreases (Figure 3.6) resulting in particle formation solely by gold polymer reduction.
Figure 3.6. pH values before and after AuNP synthesis for given HAuCl₄ concentrations ranging from 0.02 to 1mM in 0.01mM increments and from 0.1 to 0.5mM in 0.1mM increments. The x-axis is plotted on a logarithmic scale. The inset shows the pH values of the AuNP solutions from 0.01 to 0.1mM and is plotted on a linear scale. As the reduction of HAuCl₄ by CO takes place, H⁺ ions are liberated decreasing the pH of the solution. All pH measurements were taken at room temperature.

In an acidic environment, the effective monodispersed particle size threshold was reached at approximately 25nm. The effective monodispersed threshold was defined as a standard deviation below 13%. As previously mentioned, continual increase of the chloroauric concentration eventually resulted in adverse affects on nanoparticle monodispersity. To further grow particles and maintain monodispersity, HAuCl₄ hydrolysis was explored. The addition of potassium carbonate (K₂CO₃) to generate an alkaline solution for gold hydroxide polymer reduction was systematically investigated. It was found that the speciation of HAuCl₄ had great influence on the size and monodispersity of the AuNPs.

As the pH increased, speciation of aqueous HAuCl₄ occurred.

Adding K₂CO₃ raised the pH of the solution by allowing hydrolysis of HAuCl₄ to take place to form gold hydroxide solution. A 200mL aqueous HAuCl₄ solution, with a concentration of 0.1mM, was prepared by adding fresh gold to 200mL of DI water. This
solution was aged in an amber bottle, and light protected in a 4°C environment for a minimum of 72 hours prior to use. A 0.5N stock solution of K$_2$CO$_3$ was prepared and stirred for a minimum of one hour. After aging, the chloroauric acid solution was allowed to gradually rise to 22°C. The pH was measured to be 3.6. HAuCl$_4$ (0.1mM) aqueous solution with various pH values were prepared by the addition of K$_2$CO$_3$ aqueous solution into 20mL of HAuCl$_4$ aqueous solution and shaken vigorously for a minimum of one minute. This solution was allowed to age for 15 minutes before introduction of CO gas. The pH values of the aqueous solutions, measured prior to reduction, ranged from 4.25 to 11.4.

Figure 3.7 shows UV-visible absorption spectra of AuNPs prepared by reduction of hydrolyzed HAuCl$_4$ at various pH. At pH = 4.25, the acquired AuNPs exhibited a symmetric spectrum with a surface plasmon resonance (SPR) peak at 512nm.

![Figure 3.7](image_url)

**Figure 3.7.** UV-visible spectra of AuNPs produced from a 0.1mM HAuCl$_4$ aqueous solution synthesized at varying pH values.
When the pH increased to 6.6, there was a SPR shift to 527nm. When the pH increased to 7.45, the SPR peak position did not change much at 528nm, and the SPR peak remained symmetric. The SPR feature changed abruptly when the pH was 9.34 showing a broad feature originating at 559nm. The SPR peak red-shifted further when the pH increased to 10.3. Absorption in the NIR region also gained significant intensity.

Previous experimental and theoretical results demonstrated that AuCl$_4$ undergoes a pH-dependant stepwise hydrolysis which gives way to [AuCl$_{x}$(OH)$_{4-x}$]$^{-}$ [27-28]. The extent of hydrolysis in turn depends on the pH which gives an indication of the amount of OH$^{-}$ available for hydrolysis. When the pH is low, [AuCl$_4$]$^{-}$ ions dominate the solution. As the pH is increased to 4.25, [AuCl$_4$]$^{-}$ concentration is lowered and the contribution from [AuCl$_3$(OH)]$^{-}$ ions is increased. Raising the pH of the solution to 6.66 reduced the concentration of [AuCl$_4$]$^{-}$ and [AuCl$_3$(OH)]$^{-}$ significantly, and the ionic composition was primarily made up of [AuCl$_2$(OH)$_2$]$^{-}$ ions. Further increasing the pH to 8.8 resulted in large ion contribution from [AuCl(OH)$_3$]$^{-}$ ions. Additional increase to 10.3 resulted in an overwhelming ion contribution from [Au(OH)$_4$]$^{-}$ ions with an appreciable contribution from [AuCl(OH)$_3$]$^{-}$ ions. This was because [Au(OH)$_4$]$^{-}$ is amphoteric. Its solubility increased due to the formation of [Au(OH)$_4$]$^{-}$ at higher pH, thus making the soluble [Au(OH)$_4$]$^{-}$ the most dominant species at high pH instead of the precipitating [AuCl(OH)$_3$]$^{-}$ [27]. It is the control of hydrolysis to tune the speciation of [AuCl$_{x}$(OH)$_{4-x}$]$^{-}$ that subsequently influenced the nanoparticle size.

It was observed that amongst the six species of [AuCl$_{x}$(OH)$_{4-x}$]$^{-}$ discussed earlier, [Au(OH)$_4$]$^{-}$ seems to have the lower tendency to be reduced in solution to form colloidal gold. This was evident from its slow and gradual color change when reduced, taking $\sim$7
minutes for complete reduction to occur. This was in contrast to the reduction of other 
$[\text{AuCl}_3(\text{OH})_{4-x}]^-$ species formed at lower pH where it was observed that the addition of 
CO gas caused a color change within seconds and total reduction within ~2 minutes. This 
observation may possibly be attributed to a weaker reduction potential of $[\text{Au(OH)}_4]^{-}$ 
compared to other species. It was found that adjustment to pH<10 by addition of smaller 
amounts of K$_2$CO$_3$ resulted in the formation of other dominant species that had greater 
tendency to be reduced in solution to form colloidal gold. It was observed that the 
synthesis environment also affected nanoparticle stability. The stability of the 
nanoparticles was monitored for approximately 2 months to examine the pH effect on 
nanoparticle stability. As the pH increased, prior to synthesis, the nanoparticles became 
less stable. Table 1 illustrates the stability of the AuNP solutions produced at varying pH.

**TABLE 3.1: Influence of pH upon stability of AuNPs**

<table>
<thead>
<tr>
<th>pH Before Synthesis</th>
<th>pH After Synthesis</th>
<th>Color</th>
<th>Stability After 1 Hour Stored at 22°C</th>
<th>Stability After 6 Hours Stored at 22°C</th>
<th>Stability After 2 Months Stored at 4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.25</td>
<td>3.72</td>
<td>Light pink</td>
<td>Stable</td>
<td>Stable</td>
<td>Small Aggregation</td>
</tr>
<tr>
<td>5.55</td>
<td>4.75</td>
<td>Light red</td>
<td>Stable</td>
<td>Stable</td>
<td>Small Aggregation</td>
</tr>
<tr>
<td>6.6</td>
<td>5.92</td>
<td>Light Red</td>
<td>Stable</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td>7.45</td>
<td>6.11</td>
<td>Light Red</td>
<td>Stable</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td>8.8</td>
<td>6.42</td>
<td>Light Red</td>
<td>Stable</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td>9.23</td>
<td>6.55</td>
<td>Medium Red</td>
<td>Stable</td>
<td>Stable</td>
<td>Stable</td>
</tr>
<tr>
<td>9.34</td>
<td>6.32</td>
<td>Purple</td>
<td>Stable</td>
<td>Stable</td>
<td>Medium Aggregation</td>
</tr>
<tr>
<td>10.3</td>
<td>8.10</td>
<td>Blue</td>
<td>Stable</td>
<td>Some Aggregation</td>
<td>Heavy Aggregation</td>
</tr>
<tr>
<td>11.4</td>
<td>10.96</td>
<td>Light Blue</td>
<td>Crashed</td>
<td>Crashed</td>
<td>Crashed</td>
</tr>
</tbody>
</table>
It was observed that hydrolysis of $[\text{AuCl}_4]^{-}$ started to occur within minutes after the addition of $K_2\text{CO}_3$ indicating immediate formation of the $[\text{AuCl}_x(\text{OH})_{4-x}]^{-}$ species. It was further observed that Au colloid, of varying sizes, were produced when $K_2\text{CO}_3$ and $\text{HAuCl}_4$ concentrations and gas injection flow rates remained constant and only aging times varied. This indicated that aging the gold hydroxide solution, before the addition of CO gas, had a strong influence on the outcome of the reaction.

By controlling the development of the $[\text{AuCl}_x(\text{OH})_{4-x}]^{-}$ species, colloids of various sizes can be synthesized using CO as a reducing agent. When the pH is sufficiently high, the resultant aging process can generate coalescence of $\text{Au(OH)}_4$ initiating a limited nucleation process absent of a reducing agent. This nucleation process is out of favor with the requirements necessary for generating monodispersed nanoparticles. Thus proper aging times must be determined to synthesize monodispersed nanoparticles of a particular size from a given $K_2\text{CO}_3$ and $\text{HAuCl}_4$ concentration. Exploiting the control of $[\text{AuCl}_x(\text{OH})_{4-x}]^{-}$ species development, by addition of $K_2\text{CO}_3$ and aging of the solution, Au colloid in the ranges of 15 to 100nm in diameter were produced. Spectra A and B in Figure 3.8 show the UV-visible spectra of Au colloid produced from a mixture of 200mL 0.38mM HAuCl$_4$ aqueous solution and $K_2\text{CO}_3$ (2.71mM) aged at 30 and 40 minutes, respectively, in solution reduction volumes of 40mL.
Figure 3.8. UV-visible spectra of AuNPs produced from a mixture of 0.38mM HAuCl₄ aqueous solution with 2.71mM or 3.62mM K₂CO₃. A and B are 2.71mM K₂CO₃ aged at 30 and 40 minutes, respectively, at an aeration volume of 40mL. C, D, and E are 3.62mM K₂CO₃ aged for 30 minutes each at aeration volumes of 20, 40, and 50mL, respectively. All samples were aerated at a gas flow rate of 25.5 mL/min.

Both SPR peaks were well ordered with a SPR peak at 536nm for the 30 minute aged solution and 546nm for the 40 minute aged solution. Both solutions were aerated with CO gas at an inlet gas flow rate of 25.5 mL/min. The red-shift and dampening of the SPR peak indicated an increase in particle size. The effect of the solution volume being aerated was explored to determine if the amount of solution being aerated had an effect on nanoparticle size and monodispersity. Spectra C, D, and E in Figure 3.8 were produced from AuNPs synthesized from a 200mL 0.38mM HAuCl₄ aqueous solution with K₂CO₃ (3.62mM) aged for 30 minutes. The aeration volumes were 20, 40, and 50mL respectively. The amount of solution aerated had a small but noticeable effect on SPR peak position. The resulting SPR peak positions were 550, 553, and 554nm for aeration volumes of 20, 40, and 50mL respectively. Increasing the amount of K₂CO₃ in a HAuCl₄ aqueous solution of known concentration, while decreasing the aging time,
produced larger AuNPs while still maintaining monodispersity. Aqueous solutions of 200mL 0.38mM HAuCl₄ with 2.71mM and 3.62mM of K₂CO₃ aged for 30 minutes each produced AuNPs with SPR peak positions at 536 and 553nm, respectively.

By employing a combination of gold polymer reduction and gold hydrolyzed polymer reduction, particles sizes from ~4nm to 100nm can be synthesized. Figure 3.9 shows a TEM micrograph illustrating the different sizes available using CO as a reducing agent. Figures 3.9A, 3.9B, 3.9C, and 3.9D are TEM images of AuNPs synthesized without the addition of K₂CO₃. Figures 3.9E and 3.9F are AuNPs synthesized from a hydrolyzed solution of aqueous HAuCl₄ via the addition of K₂CO₃. The corresponding sizes of the AuNPs are 4, 6, 15, 25, 50, and ~100nm with standard deviations of 7, 13, 8, 8, 10, and 11%, respectively.
3.6 Summary

In this chapter we showed how sensitive synthesis of metal nanostructures can be to the synthesis environment. These results discussed show that AuNPs can be synthesized using carbon monoxide as a reducing agent. Carbon monoxide offers tunability of nanoparticle sizes via altering HAuCl₄ concentration and flow rate. The fast synthesis rates, ease of tunability, and absence of cytotoxic by-products allow for these CO-based AuNPs to be optimized and readily produced for biomedical and industrial applications. The manipulation of the solution pH and speciation of HAuCl₄ to control particle morphology may also be used as a means to tune the particle size. TEM micrographs and

**Figure 3.9.** TEM images of AuNPs synthesized by CO reduction of HAuCl₄. A, B, C, and D are TEM images of AuNPs synthesized without the addition of K₂CO₃. E and F are AuNPs synthesized from a hydrolyzed solution of aqueous HAuCl₄ via the addition of K₂CO₃. The corresponding sizes of the AuNPs are 4, 6, 15, 25, 50, and ~100nm respectively.
UV-Visible spectral analysis confirm that the CO-based AuNPs are monodispersed upon synthesis.

3.7 References


Chapter 4: T cell Delivery of Theranostic Agents

Chapter 4 of this thesis discusses the delivery of AuNPs in in vivo environments. In this chapter, we discuss the capacity of activated T cells to function as delivery vehicles for AuNPs. Here, we have explored whether T cells can be used as AuNP carriers to increase delivery to tumor sites in vivo using AuNPs 40-45 nm in diameter. The AuNPs are synthesized via the aforementioned carbon monoxide reduction method. The results demonstrate that T cells can be used to enhance AuNP delivery to a tumor in vivo. The use of AuNPs and T cells together combines the photothermal therapy and imaging advantages of AuNPs with the immunotherapy and biodistribution advantages of T cells leading to a novel theranostic approach.

As previously mentioned, AuNPs can be designed to absorb light in the NIR, where light is maximally transmissive in tissue. AuNPs, optically responsive in the NIR region can be used in such applications as PTT and imaging. When AuNPs are used as a therapeutic agents it is important that the particles are delivered effectively to the tumor site. NP delivery is often accomplished by intravenous injection and will accumulate within the tumor via its irregular vasculature. This type of accumulation is known as the enhanced permeability and retention (EPR) effect [1-2]. Yet, the percentage of the injected NPs delivered to the tumor is low compared to the injected dose. There is also high accumulation in non-target sites such as the liver and spleen when injected intravenously [3-5].

To address these delivery issues new methods aimed at improving tumor delivery and specificity to increase the tumor concentration of NPs has been explored. Efforts to enhance AuNP tumor delivery have included a variety of NP surface modifications, yet
inclusion of targeting ligands has only modestly improved tumor accumulation and specificity [6, 7]. Therefore, tumors are less likely to be effectively targeted using the above mentioned NP schemes, ultimately limiting their therapeutic use. Choi et al. [8] demonstrated that macrophages could be used as a cellular delivery vehicle to deposit AuNPs in tumors. These results suggested that other immune cells might be used as AuNP delivery vehicles. Unlike macrophages, T cells are readily isolated and expanded in vitro, and upon infusion, circulate throughout the body and migrate into tumors in response to tumor-associated chemokines. This tumor-tropic property permits their use as cellular vehicles for the delivery of molecular therapeutics [9-12].

We demonstrated that CO-AuNPs are readily taken up by activated human T cells without impairing their viability or cellular functions, and that following intravenous infusion into tumor bearing mice can more efficiently deliver AuNPs to distant tumor sites. Although CO-AuNPs in this size range have maximal absorbance in the visible wavelengths, there are several variants of AuNPs that are of similar size (30-60 nm) that absorb optimally in the NIR region, permitting translation of this delivery method for PTT. These AuNP variants include gold-gold sulfide nanoparticles, hollow gold nanoshells, and gold nanocubes, all of which have demonstrated efficacy as PTT-mediating agents in mouse studies [13]. Additionally, there have been studies demonstrating photothermal therapy using AuNPs that have been strategically aggregated to red-shift the peak absorbance into the NIR [14-15]. Therefore, the use of CO-AuNPs in the 40-45 nm size regime served as a particle model to explore the potential of combined T-cell/NP therapeutics.
4.1 T cell loading with CO-AuNPs

Synthesized Co-AuNPs sizes were determined to be 40-45 nm in diameter by transmission electron microscopy (TEM) Figure 4.1A. Activated and expanded human T cells were cultured in the presence of CO-AuNPs for a period of 1 to 24 h to permit CO-AuNP internalization. CO-AuNP loading was confirmed using bright field and dark field microscopy demonstrating that T cells co-localize with CO-AuNPs (Figure 4.1B). To determine the number of nanoparticles present per T cell, an inductively coupled plasma optical emission spectrometry (ICP-OES) analysis was used. T cells from three different human donors were first cultured with concentrations of CO-AuNPs ranging from 0.05 to 0.5 nM for a period of 24 h to evaluate for variability in gold nanoparticle loading due to differences in T cells from different donors (Figure 4.1C). A maximum of 14,900 ± 2,400 AuNPs was internalized per T cell using a AuNP loading concentration of 0.5 nM (Figure 4.1C). We then performed a time course study using T cells from a single donor to determine the minimum amount of time required to load the T cells with the maximum number of AuNPs (Figure 4.1D). For this study, we incubated T cells with nanoparticle concentrations ranging from 0.05 to 1 nM. At 24 h, the 0.5 and 1 nM groups have similar gold content, suggesting that there is a maximum amount of AuNPs that can be internalized by T cells. These results demonstrate that maximal CO-AuNP loading of T cells can be achieved using a concentration of 0.5 nM CO-AuNP and an incubation period of 24 h.
Figure 4.1. Gold nanoparticle uptake by human T cells. (A) TEM imaging of gold colloid (diameter = 40-45 nm). (B) Brightfield (upper) and darkfield (lower) images of human T cells demonstrate gold nanoparticle uptake by the increased light scattering seen in the AuNP-T cell group compared to T cells alone. (C) ICP-OES analysis of T cell gold content at 24 h using different nanoparticle loading concentrations. Each point is a composite of data acquired from three different T cell donors. (D) Time course data for T cells from a single donor loaded with different concentrations of gold nanoparticles. Optimal loading occurred after 24 h at a concentration between 0.5 and 1.0 nM.

4.2 CO-AuNP loading and T cell viability

We next measured T cell viability and function post-AuNP loading to assess potential toxicity that may inhibit T cell performance as an in vivo delivery vehicle. Loading T cells with AuNPs had no immediate effect on T cell viability as determined by Annexin-V/7-AAD staining (Figure 4.2A) and did not alter the phenotype of the cells. Furthermore, there were no prolonged effects on T cell proliferation as measured by thymidine incorporation (Figure 4.2B). Importantly, AuNPs did not affect migration
when tested in a transwell chemotaxis assay against supernatant produced from human LCL tumors, suggesting that T cells retain their migratory behavior post-AuNP loading (Figure 4.2C). Finally, production of IFN-g following mitogen activation (PMA-I) was not impaired by AuNPs (Figure 4.2D). These results show that AuNPs have no detrimental effects on T cell viability and function in vitro and indicate that T cell migration in vivo will likely be retained following loading.

**Figure 4.2.** AuNP loading has no significant effect on T cell viability or function. T cells were loaded in the presence of 0.5 nM AuNPs for 24 h or cultured in medium alone and then measured for viability using Annexin-V/PI staining (A), proliferation using thymidine incorporation (B), migration through a transwell membrane in response to tumor (LCL) produced supernatant (C) and intracellular analysis of IFN-g cytokine production following mitogen stimulation (D).
4.3 T cells transport CO-AuNPs to tumors in vivo

In vivo CO-AuNP-T cell migration to tumor sites was first examined using bioluminescent imaging and histology. T cells were first genetically modified to express firefly luciferase and then subsequently loaded with CO-AuNPs. Bioluminescent imaging 48 h post-intravenous injection of CO-AuNP-T cells demonstrate specific migration of the T cells to subcutaneous LCL tumors in immune deficient SCID mice (Figure 4.3A). This time point was selected based on previous studies that have demonstrated T cell localization to tumor sites 48 h post-infusion [16-17]. We next resected the tumors and performed histology to determine if CO-AuNPs and T cells co-localized within the tumor. Immunohistochemical staining using CD3 antibody (a pan-T cell marker) demonstrated infiltration of T cells into the tumor (Figure 4.3B). In addition, areas of increased scatter in the darkfield images correlated well with areas of CD3+ staining. This observation demonstrates that the T cells maintain internalized CO-AuNPs during in vivo migration to the tumor site.
Figure 4.3. AuNP-loaded T cells migrate to tumors in vivo. (A) T cells were retrovirally modified to express firefly luciferase then loaded in the presence of 0.5 nM AuNP for 24 h. Cells were subsequently injected intravenously into SCID mice bearing subcutaneous xenografted LCL tumors. Bioluminescent imaging of AuNP-T cell biodistribution at 48 h post-injection showing AuNP-T cell localization at the tumor site (red circle) and within the spleen. (B) Resected tumors were analyzed by bright field imaging (top row) and immunohistochemistry for human CD3 expression and dark field imaging (bottom row) to indicate the presence of AuNPs. Red arrows indicate the colocalization of CD3+ T cells and AuNPs within the tumor.

4.4 T cell effect on CO-AuNPs biodistribution

We next performed a comprehensive in vivo biodistribution study using inductively coupled plasma mass spectrometry (ICP-MS) and ICP-OES to map the location of free PEGylated CO-AuNPs (40-45 nm gold colloid coated with 5000 MW PEG) and CO-AuNPs delivered by T cells. Prior to injection, ICP-OES was performed to determine the absolute gold dose for PEG-CO-AuNPs and CO-AuNP-T cells. Following intravenous injection with PEG-CO-AuNPs or CO-AuNP-T cells, tumors, and organs (bone, brain, heart, intestine, kidney, liver, lungs, muscle, plasma, and spleen) were harvested and analyzed for gold levels using ICP-MS. For PEG-CO-AuNP treated mice, organs were harvested at 4, 8, 24, and 48 h post-injection, while for CO-AuNP-T cell treated mice, organs were harvested at 24 and 48 h (Figure 4.4A). Predictably, the biodistribution of CO-AuNP-T cells is altered when compared to that of the PEG-CO-AuNPs. As observed
in previous studies [3,5], the highest percentages of CO-AuNPs using PEG coating were delivered to the liver and spleen (5.65 and 17.03%, respectively, at 48 h, Figure 4b,c). In comparison, T cells delivered CO-AuNPs to the lung, liver, and spleen, which received 4.76, 33.5, and 2.69% at 48 h, respectively (Figure 4.4B and 4.4C). The plasma half-life of the PEG-CO-AuNPs was calculated to be 6.05 h, and no gold was detected in the plasma for the CO-AuNP-T cell group, suggesting no significant CO-AuNP leakage from the T cells during in vivo migration. The CO-AuNP-T cell biodistribution over time correlates with the normal biodistribution of human T cells, suggesting that the presence of internalized CO-AuNPs does not significantly change the T cell biodistribution [32]. These data suggest that cellular delivery of CO-AuNP will result in a unique biodistribution pattern that is dependent on the cell type used for delivery. T cell delivery increases tumor accumulation of CO-AuNPs. Closer examination of LCL tumors following treatment with either PEG-CO-AuNPs or CO-AuNP-T cells showed an increase in CO-AuNP delivery to tumors following cellular transport. For PEG-CO-AuNPs, the highest level of accumulation in tumors was observed at 24 h post-injection, while peak tumor gold accumulation following T cell delivery was seen at 48 h. Using PEG-CO-AuNP, ICP-MS analysis of gold content of excised tumor tissue showed that 0.39 ± 0.33% of ID reached the tumor at 24 h. Whereas, using CO-AuNP-T cells, 1.55 ± 0.72% of the ID localized to the tumor at 48 h (P < 0.01) (Figure 4.5). This represents a four-fold increase in the efficiency of CO-AuNP delivery to the tumor site using T cells as vehicles.
Figure 4.4. Biodistribution comparison of AuNPs and AuNP-T cells in mice. Mice were injected with PEG-AuNPs (60-65 nm hydrodynamic diameter), AuNP-T cells, or PBS and subsequently sacrificed at various time points to determine biodistribution. PBS gold levels were negligible in comparison to AuNP and AuNP-T cell groups for all organs. Values are percentage of the injected gold dose (%ID) were calculated from ICPMS and are normalized for dry weight differences. The AuNP-T cell group exhibited significantly higher gold delivery to the lungs, liver, and bone, while the AuNP group demonstrated higher gold levels within the small intestine. No significant differences were seen in the spleen, kidney, muscle, or brain. An asterisk indicates statistically significant (P < 0.05) differences.
Figure 4.5. AuNP-T cells enhance the delivery of gold nanoparticles to the tumor site in vivo. Tumor-bearing mice were injected i.v. with PEG-AuNPs or AuNP-T cells. Tumors were subsequently resected at various time points and measured for AuNP content using ICP-MS. Values displayed represent the percentage of injected gold normalized for tumor dry weight differences (mean ± SEM). The percentage of gold delivered by the AuNP-T cells at 48 h represents a significant, four-fold increase over the PEG-AuNP group at 24 h (P < 0.01).

4.5 Results of T cells delivery approach

One of the biggest obstacles impeding use of nanostructures in clinical applications is effective in vivo delivery. Maximizing CO-AuNP accumulation at the tumor site has the potential to enhance photothermal cancer therapy, as well as other applications such as optical imaging. In this study, we showed that human T cells can be used to transport AuNPs to distant tumor sites following intravenous administration. Following short term incubation with AuNPs, T cells were efficiently loaded with over 14,000 CO-AuNPs per cell without affecting cell viability, proliferation, and cytokine production. Importantly, T cells loaded with CO-AuNPs retained their ability to migrate in vitro, and demonstrated tumor-specific homing in mice. Using T cells as a vehicle to deliver CO-AuNPs resulted
in a four-fold increase in the efficiency of AuNP tumor accumulation, demonstrating that active transport of AuNPs by cellular chaperones is superior to that of passive accumulation through the EPR effect. Our study conclusively demonstrated in vivo that internal loading of AuNPs in T cells can improve tumor localization, and thus may be a useful technology for a variety of nanoparticle based therapies.

We selected the size of our AuNPs for this proof-of-concept delivery study to optimize nanoparticle cellular uptake. We modulated the degree of nanoparticle internalization by altering the concentration of nanoparticles incubated with the T cells (Figure 4.1C). We also evaluated nanoparticle uptake using T cells isolated from three different human donors (Figure 4.1C) and saw only small variation, suggesting that this technique could be extrapolated to the T cells of any patient. The internalized CO-AuNPs used in this study also had no detrimental impact on the viability or function of activated human T cells in vitro (Figure 4.2), and the T cells were able to migrate to tumors in vivo while maintaining their AuNP payload (Figure 4.3). In addition to their ability to carry AuNPs to tumors, T cells can be selected for tumor-specificity for adoptive immunotherapy studies [18-20]. Furthermore, T cells may be genetically engineered to improve their function [21-22] or enhance their ability to migrate to tumors in vivo [23-24]. It has been demonstrated that systemically administered AuNPs tend to accumulate mainly in the perivascular regions of the tumor [20], limiting passive accumulation of nanoparticles by the EPR effect to well vascularized regions of the tumor. T cells may naturally localize to tumors, and tumor specific T cell clones have been demonstrated to penetrate into the hypoxic cores of the tumors in vivo [25]. The more extensive infiltration of tumor sites by antigen-specific T cells may permit enhanced penetration of
the tumor when compared to freely-injected nanoparticles, potentially augmenting therapeutic efficacy. The use of T cell vehicles also significantly affected nanoparticle biodistribution (Figure 4.4). Freely injected nanoparticles (40-45 nm gold colloidal nanospheres coated with 5000 MW PEG) accumulated most in well-vascularized organs such as the liver, spleen, kidney, and gut (Figure 4.4). Maximal AuNP tumor accumulation for the freely injected PEG-AuNP group is seen at 24 h (Figure 4.5). After 24 h, increased gold content for the PEG-CO-AuNP group was seen in the spleen, liver, and kidney with a corresponding decrease in gold content within the tumor and other organs, which represents a shift towards AuNP clearance. CO-AuNP-T cells presented a much different biodistribution from the systemically administered nanoparticles. After adoptive transfer of AuNP-T cells, a large percentage of the ID was seen within the liver and lungs at 24 h. T cells are known to accumulate within the liver and lungs after administration due to the vascularity and number of adhesion molecules present in these organs [26]. This pattern of T cell migration is consistent with the biodistribution of adoptively transferred T cells seen in previous studies [17, 26]. CO-AuNP-T cells are also seen accumulating in the spleen and bone of the mice; these locations are also normal reservoirs of T cells [27]. The large number of CO-AuNP-T cells seen in the liver likely represents apoptotic T cells. This large accumulation is not observed by bioluminescence imaging in Figure 4.3, and the liver is a known site where apoptotic T cells are entrapped [28]. Tumor accumulation of AuNP-T cells increases from 24 to 48 h as T cells escape from the lungs and migrate to the tumor (Figure 4.5). The biodistribution of AuNP-T cells matches the expected biodistribution of normal activated T cells, suggesting that AuNP biodistribution can be modulated based on the selection of
the cellular vehicle. In the case of T cells, it is possible that the biodistribution may be altered to further favor tumor accumulation and persistence by manipulating cell culture conditions [26] or by genetic modification of T cells [24]. Using T cells as cellular vehicles for AuNP delivery, we achieved a four-fold increase in tumor delivery efficiency at 48 h when compared to freely injected PEGcoated AuNPs at 24 h (Figure 4.5). This represents a significant increase in delivery efficiency (P < 0.01, Student’s t-test) using T cells. These results demonstrated for the first time that T cells can be used to enhance AuNP delivery to a tumor in vivo. The use of AuNPs and T cells together combines the photothermal therapy and imaging advantages of AuNPs with the immunotherapy and biodistribution advantages of T cells.

4.6 Summary

In this study we demonstrated the internalization of CO-AuNPs into activated human T cells for the delivery of nanoparticles in vivo. CO-AuNP uptake had no negative impact on T cell viability, proliferation, or immune function, and T cells were able to transport the AuNP payload to tumor sites in vivo. Furthermore, the use of T cells as a AuNP vehicle enhanced in vivo delivery efficiency by four-fold. This delivery method altered the biodistribution of AuNPs compared to freely injected AuNPs, and demonstrated that the selection of a particular cellular vehicle may dictate AuNP biodistribution.

4.7 References


Chapter 5: Au Islet Decorated Chalcogenide Structures for NIR and SWIR Optical Responses

Chapter 5 of this thesis discusses the use of chalcogenides as substrates for nanocomplexes. In particular, this chapter discusses the use of spherical metal-based chalcogenides as substrates for Au islet growth. We have designed a sub-30 nm diameter NP complex with optical properties in the NIR and SWIR. Our novel NIR and SWIR optically responsive Au isleted chalcogenide nanostructures are produced via a controlled growth method. Using discrete dipole approximation, we found that the optical properties of the nanoparticle complex depends strongly on the coupling effect of the Au islets and the refractive index of the chalcogenide substrate. TEM results also indicated that Au islets decorate the surface of the chalcogenide nanoparticles and do not form a complete shell. The work presented in chapter 5 will be published at a later date.

5.1 Chalcogenides as substrates

As previously mentioned in the introduction chapter of this thesis, chalcogenide based nanostructure complexes are of particular interest, since they offer the ability to combine linear and nonlinear optical responses or to design surface plasmon resonance effects in nonconventional wavelength ranges. Chemically, chalcogenides consist of at least one chalcogen ion and at least one more electropositive elements. Of particular interest are metal chalcogenides, compounds that consist of a metal and an element from the chalcogen group such as sulfides, selenides, and tellurides. Focus has recently been placed on chalcophilic elements such as Ag, Au, Cu, Ni, Co and Fe to form metal chalcogenide nanoparticles.
Metal-based chalcogenide nanostructured systems can exhibit strong optical responses ranging from the UV to the shortwave infrared (SWIR) region of the electromagnetic spectrum due to the extinction of the surface plasmons by an alternating electromagnetic field [1-2]. We are particularly interested in using metallic-based chalcogenides for biological applications so we have focused our attention on nanostructures with extinction maxima in near-infrared (NIR) and SWIR window in biological tissue, also known as the transparency window. The difficulty of tuning optically responsive nanostructures to fall within the transparency window of tissue (650 – 1300 nm) presents a challenge in the development of optical-based biological applications. Nanostructures that are responsive in the transparency window affords for applications in areas such as tissue imaging, diagnostics, and therapy. Furthermore, there is a pursuit to produce complexes with an overall diameter less than 50 nm that absorb in the NIR for biological applications [3]. Sub-50 nm NIR particles would be sufficiently small to enable longer circulation times in vivo, larger cargo capacity, allow for greater cellular uptake, and tumor penetration compared to larger NPs [4-6]. The design of the NP complex should also incorporate materials that allow it to remain chemically inert. Gold is of particular interest because it is chemically inert, affords for easy surface modification, and when combined with other materials can potentially lead to novel materials and nanostructures that allow optical responses in the NIR to shortwave infrared (SWIR).
5.2 Au islet chalcogenides as alternative NIR responsive NPs

Several existing Au-based NP complexes are tunable to the transparency window. AuNS are one such complex. AuNS are particles that consist of a spherical dielectric core coated with a concentric layer of Au. This technology has become ubiquitous in applications such as photothermal therapy, biological imaging, controlled drug release and biological sensing [7-11]. Traditional silica cored AuNS can be considerably large with diameters of more than 140nm [4]. Upon further surface modification for biocompatibility, these AuNS extend out to an overall diameter greater than 160 nm. While smaller AuNS systems, less than 120 nm but greater than 50 nm in diameter have been realized, these systems are strongly dependent on concentric shell formation, in which case an incomplete shell leads to particle instability and inability to tune the plasmon resonance. Here, we have designed a NP complex with optical properties independent on the formation of a metallic shell. Sub-30 nm Au chalcogenide cores, in the form of Au$_2$S, are decorated with Au islets which combination enabled optical responses extending from the visible to the SWIR region of the spectrum and a smaller particle size suitable for biological applications. Furthermore, the optical properties are not dependent on the formation of a metallic shell. This adds flexibility to the system design, offering many applications for biosensing, plasmon mediated optical response enhancement and phototherapy.

5.3 Chalcogenide synthesis and core tunability

Traditionally, Au chalcogenides are synthesized and coated with Au using a one or two step reaction of Na$_2$S with tetrachloroauric acid (HAuCl$_4$). A solution of 1mM of Na$_2$S is
typically mixed in a solution of 2mM of HAuCl₄ to create the Au₂S cores [2, 12]. An optional second addition of Na₂S is injected at a later time to reduce the remaining free Au ions to form the shell layer. The above-mentioned method produces byproducts in the final synthesis in the form of pure gold colloids and other pure gold particulate matter. It is also difficult to obtain a pure sample by separation of the Au coated Au₂S NPs from the Au impurities in the final synthesis due to similar size distributions[12]. Additionally, this process has the disadvantage of having a time dependent dual core/shell growth mechanism.

In order to manipulate the diverse properties of these materials it is necessary to control the interface, therefore being able to synthesize and tune the sizes of pure Au chalcogenides is imperative. To date, only a few methods have been established to synthesize pure uncapped Au chalcogenide cores. Szulczewski et al. used an aqueous mixture of Na₃Au(SO₃)₂ and Na₂S to produce particle sizes from ~3 to ~6 nm. However, byproducts of pure Au colloid were also produced during synthesis when the gold to sulfur ratio was stoichiometric [13]. Both Suzuki and Treguer [14-15] used H₂S gas to reduce KAu(CN)₂ to Au₂S yet both procedures were limited in their ability to tune the NP sizes. They also used ionizing radiation to reduce Au(CN)₂ onto the surface of the Au₂S NPs for shell coating [15]. This method resulted in a lack of control over shell growth and overall plasmon tunability. Augmenting and extending the methods used by Suzuki and Treguer, we developed a synthesis process that affords size tunability and controlled surface Au islet functionalization for optical responses in the NIR and SWIR.
In this work, we synthesized pure monodispersed chalcogenide cores by reducing KAu(CN)₂ with H₂S gas in a sodium citrate saturated aqueous environment. The size of Au₂S cores is tunable by altering the concentration of KAu(CN)₂, inlet H₂S gas-injection flow rate, and solution temperature similar to the CO gas reduction method we have reported for AuNP tunability [16]. Au₂S cores, synthesized with H₂S gas, with an average diameter ranging from 10 to ~90 nm were prepared as described below. A set of 100 mL KAu(CN)₂ solutions ranging from 0.1mM to 1M were used. Each of the 100 mL sample volumes were aerated at different flow rates regulated by a control valve. The solutions were aerated for a minimum of 30 minutes to ensure all of the KAu(CN)₂ was reduced. For monodispersed particle sets we observed a correlation between the KAu(CN)₂ concentration and H₂S gas inlet flow rate. For example, when the KAu(CN)₂ concentration was 0.2 mM the minimum and maximum gas injection flow rate to produce monodispersed particle sets was ~31 mL/min and ~50 mL/min respectively. The optimum flow rate that produced the most monodispersed particle set was determined to be ~37 mL/min. We hypothesize that the gas injection flow rate, with respect to reagent concentration, controls the nucleation rate and subsequently precursor concentrations that dictate further growth. In addition, the effect of stir speed was also examined, and it was found that the number of revolutions per minute (rpm) by which the solution was stirred played a negligible role in particle size and morphology. For the purpose of this discussion, the stir speed was maintained at 800 rpm. Solution temperature, however, played a critical role in particle size and morphology. The optimum temperatures to produce monodispersed chalcogenide nanoparticles fell between 65 and 75°C.
When the temperature was below 40°C the synthesis resulted in polydispersity and the polydispersity increased with a decrease in temperature. When the temperature was above 90°C, we noticed the synthesis took place faster with the solution turning dark brown in less than 6 minutes as opposed to light brown after 15 minutes as seen between 65 and 75°C. We concluded that the lower temperatures reduce the available energy for hydrolysis, resulting in slower condensation. This allowed the precursor concentration to continue to increase. Alternatively, the higher temperatures increased the speed of the reaction and caused particle aggregation. By varying the concentration of reagents and controlling the reduction rate monodispersed chalcogenide cores ranging from 10 nm to ~90 nm in diameter were realized. Figure 5.1A, B, and C demonstrates monodispersed chalcogenide nanoparticles, synthesized under varying precursor conditions, 11, 52 and 70 nm in diameter, respectively.

![Figure 5.1](image_url)

**Figure 5.1.** Transmission electron micrographs of pure Au chalcogenide nanoparticles 11 nm (A), 52 nm (B), and 70 nm (C) in diameter. The insets for (A), (B) and (C) are histograms showing the particle size distribution. Solid lines are best fits showing a Gaussian distribution profile.

The histogram inset for each subfigure displays the size distribution of the nanoparticles. Solid lines are best fits showing a Gaussian particle distribution profile. The H₂S gas
reduction method offered excellent core tunability over a broad range of sizes while maintaining a high level of monodispersity.

5.4 Chalcogenide purity and structural properties

When synthesizing the chalcogenide NP cores it is imperative that no gold byproducts are produced. The purity of the chalcogenide NPs were examined by spectral analysis and diffraction. Figure 5.2 displays the absorption spectra of synthesized Au chalcogenide nanoparticles, which is in good agreement with literature [15]. Note that no absorption from gold nanocrystals is observed, indicating that no Au colloidal byproducts were produced during synthesis.

![Figure 5.2](image1.png)

**Figure 5.2.** UV-Vis absorption spectra of as synthesized pure Au chalcogenide nanoparticles.

Figure 5.3 exhibits the diffraction pattern of the as synthesized pure Au chalcogenide sample with no byproducts. The rings correspond to plane spacing of cuprite-type Au₂S
crystal structure. To confirm the chalcogenide nanoparticles are covalent crystalline structures, X-ray photoelectron spectroscopy (XPS) measurements were conducted.

Figure 5.3. Electron diffraction pattern of nanoparticle sample. Sharp Bragg peaks correspond to various plane spacings of the Au chalcogenide complex, as labeled.

Au$_2$S (311)
Au$_2$S (222)
Au$_2$S (220)
Au$_2$S (111)
Au$_2$S (200)

Figure 5.4 shows the binding energy of the S(2p) region with a S(2p$_{3/2}$) peak at 162 eV. This value is in close agreement with other literature for covalent bonds between Au and S groups [14,15,17]. XPS measurements support that Au$_2$S crystalline structure consists of covalent bonds and has a similar cuprite type structure to $\alpha$-Ag$_2$S [15,17]. This proves that the chalcogenide nanoparticles are not formed by electrostatic aggregates of nuclei during growth after the nucleation phase and that nanoparticle formation is covalent in nature.
5.5 Au islet chalcogenide decoration

The controlled nucleation and growth of the Au islets onto the surface of the Au chalcogenide nanoparticles is achieved by using a precise dose of HAuCl₄ and hydroxylamine hydrochloride (NH₂OH HCl). NH₂OH is considered a “soft” reducing agent and has been shown to preferentially reduce HAuCl₄ onto existing Au structures as opposed to self-nucleation. This results in all of the HAuCl₄ in solution being reduced on the Au chalcogenide nanoparticles with no Au colloidal byproducts. Figure 5.5A illustrates the high-resolution transmission electron micrographs of Au isleted 27 nm Au chalcogenide nanoparticles. Figure 5.5B reveals the accumulation of Au onto the preferential chalcogenide facets and, in agreement with work by Treguer et al. in which they showed the growth of the Au islets always occurs according to epitaxial
relationships [15]. The formation of the Au islets on the surface of Au chalcogenide nanoparticles is shown to depend strongly on the concentrations of HAuCl₄ and NH₂OH.

Figure 5.5. (A) Transmission electron micrographs of ~27 nm Au isleted chalcogenide nanoparticles showing typical morphologies of chalcogenide nanoparticles decorated with Au islets. (B) High resolution transmission micrograph illustrating the epitaxial growth of Au islets on Au chalcogenide nanoparticles.

Increasing the amount of HAuCl₄ injected into the solution alters the growth dynamics and further grows the surface islets. Adding too much HAuCl₄ produced aggregates. The absorption spectra of these particles show a surface plasmon whose peak wavelength shifts to the red as the Au islets are formed and grow in size to encompass the Au chalcogenide surface, Figure 5.6.
Figure 5.6. UV-Vis extinction spectra of Au isleted chalcogenide nanoparticles as a function of HAuCl₄ injection concentration. “Control” represents no HAuCl₄ injection.

Figure 5.7 demonstrates the plasmon peak location and absorbance (inset) for ~27 nm Au chalcogenide nanoparticles as a function of increasing HAuCl₄ addition. As the HAuCl₄ concentration increases, the plasmon shifts towards lower energies. As the islets continue to grow and merge with one another on the surface a continuous shell is formed around the chalcogenide nanoparticles and the plasmon blue shifts towards higher energies.
5.6 Au islet modeling using DDA

To study the optical mechanics involved during Au surface formation we examined how the visible and NIR spectrum behave as a function of Au islet growth. Specifically, we focused our attention on the region where the islet growth is significant yet the shell formation still is incomplete. It is at this point where we observed the furthest red shift in plasmon location. We hypothesize that the Au islets on the surface begin to couple between nearest neighbor, as they grow larger. This coupling effect causes a shift in the plasmon from individual islet higher energy states to that of coupled lower energy states. While the optical properties of spherical particles have been extensively studied, to our knowledge the optical properties of Au islets on sub 50nm metal chalcogenide structures have never been investigated.

We modeled spectral dependence on Au islet morphology on the surface of the chalcogenide nanoparticles using DDA [18-21]. DDA is a useful method for describing
scattering and absorption from targets with arbitrary shape and a complex surrounding environment and has been described in chapter 2 of this thesis. When modeling the optical properties of the individual Au islets, the shapes of the individual islets are described as nanodisks or hemispheres. The study of the shape of the nanodisk and hemisphere is motivated by observations in TEM micrographs. The electromagnetic extinction of the Au islet-chalcogenide nanostructure is solved using a target array of point dipoles, \( j \), with polarizabilities, \( \alpha_j \), located at positions \( u_j \). Each dipole has a polarization, \( P_j \), which is determined from:

\[
P_j = \alpha_j E_j(u_j)
\]

where \( \alpha_j \) and \( u_j \) are the polarizability and location, respectively, of the \( j \)th dipole, and \( E_j \) is the local electric field \( E_{inc,j} = E_0 \exp(ikr_j-\omega t) \). The local field at each dipole is given by

\[
E_j = E_{inc,j} - \sum_{k \neq j} A_{jk} P_k
\]

where \(-A_{jk} P_k\) is the electric field including retardation effects at \( u_j \) that is due to dipole \( P_k \) at location \( u_j \). \( A_{jk} \) is a 3 x 3 matrix. We generate the system of equations

\[
E_{inc,j} = \sum_{k \neq j} A_{jk} P_k
\]

Once equation 5.3 has been solved for the unknown polarizations \( P_j \), the extinction cross section can be evaluated:

\[
C_{ext} = \frac{4\pi k}{|E_{inc}|^2} \sum_{j=1}^{N} Im(E_{inc,j} \cdot P_j)
\]

The core refractive index is taken to be 2.332 for AuS and the refractive index of the surrounding aqueous media is taken to be 1.33 [14]. The frequency dependent dielectric function of Au is taken from Johnson and Christy [22]. Since the Au chalcogenide
nanoparticle size is ~27 nm we chose individual islet nanodisk diameters between 2 and 8 nm. We assume that islet sizes larger than 8 nm in diameter would touch nearest neighbor and begin to merge on the surface to form complete shells. The height of each Au islet was taken to be between 1.5 and 4 nm. With islet diameters between 2 and 8 nm, the chalcogenide surface is taken as a planar structure with a refractive index of 2.332.

To model the Au islet decorated surface of the chalcogenide nanoparticle as observed via TEM, we varied the Au islet diameters from 2 to 8 nm in 1 nm increments while adjusting the spacing (d) in between the Au islets from 0 to 6 nm. Where spacing (d) is defined as the measured distance from the edge of one islet to the nearest edge of the adjacent islet. We also explored the effect the chalcogenide dielectric core refractive index had on the system. We simulated Au islet coupling in both a uniform surrounding media minus the chalcogenide substrate, as well as dual dielectric environment inclusive of the chalcogenide substrate. For the uniform environment model, we noticed that for d > 4 nm the individual Au islets produced optical responses similar to pure AuNPs and no coupling phenomena was observed. For d ≤ 4 nm, the two Au islets couple and reduce the plasmon frequency towards lower energies. In the dual dielectric environment, the Au islets were affixed on the surface of a substrate with a refractive index of 2.332. The substrate surface area was large compared to the Au islets such that the edges of the islets did not extend beyond the boundary of the substrate. The substrate thickness, representing increasing chalcogenide diameter, was varied from 1 to 60 nm. We observed that for substrate thicknesses varying between 1 and 3 nm, the change in thickness had a noticeable effect on the spectrum for fixed islet geometry and spacing. Varying substrate thickness from 10 to 60 nm had little to no effect on the optical properties of the system.
when the islet geometry and spacing remained constant. Since the minimum chalcogenide diameter for generating an NIR spectrum is 22 nm, the substrate thickness was held constant at 10 nm for the theoretical calculations described below. A larger red-shift was observed for the substrate-based system compared to the uniform surrounding media system. The larger red-shift can be attributed to the high refractive index mismatch between the surrounding media and the chalcogenide substrate which contributes to the greater shift of the plasmon into the NIR. Figure 5.8 shows a schematic of the Au islet decoration of the chalcogenide core. The polarization of the modeled Au islets is shown in the schematic. The enhancement is greatest in between the two islets as a result of islet coupling.

Figure 5.8. Schematic showing the Au islet decoration of the chalcogenide core.

Figure 5.9A and B show the location of the plasmon extinction maximum for a pair of Au islets as a function of geometry and islet spacing (nm) for both a uniform and substrate-based dielectric environment respectively. As seen in Figures 5.9A and B, when the Au
islet size is held constant and spacing decreases, the plasmon shifts towards the red. The plasmon also shifts as a function of Au islet geometry as indicated in Figures 5.9a and b.

**Figure 5.9.** (A) 3D plot of the plasmon absorption maximum wavelength as a function of islet spacing (nm) and diameter (nm) for uniform dielectric environment. (B) 3D plot of the plasmon absorption maximum wavelength in a dual dielectric environment inclusive of chalcogenide substrate.

As the Au islet diameter increases and the spacing is held constant, the plasmon red shifts towards longer wavelengths. For the dual dielectric system, the lower energy shift extends out towards \( \sim 1070 \) nm as the spacing between the islets approaches zero. For the uniform system, minus the substrate, the lower energy shift extends out only to \( \sim 680 \) nm.

The systematic calculations performed here tell us where to expect resonance for a coupled Au islet system. For a closely spaced system of islets, the extinction maximum are much more sensitive to changes in spacing than changes in islet dimensions. To confirm that the theoretical model correlates to the observed experimental results several TEM samples were analyzed. The Au isletted cores were grouped based on concentration of HAuCl\(_4\) added during Au islet growth. Upon examining the TEM samples and determining surface morphology each experimental sample set was characterized and matched with the corresponding theoretical model parameters. Figure 5.10 displays the experimental and theoretical plasmon peak locations based on decreasing islet spacing (x-
axis) and increasing islet size (y-axis) for a 10 nm substrate thickness (theoretical) and 22 nm diameter (experimental). The experimental and theoretical plots follow a similar curvature based on Au islet surface growth.

![Plasmon peak locations plot](image)

**Figure 5.10.** Plot of the experimental and theoretical plasmon peak locations based on islet spacing (x-axis) and size (y-axis). The experimental and theoretical plots follow a similar curvature based on Au islet surface growth.

The transitions between higher and lower energy optical systems during Au islet growth is further explained. When the islets are initially formed on the chalcogenide surface, the nearest neighbor is sufficiently distant to prevent particle coupling. As the spacing between islets decreases, the plasmon is shifted towards longer wavelengths and lower energies. Eventually, the islets merge on the surface of the chalcogenide nanoparticle to form a completed shell. As the shell thickness increases the nanoparticle complex starts to resemble a pure AuNP and the plasmon is significantly blue shifted to higher energies.
5.7 Optical dependence on chalcogenide refractive index

To further examine refractive index effects on the optical response of a coupled Au isleted system, we used a DDA theoretical model that varied the refractive index of the substrate while also varying the geometric parameters. Two 5 nm diameter Au islets were placed apart with spacings between the islets ranging from 0.5 to 4 nm on a 10 nm thick chalcogenide substrate. The refractive index of the substrate was varied from 2.3 to 4.2 for each islet spacing condition. Figure 5.11A shows linear fits to the plasmon peak position as a function of substrate refractive index in a complex dielectric environment. As the refractive index increases, the plasmon peak position red shifts to lower energies. The linear profile is maintained as the spacing between islets decreases. This observation is in agreement with results reported by others [23]. Other metal chalcogenide complexes were modeled to further examine the refractive index effect on optical response. Figure 5.11B shows DDA calculations of the plasmon peak position for two Au islets as a function of substrate refractive index for Au$_2$S, Ag$_2$S and CuS crystalline structures. It was found that metallic chalcogenide structures with higher refractive index further shifts the plasmon towards lower energies when all other geometrical parameters are held constant. This is in agreement with theoretical results shown in Figure 5.11A. Although these additional metal chalcogenide complexes produce NIR and SWIR plasmon resonances they do not serve as viable alternatives to Au isleted Au$_2$S. Based on our experimental results, it is difficult to synthesize suitable Ag$_2$S or CuS nanostructures for Au islet decoration.
Figure 5.11. DDA calculations of Au isleted substrates. (A) Plasmon peak position as a function of refractive index for islet spacings 0.5, 1, 1.5, 2, 3, and 4 nm. (B) Plasmon peak position for different metallic chalcogenides Au$_2$S, CuS and Ag$_2$S as a function of islet spacings 0.5, 1, 1.5, 2, 3, and 4 nm.

5.8 Summary

In this chapter we reported the synthesis of novel NIR and SWIR optically responsive Au isleted chalcogenide nanostructures via a controlled growth method. Our controlled growth method offered monodispersed core size tunability from 10 nm to ~ 90 nm in diameter. We designed a sub-30 nm diameter NP complex with optical properties in the NIR and SWIR. Furthermore, the optical properties are not dependent on the formation of a metallic shell. Using discrete dipole approximation, we found that the optical properties of the nanoparticle complex depends strongly on the coupling effect of the Au islets and the refractive index of the chalcogenide substrate. These results are consistent with previous studies of metallic nanoparticle coupling in other similar and alternative geometric configurations [21]. TEM results also indicated that Au islets decorate the surface of the chalcogenide nanoparticles and do not form a complete shell. This adds flexibility to the system design, offering many applications for biosensing, plasmon mediated optical response enhancement and phototherapy.
5.9 References


Chapter 6: Triple Theranostic Agents

Chapter 6 of this thesis introduces a novel nanoparticle complex design that serves as a photothermally active triple theranostic agent. The NP complex serves as a triple theranostic agent by affording detection, treatment, and conformational functionality. The NP complex structure consists of a plasmonically active AuNP core surrounded by quantum nanocrystals. The quantum nanocrystals are chalcogenic in nature yet differ from the chalcogenides substrates introduced in chapter 5. The chalcogenides used in this work are photoluminescent and serve as an intrinsic component in the nanoparticle complex theranostic capability. The AuNP core exist in the form of a HGN. The synthesis, optical properties and functionality of the HGNs will also be discussed. The work presented in chapter 6 was done in collaboration with Adam Y. Lin and will be published at a later date.

6.1 Case for a triple theranostic agent

As previously mentioned, metallic NPs have been used to combat cancer by use of image enhancement scattering mechanisms and plasmonically generated photothermal effects for ablation. Novel multifunction NP complexes have been recently developed that combine mesoscopic properties related to morphology and surface modification for a variety of biological applications. Some of the biggest difficulties in photothermal treatment or surgical removal of cancerous tissue are locating of satellite tumors, identification of tumor margins, and confirmation of complete tumor site ablation. A critical challenge in design revolves around the separation between diagnosis, treatment and subsequent imaging to confirm therapeutic effect. The ideal NP complex would be
capable of several tunable functions ranging from imaging to treatment to monitoring with accurate targeting of cancer specific cells. This ideal NP complex would serve as a multifunction theranostic agent. A NP complex capable of combining three phases of medical treatment, diagnosis, therapeutics and confirmation would greatly decrease time and improve efficacy of treatment. Here we report the design and application of a photothermally active photoluminescent nanoparticle complex for use as a multimodal theranostic agent. This novel NP complex would also serve as a confirmation agent.

6.2 Rational for the quantum nanocrystal HGN hybrid design

Our nanoparticle complex is deliberately designed to function in the optical regimes that maximize tissue transmissivity and minimize tissue absorption (~ 800 nm and 1100 nm) [1] as shown in Figure 6.1. The NP complex structure consists of a plasmonically active AuNP core surrounded by photoluminescent QNs in the form of PbS. The QNs are excited at the same wavelength used to activate the photothermal properties of the HGNs. Upon irradiation of the complex with a lower power 800 nm laser the QNs fluoresces, indentifying tumor sites of interest. Once tumors sites are indentified through PL of the QNs the optical laser power is increased to ablate the tissue. At ablative temperatures the intrinsic fluorescent properties of the QNs is altered and the fluorescent output significantly reduced.
Figure 6.1. Absorption spectra for oxygenated haemoglobin (HbO$_2$), deoxygenated haemoglobin (Hb), melanin, and water (H$_2$O) over wavelengths in NIR range. Diagram describes the optical functionality of the NP complex and absorption profile of tissue. The QNs are excited at the same wavelength used to photothermally activate the HGNs. Once excited, the QNs emit at 1100 nm avoiding the absorption peak of water located at 1000 nm. Upon further increase in laser power to ablate, the surrounding tissue temperature rise results in photoluminescent quenching of the QNs.

This QN fluorescence response is temperature dependent and a result of the plasmonic thermal response of the HGNs. The required ablative temperature of cancerous tissue (~44 to 60°C) is sufficient to significantly decrease the luminescence of the QNs. When the cancerous tissue is ablated there will be a visible decrease of the QN emission indicating successful ablation. This complex can serves as a "real time" identifier of satellite tumors and allow monitoring of cancerous tissue during photothermal therapy and/or surgical removal of cancerous tissue.
A schematic representing the fabrication procedure of the silica encased quantum nanocrystal-hollow gold nanoshell complex (SQN-HGN) is shown in Figure 6.2.

**Figure 6.2.** Schematic representing the fabrication steps for the silica encased quantum nanocrystals-hollow gold nanoshell complex.

The NP complex design is based on a ~40 to 45 nm diameter HGN tuned to 800 nm which falls within the transparency region of the skin ~700 to 1300 nm (Figure 6.1). Gold-based plasmonic NPs are a proven technology for use in photothermal applications and the HGNs serve as the photothermal component within the NP complex when excited at 800 nm. The surface for the HGNs is modified with a 7 to 13 nm silica shell. This silica layer serves as both a protective shell to reinforce the structural integrity of the HGNs upon laser irradiation and as a spacer layer between the HGN and QNs.

QNs have been used as tissue imaging agents and are ideal for experiments requiring long-term photostability and high quantum yields. PbS QNs are highly tunable between 950 – 1500 nm [2]. This broad tunability makes PbS QNs advantageous in applications where deep optical tissue penetration is of concern. The QNs used in this NP complex are tuned to 1100 nm wavelength to avoid the 1000 nm absorption line of water.
in tissue (Figure 6.1). QNs are covalently attached to the surface of the silica layer surrounding the HGNs. The thickness of the silica layer is tunable yet priority is given to the minimum thickness required to prevent fluorescence quenching of the QNs by the HGNs. Therefore the thickness of the silica spacer is tuned to a minimum of 7 nm in order to prevent fluorescence quenching by the HGNs. The plasmonic response of the HGNs and proximity of the QNs to the gold surface also serve to enhance the luminescent properties of the QNs. The luminescent enhancement is at a maximum at an optimum QN distance thus silica spacer layer thicknesses were varied from 2 to greater than 20 nm. After QN conjugation another 3 to 5 nm layer of silica is grown on top encapsulating the QDs/HGN complex. The final overall NP complex size was below <90 nm in diameter. The outer silica layer is easily functionalized with biological agents for diagnosis and targeting of cancerous tissue. The plasmonic response of the HGNs enables photothermal applications and the luminescent properties of the QNs enables diagnostic and imaging capabilities. By combining the NIR and photothermal properties of HGNs with the luminescent properties of NIR-based QNs we have developed a triple function theranostic NP complex for use as a diagnostic, treatment, and therapeutic confirming agent.

6.3 HGN synthesis and plasmonic properties

Hollow gold nanoshells (HGNs), tuned to the NIR, served as the core of the nanoparticles complex. HGNs were synthesized via a galvanic reaction in which gold tetrachloroauric acid (HAuCl₄) was reduced onto silver nanoparticles serving as sacrificial templates following previous procedures [3-5]. Briefly, in a typical synthesis an aqueous solution of
AgNO₃ and sodium citrate is heated and NaBH₄ is then added to the solution to initiate the nucleation and growth of Ag seed particles. To improve the Ag core monodispersity, the Ag seed was centrifuged at 11000 g for 3 hours. This centrifuge step removed the larger seed particles producing a very monodispersed Ag seed set. If this step is not included the final nanoparticle product would resemble the polydispersed HGNs with nonuniform shells as seen in reference [3]. The monodispersed Ag seed particles were further grown by the addition of NH₂OH and additional AgNO₃ injection. The gold/silver galvanic reaction was then initialized by the aliquot addition of 1% HAuCl₄ into the solution resulting in HGNs. Figure 6.3 shows a schematic of the HGN synthesis process for a HGN.

![Figure 6.3](image)

**Figure 6.3.** Schematic representing the galvanic replacement of the silver core with gold. As gold reduces to the surface of the silver core, silver atoms are exchanged with gold atoms that eventually leads to complete etching of the silver core.

This synthetic procedure uses the redox potential between silver and gold salt in solution. When Ag³⁺ ions come in contact with the silver atoms, there is an electroless plating that reduces the Au³⁺ ions to gold atoms and oxidizes the silver to Ag¹⁺ ions. For every 3 Ag atoms oxidized, a single Au atom is reduced [5]. This 3 to 1 atom replacement leads to a
gold shell hollow core structure. TEM images show the as synthesized HGNs (Figure 6.4).

![Figure 6.4. TEM image of HGNs synthesized via galvanic replacement exchange of silver and gold atoms.]

Varying the amount of gold injection controls the gold shell formation and subsequent growth. As the shell thickness increases the optical properties of the HGNs change. The optical properties of the HGNs were characterized using UV-vis-NIR spectroscopy. Figure 6.5 shows the absorbance spectra of aqueous HGNs as a function of gold addition.
Figure 6.5. UV-Vis-NIR extinction spectra of HGNs with varying amounts of gold salt addition.

The optical properties of the HGN structure can be understood by examination of plasmon hybridization theory. As discussed in chapter 2 of this thesis, the optical properties of Au nanostructures are determined by the collective oscillations of their conduction electrons with respect to the restoring force of the positive ion background. For solid AuNPs there exist a collective oscillation of conduction electrons in response to an external field (Figure 6.6).
When the NP under examination contains of a complex structure or morphology there is the potential of plasmons interacting with neighboring plasmons and surfaces of opposing structures leading to plasmon mixing and hybridization (Figure 6.6).

The general assumption of a uniform collective oscillation of conduction electrons across the surface of the HGN does not hold true as in the case of a solid AuNP. This plasmon mixing is analogous to the electron wave functions of simple atomic and molecular orbitals. This property has been termed plasmon hybridization and governs the optical properties on nanostructures with complex geometries.
Plasmon hybridization theory uses deconstruction methods to reduce complex nanostructures into more elementary components that interact with one another to produce the hybridized plasmon modes [6-9]. Upon examination of our HGN structure the optical properties are determined by the inner and outer radius of its metallic shell layer. The core-shell dependent optical properties result from the plasmon response from a sphere and that of a cavity.

Figure 6.8. Geometric description of HGN and energy level diagram depicting plasmon hybridization in HGNs. The plasmon hybridization is a result of sphere cavity coupling that splits the plasmon into an antisymmetric "antibonding" and symmetric "bonding" mode.

Figure 6.8 shows the plasmon hybridization model for our HGN. The cavity and sphere are electromagnetic excitations at the inner and outer interfaces of the HGN Au shell, respectively. When the sphere and cavity plasmons interact a splitting of the plasmon resonance occurs. The two new resonances consist of a higher energy antisymmetric plasmon ($\omega_-$) and a lower energy symmetric plasmon ($\omega_+$) (Figure 6.8). These two
energies are termed the antibonding and bonding modes respectively. Classical Mie
type theory predicts that the oscillation frequencies associated with the sphere and cavity are
\[
\omega_{sp} = \frac{\omega_B}{\sqrt{3}} \quad \text{and} \quad \omega_{cav} = \sqrt[3]{2} \omega_B
\]
respectively where \(\omega_B\) is the bulk plasmon frequency. A more detail derivation can be found here [8]. From examining Figure 6.8 one can intuitively determine that the surface charges couple the sphere and cavity modes and the resultant hybridization depends on the difference in energies and on the Au shell thickness. The hybridization picture of the HGN describes why there is a blue shift after the initial Au shell is formed and gold is further reduced to the surface (Figure 6.5). When the concentric shell is initially formed it is at its thinnest. This initial shell thickness dictates the coupling dynamics between the sphere and cavity plasmons which lead to plasmon splitting. For a thin shell, there is a strong coupling between the sphere and cavity that corresponds to the lower energy bonding mode. As the shell continues to grow and thicken weight is given to the higher energy antibonding mode reducing the coupling between the cavity and sphere and shifting the spectrum towards higher energies. For the final nanoparticle complex, HGNs tuned to an 808 nm wavelength were selected.

6.4 Silica coating of HGNs

Since the nanocrystals need to be placed at a minimum distance to prevent fluorescence quenching an initial effort was focused on uniformly coating HGNs with silica shells of varying thickness. The surfaces of the HGNs could be directly coated with conformal shells of amorphous silica using the sol-gel process previously reported [10]. In this process, the formation of silica coatings involved base-catalyzed hydrolysis of tetraethyl
orthosilicate (TEOS) in the presence of ammonia to generate silica sols, followed by nucleation and condensation of these sols onto the surfaces of the HGNs. Ammonia was added as a catalyst to speed up the hydrolysis of the TEOS precursor. Due to the surface charge of the HGNs the silica preferentially reduced to the surface of the gold shell yet silica byproducts were persistently generated during synthesis. To help facilitate the reduction of silica onto the surface of the HGN and reduce or eliminate the self nucleation and subsequent growth of pure silica nanoparticles in solution, various functional surface ligands were studied.

HGNs were incubated with various amounts of thiol or amine terminated ligands to study the effect of silica growth using different moieties. Varying concentrations of (3-mercaptpropyl) trimethoxysilane (MPTMS), 11-mercaptopoundecanoic acid (MUA), 6-mercaptohexanoic acid (MHA), and (3-Aminopropyl) triethoxysilane (APTES) were added to HGNs for surface functionalization. Data here is presented for MUA and MPTMS functionalized HGNs and subsequent silica growth. Each surface ligand was evaluated by attempting to tune the silica shell thickness from 2 to greater than 30 nm in thickness. Although each ligand species formed self assembled monolayers (SAMs) on the surface of the HGNs, the various surface moieties demonstrated different effects on the growth of the silica layer (Figure 6.9A-H).
It should be mentioned that great care was taken to ensure all excess thiols where removed from solution prior to silica growth. If an abundance of free thiols were present in solution during silica growth, there is a high chance that the thiols would have cross linked and served as silica nucleation sites leading to pure silica byproducts in solution. HGNs functionalized with MPTMS produced a uniform shell thickness at 1 nm growth. As the amount of necessary silica precursor increased to grow thicker silica layers, the surface of the MPTMS functionalized HGNs roughened. As the silica layer approached 5 nm in thickness the surface of the NP complex became jagged and non-uniform (Figure 6.9B). At 15 nm thickness large portions of the NP surface contained shoulders and long protrusions from the surface (Figure 6.9C). In contrast, MUA surface fictionalization produced relatively uniform silica surfaces throughout the growth process (Figure 6.9E-H). MUA did however lack the ability to produce uniform silica layers below 2 nm in
thickness where as MPTMS formed uniform silica layers at 1 nm (Figure 6.9A and E). MUA was chosen as the optimum ligand to help facilitate the controlled growth of the silica shell. Upon using MUA and by controlling experimental conditions, for example the coating time and the concentration of catalyst, water, or precursor, it was possible to uniformly vary the silica shell thickness from 2 to 40 nm. Since the silica functionalized HGNs are spherical in shape and relatively monodispersed they serve as an effective foundation for the precise placement of nanocrystals within the NP complex. The extinction spectra of the bare HGNs and the HGNs with a 7 nm silica are shown in Figure 6.10. Upon examining the spectra, it was observed that the addition of the silica layer had a small but noticeable effect on the optical properties of the HGNs. There is an observable shift in plasmon resonance from 808 to 814 nm upon addition of a 7 nm silica layer. The plasmon shift is related to the change in the local index of refraction at the HGN surface and is similar to shifts observed for other nanoparticle complexes in literature [11].
As previously mentioned, the silica layer not only serves as a spacer layer but also as a protective layer to reinforce the structural integrity of the HGN upon irradiation. Prevo et al. demonstrated that upon laser irradiation, HGNs maintained composition and original spectral profile. Yet, with increased total energy input, the HGNs underwent particle morphological changes losing resonance at 800 nm towards higher energy wavelengths and physically resembling colloidal gold [3]. The effect on structural integrity by adding a silica layer was studied by irradiating bare HGNs and HGNs encapsulated in a 7 nm thick silica layer. The particles were irradiated at different power densities for a maximum of 2 minutes in 1.5 mL volumes. After each irradiation the extinction spectra was taken and the solution visually checked to account for aggregates due to lose of particle stability. Figure 6.11 shows the thermal effects on bare and silica protected HGNs irradiated with an 808 nm NIR laser at a power density of ~ 2 MW/cm² for 1 minute. The irradiation powers used to demonstrate the protective functionality of a
thin silica layer are a lot larger than the irradiation power range used in vivo to ablate tissue ~ 1 to 80 W/cm².

As can be seen, the bare HGNs underwent a morphological change and lost resonance. The higher the irradiation energy the more the bare HGNs experienced a collapse of the hollow cores. At really high energies the bare HGN spectra resembled that of pure gold colloids indicating a total and complete collapse of the shell structure (Figure 6.12A and B). However, the silica encapsulated HGNs maintained structural integrity upon irradiation and demonstrated a persistent plasmon resonance peak position. This data shows that the HGN-silica construct would withstand the repeated irradiation at high power densities for photothermal applications.
6.5 Quantum nanocrystal synthesis and tuning

Under normal conditions PbS nanocrystals are not photoluminescent above a certain size regime. In order to generate PbS nanocrystals smaller than their Bohr radius (~18 nm) capping agents are implemented. Zhao et. al. demonstrated the synthesis of water soluble PbS nanocrystals stabilized with a mixture of thiols [2]. They used a combination of 1-Thioglycerol (TGL) and dithioglycerol (DTG) to produce nanocrystals possessing stable photoluminescence in the NIR spectral range of (1000-1400 nm). An alternative method to synthesize PbS nanocrystals using Zhao’s work as a template was explored. Since the nanocrystals would need to be attached to the surface of the silica layer, the use of alternative thiol and amine-based capping agents possessing an end terminated silanol functional group was investigated. The use of APTES and MPTMS as a capping agent was explored individually and in combination with DTG and TGL. Briefly, PbS nanocrystals were synthesized by adding Na₂S to an oxygen expelled aqueous solution of lead (II) acetate in the presence of capping agents. By adjusting the ratio of thiols and/or
amines to lead acetate used to synthesize the nanocrystals, the PbS size and emission spectrum can be tuned. Several ratios of TGL/DTG/MPTMS and TGL/DTG/APTES were used and the ratio dependent QN morphology and photoluminescent properties were studied.

The photoluminescent properties of the QNs were evaluated using a Jobin-Yvon Fluorolog-3 spectrometer equipped with a near-infrared photomultiplier tube. Two excitation sources and three irradiation wavelengths were used to independently excite the PbS NCs. Wavelength lines of 480 nm and 823 nm were isolated using a tungsten lamp as the excitation source. A 808 nm NIR laser was also used in a homemade setup to study the photoluminescent properties of the PbS NCs. It was concluded that APTES served as a poor capping agent producing large aggregates of nanocrystals with little to no photoluminescent properties. This poor result for APTES can be attributed to the low affinity of the amine group for the Pb-S lattice structure in comparison to the fast processing of solution precursors during crystal growth. Thiols on the other hand readily bonded to the Pb-S lattice structure of sufficient size after nucleation and condensation of solution precursors. However when MPTMS was used as the sole capping agent highly polydispersed PbS nanocrystals were produced with no observable photoluminescent properties in the 950 to 1500 nm wavelength range when excited at either 480 or 823 nm. When different concentrations of MPTMS was used in conjunction with DTG and no TGL, PbS nanocrystals were produced that presented weak photoluminescent outputs ranging from 1245 to 1255 nm wavelength. Yet, when MPTMS was used with TGL alone, a MPTMS/TGL ratio dependent spectra was produced with a strong photoluminescent output. As the ratio of MPTMS/TGL increased from 0 to 0.5 the PL
peak position shifted from 960 to 1100 nm. As the ratio continued to increase from 0.5 to 0.8 the peak position extended out to 1150 nm then blue shifted back towards 1000 nm. As the ratio approached 1 the PL intensity dramatically decreased and the peak position began to blue shift further towards 960 nm. There was also an observable change in PL intensity as a function of MPTMS/TGL ratio (Figure 6.13).

![Figure 6.13](image)

**Figure 6.13.** Quantum nanocrystal PL intensity and wavelength peak position as a function of MPTMS/TGL ratio.

When a ratio of MPTMS/TGL greater than 0.2 and less than 2 was used, PbS crystals were produced that possessed significantly higher PL intensities than the optimum ratio of DTG/TGL/Pb presented in [2]. Figure 6.14 shows the QN PL intensities as a function of MPTMS/TGL ratio with DTG concentration held constant.
It was also observed that replacing short-ligand TGL and DTG thiols (Figure 6.15) with longer-ligand MPTMS thiols altered the luminescent properties of the PbS nanocrystals and in some ratio cases increased the emission intensity.

**Figure 6.14.** Quantum nanocrystal PL intensity and wavelength peak position as a function of MPTMS/TGL ratio with DTG concentration held constant. A MPTMS/TGL ratio of 0.0 corresponds to the optimum ratio of TGL and DTG according to literature.

**Figure 6.15.** (A) Diagram of PbS QNs functionalized with DTG/TGL alone (left) and MPTMS/TGL alone (right). (A) Serves as a quality illustration of the surface dynamics of a shorter ligand (DTG) vs. a longer ligand (MPTMS) thiol molecule. (B) Bar graph shows the PL intensity difference when using MPTMS in place of DTG. The DTG/TGL ratio is the optimum ratio used to synthesize PbS QNs taken from literature.
A final MPTMS/TGL ratio 0.287 was selected as it produced PbS NCs with a peak position of 1100 nm and the largest PL intensity (Figure 6.16).

**Figure 6.16.** Normalized PL measurement of quantum nanocrystals displaying sharp peak width. MPTMS/TGL ratio 0.287 was used to tune PL output to 1100 nm wavelength.

### 6.6 Quantum nanocrystal-HGN complex formation

After the MPTMS capped PbS were synthesized, the PbS NCs were incubated with the silica coated HGNs (Figure 6.17). Silanol groups exposed on the surface of the PbS nanocrystals served as anchors to covalently affix the nanocrystals to the surface of the silica functionalized HGNs. The concentration of PbS NCs injected into the HGN solution was varied.
This was in an effort to find the optimum PbS NC concentration that would provide the most surface coverage while not generating large aggregates of HGN complexes. Figure 6.17B, E show the result of incubating a low dose and high dose concentration of PbS QNs with silica coated HGNs. Since the PbS NCs had exposed silanol groups it was possible for a single PbS NC to covalently bind more than one HGN complex resulting in large HGN complex aggregates. Several experimental conditions were implemented in order to control the binding rate of the NCs to the silica surface and prevent aggregation. It was found that a maximum of NC coverage could be achieved when the HGNs and PbS NCs were incubated in the presence of a low energy ultrasonic pulse (Figure 6.17E).
The remaining exposed silanol groups on the other side of the PbS nanocrystals, not attached to the silica shell, helped facilitate the subsequent preferential reduction of silica to the surface of the nanoparticles complex, forming the final protective layer (Figure 6.17C, F). During the growth of the additional protective silica layer some silica byproducts were produced and observable via TEM (Figure 6.17F). Yet after centrifugation the byproducts were successfully removed from the samples.

6.7 Quantum nanocrystal temperature effects

Since the QN-HGN complex is designed to have thermally sensitive photoluminescent properties the effect of temperature T on the optical properties of the PbS NCs is of great importance for the proposed theranostic application. The temperature effects were studied by first exposing MPTMS/TGL capped PbS nanocrystals in aqueous solution to various temperatures. This PbS QN study was conducted independent of the QN-HGN complex. Several solutions of PbS NCs were heated in a controlled environment using a homemade heating and temperature monitoring setup. The QNs were studied over an extended temperature range (26 to 90°C). The optical excitation was provided by a tungsten lamp with a selected line of 823 nm. The photoluminescent properties of the heated QNs in aqueous solution were evaluated using a Jobin-Yvon Fluorolog-3 spectrometer equipped with a near-infrared photomultiplier tube. Care was taken to ensure the solution cell holder did not act as a heat sink during optical measurements. Figure 6.18 shows the PL intensities of the NCs at various temperatures for independent samples. The inset illustrates the near linear decay PL profile as a function of temperature for temperatures 50 to 80°C which corresponds to ablation temperatures responsible for cell necrosis.
The PL intensity at 26°C is taken to be the maximum PL output. As the temperature approaches 50°C the PbS NC PL intensity decreases by 70%. At 70°C the PL intensity is less than 5% output with total thermal quenching occurring at 80°C.

After careful study it was determined that several mechanisms are involved in the QN thermal quenching phenomena [12-15]. Radiative decay rate differences along with thermal trapping of carriers to states outside the core of a QD are assumed to be the main reasons for the observed PL thermal quenching at increased temperatures. The above mentioned can be attributed to processes involved in the lowest 1S-1S exciton state, which is represented by the bright and dark states. During temperature increase of the PbS NQ lattice, there is a thermal escape of carriers during multi-state transitions. As the PbS lattice temperature increases there is a thermally activated transition between the emitted states separated by an energy gap, which is a result of the exciton dark-bright-state splitting [14]. This thermally activated transition induces nonradiative relaxation.
into trap states. Basically, in quantum nanocrystals, the escape of carriers reduces the recombination rate of radiating electron-hole pairs. The PL temperature response of the QNs make them adequately sufficient for use as temperature monitoring elements within the NP complex.

6.8 Quantum nanocrystal-HGN photothermal response

The PL and photothermal response of the entire NP complex system was evaluated by use of a CW NIR 808 nm laser, micro-thermal couple, and an augmented spectrometer detection scheme with NIR photomultiplier tube. A micro-thermal couple was used to monitor the temperature of the solutions during irradiation. Care was taken in the placement of the micro-thermal couple lead to ensure avoidance of the irradiation beam cylinder. Stable suspensions of QN-HGN complexes were synthesized and placed in quartz cuvettes and stabilized to 26°C prior to irradiation. QN-HGN complexes were irradiated at different power densities while monitoring the temperature and PL output of the complex. Additionally, individual solutions of bare HGNs and bare PbS QNs were irradiated at the various irradiation powers. The PL output intensity of the QN-HGN complex was taken at room temperature at an irradiation power density low enough as to not generate a photothermal response that would increase solution temperature. The QN-HGN complex solution was then irradiated at a power density of 20 W/cm^2 for 45 minutes, simulating an ablative environment. Figure 6.19 shows the measured temperature rise of a 1 mL suspension of QN-HGN complexes during irradiation. As expected, the initial heating rate is much larger than the rate after 15 minutes with the solution reaching a steady state temperature at 25 minutes. The solution temperature
reached the ablative temperature zone within 2 minutes. It was also observed that the initial heating rate increased with an increase in power density.

![Graph showing temperature over time](image)

**Figure 6.19.** Experimental calorimetric data showing the degree of heating of a 1mL volume of QN-HGN solution at a power density of 20 W/cm$^2$ for 45 minutes.

The QN-HGN complex was irradiated with a CW 808 nm NIR laser at 200 mW to determine the PL profile of the complex prior to thermal quenching. A relatively low laser output power of 200 mW was chosen to prevent plasmonic photothermal activation of the HGN cores. The solution temperature remained relatively unchanged when irradiate with 200 mW. The PL profile was measured and is shown in Figure 6.20 (solid black line). The sample was then irradiated at a power density 20 W/cm$^2$ for 5 minutes. The solution temperature reached 55°C within the 5 minute irradiation window. The PL output intensity for the QN-HGN complex before and after irradiation with a power density of 20 W/cm$^2$ is shown in Figure 6.20.
One could assume that the placement of the QNs within a 10 nm radial distance of the HGNs would result in rapid thermal quenching on a time scale much shorter than that of bare NCs in bulk, yet the observed temperature dependent PL decay followed that of bare NCs. This observation can be explained by examining the temperature distribution around the optically-stimulated complexes. To explicate the mechanism of the temperature rise, a simple calculation was conducted on the basis of the steady-state heat conduction from the nanoparticle to the surrounding media. The nanoscale silica layer and surrounding QNs were taken to be thermally transparent and assumed not to impede or contribute to the conduction of thermal energy into the surrounding media. Therefore, the thermal conduction was assumed to take place from the plasmonically active HGN at the complex center [16]. This approximation leads to the one-dimensional heat conduction equation as represented by equation 6.1 for a steady-state system.
\[ \frac{dT}{dr} = - \frac{Q_{\text{abs}}}{4\pi C} \frac{1}{r^2} \]  \hspace{1cm} 6.1

Here \( Q_{\text{abs}} \) is the photon energy absorbed by the HGN, \( C \) is the thermal conductivity of the surrounding medium, \( T \) is the temperature (\( K \)), and \( r \) is the distance from the center of the HGN. Do to the HGN size regime the temperature from the center to the surface of the HGN is considered constant. By implementing the following boundary conditions \( T = T_1 \) at \( r < r_1 \) and \( T = T(r) \) at \( r = \infty \), the temperature distribution is derived as eq 6.2.

\[
T(r) = T_1 = \frac{Q_{\text{abs}}}{4\pi r_1 C} + T_{\text{room}} \hspace{1cm} (0 \leq r \leq r_1)
\]

\[
= \frac{Q_{\text{abs}}}{4\pi r C} + T_{\text{room}} \hspace{1cm} (r_1 < r)
\]  \hspace{1cm} 6.2

The photon energy absorbed by the single HGN, \( Q_{\text{abs}} \), is represented by \( Q_{\text{abs}} = C_{\text{abs}} I \), where \( C_{\text{abs}} \) and \( I \) are the absorption cross-section of the particle and the laser fluence. The absorption cross section of the HGN was tabulated using MIE theory.

When the HGNs undergo optical excitation the laser electric field strongly drives mobile carriers within the metal lattice, which turns to heat. The heat generated by the plasmon diffuses away from the HGN and leads to an elevated temperature of the surrounding environment \([3, 17-19]\). Figure 6.21 shows the calculated temperature distribution around a single QN-HGN complex under photoexcitation at a laser fluence of 20W/cm\(^2\).
The results show a temperature change $\Delta T = 0.06$ at the surface of the HGN nanoshell. The temperature generated by the HGN decays exponentially with increasing distance from the center. The local increase in temperature at the surface of the HGN is very small. The overall solution temperature increase and resultant solution steady-state temperature of 62°C originates from the collective release of energy from many HGN heat sources. These results are similar to other research observations [20]. This result shows that there is no instantaneous quenching of the QNs due to the close proximity of the heat generating source and that the observed quenching is due to the elevated temperature of the total ablative environment.

### 6.9 Summary

In this chapter we introduced a novel triple theranostic NP agent. The NP complex structure consists of a plasmonically active AuNP core surrounded by photoluminescent
QNs in the form of PbS. The plasmonic response of the HGNs enables photothermal applications and the luminescent properties of the QNs enables diagnostic and imaging capabilities. Our nanoparticle complex is deliberately designed to function in the optical regimes that maximize tissue transmissivity and minimize tissue absorption (~ 800 nm and 1100 nm). The QNs are excited at the same wavelength used to activate the photothermal properties of the HGNs. Upon irradiation of the complex with a lower power 800 nm laser the QNs fluoresces, indentifying tumor sites of interest. Once tumors sites are indentified through PL of the QNs the optical laser power is increased to ablate the tissue. We have shown that at ablative temperatures the intrinsic fluorescent properties of the QNs is altered and the fluorescent output significantly reduced. The final overall NP complex size was below <90 nm in diameter. The outer silica layer is easily functionalized with biological agents for diagnosis and targeting of cancerous tissue. By combining the NIR and photothermal properties of HGNs with the luminescent properties of NIR-based QNs we have developed a triple function theranostic NP complex for use as a diagnostic, treatment, and therapeutic confirming agent.

6.10 References


Chapter 7: Magnetism\cite{1, 5, 6}

In order improve the theranostic ability of contrast agents we designed a dual function magnetic and plasmonic nanoparticle complex. Having discussed plasmonics in detail in previous chapters, it would be beneficial to first explore the basic concepts of magnetism before discussing the specific properties of our novel magnetic nanoparticle complex. Chapter 7 of this thesis serves as a brief introduction into magnetism and MRI principles. The bulk of the introduction was sourced from reference \cite{1}. For more detailed explanation and equation derivations please see reference \cite{1, 5, 6}.

7.1 Magnetism

Magnetic dipoles exist in magnetic materials and can be thought of as small quanta with north and south poles. Magnetic dipoles can be influenced by magnetic fields in a manner similar to the way in which electric dipoles are affected by electric fields \cite{1}. Within a magnetic field, the force of the field itself applies a torque that predisposed to orient the dipoles with the field. The magnetic behavior can be described in terms of multiple field vectors. The externally applied magnetic field is designated by $H$. The magnetic flux density, denoted by $B$, represents the magnitude of the internal field strength within the substance that is subjected to an $H$ field. The magnetic field strength and flux density are related according to $B = \mu H$. $\mu$ is called the permeability where $\mu_r = \mu / \mu_0$ and $\mu_r$ is the relative permeability. Another field quantity, $M$, called the magnetization of the solid, is defined by the expression

\[ B = \mu_0 H + \mu_0 M \]  \hspace{1cm} 7.1
In the presence of an H field, the magnetic moments within a material tend to become aligned with the field and the term $\mu_0 M$ is measured by this contribution. The magnitude of $M$ is proportional to the applied field as follows:

$$M = X_m H$$  \hspace{1cm} 7.2$$

where $X_m$ is the magnetic susceptibility. The magnetic susceptibility and the relative permeability are related as by the following

$$X_m = \mu - 1$$  \hspace{1cm} 7.3$$

The magnetic properties of materials are a consequence of the magnetic moments associated with individual electrons. Each electron in an atom has magnetic moments that originate from two sources. One is related to its orbital motion around the nucleus and the other from the electron spin, which can only be in an up or down state. Thus each electron in an atom may be thought of as being a small magnet having permanent orbital and spin magnetic moments. In each individual atom, orbital and spin moments of some electron pairs cancel each other. The net magnetic moment for an atom is the sum of the magnetic moments of each of the electrons taking into account moment cancellation. For some solid materials, each atom possesses a permanent dipole moment by virtue of incomplete cancellation of electron spin and/or orbital magnetic moments. In the absence of an external magnetic field, the orientations of these atomic magnetic moments are random. These atomic dipoles are free to rotate and paramagnetism results when they preferentially align, by rotation, with an external field [1].
7.2 The foundation of MRI: Nuclear magnetic resonance

Imaging of human internal organs with high spatial resolution is very important for medical diagnosis, treatment and follow-up [2-4]. MRI is an excellent technology as it offers the ability to achieve high temporal and spatial resolution in addition to its non-invasiveness. The foundation of MRI is nuclear magnetic resonance (NMR). NMR can be described as follows. When a sample is placed in a magnetic field and is subjected to radiofrequency (RF) radiation (energy) at the appropriate frequency, nuclei in the sample can absorb the energy. The frequency of the radiation necessary for absorption of energy depends on three things. First, it is characteristic of the type of nucleus. Second, the frequency depends on chemical environment of the nucleus. Third, the NMR frequency also depends on spatial location in the magnetic field if that field is not uniformly located everywhere [5]. The last variable provides the basis for MRI.

Nuclei have positive charges and many behave as though they were spinning. Therefore, a spinning nucleus acts as a tiny magnet which is called a nuclear spin. If this nuclear spin is subjected to a larger field, its orientation will no longer be random. The small nuclear magnet may spontaneously "flip" from one orientation to the other as the nucleus sits in the larger magnetic field. These orientations are called the low-energy and high-energy states. Yet, if the energy equal to the difference in energies (ΔE) of the two nuclear spin orientations is applied to the nucleus, much more flipping between energy levels occur [6]. The irradiation energy is in the RF range and is typically applied as a short pulse. The absorption of energy by the nuclear spins induces a voltage that can be detected by a MRI coil. Relaxation processes eventually return the spin system to thermal equilibrium, which occurs in the absences of an further perturbing RF pulses.
The energy required to induce flipping and obtain an NMR signal is just the energy difference between the two nuclear orientations and is shown to depend on the strength of the magnetic field $B_0$ in which the nucleus is placed. The fastest way to obtain an NMR signal is to detect the signal following a strong RF pulse applied at the resonance frequency. This signal is referred to as the free induction decay (FID) signal. An observable FID can be produced by using a rotating coordinate system (x, y, and z) in which x and y-axis are rotating about the z-axis at the NMR instruments operating frequency. The use of this coordinate systems enables consideration of the effect of applying an RF pulse $B_1$, along the x-axis and observing magnetization along the y-axis. Magnetization will be rotated in a plane perpendicular to the applied $B_1$ pulse with $B_1$ matching the appropriate frequency. The angle of rotation depends on the gyromagnetic ratio of the nucleus, the amplitude of $B_1$ of the RF pulse, and the length of time $t_w$ the RF pulse is applied: equation $\theta = \gamma B_1$ [6].

### 7.3 Magnetism and relaxation dynamics

Relaxation was already alluded to as the process by which a nuclear spin system returns to thermal equilibrium after absorption of RF energy. The time constants for the return to thermal equilibrium are $T_1$ for longitudinal magnetization ($z$-magnetization) and $T_2$ for the transverse magnetization ($x,y$-magnetization). $T_1$ is also called the spin lattice and $T_2$ is called the spin-spin relaxation time [6].

For relaxation to take place, internal forces must act on the individual spins. In solution, the main source for these forces are random fields caused but he random (Brownian) motion of the molecule. The forces resulting from these random fields are
rather weak compared to the force that is exerted on the spins by the external magnetic field. These weak forces lead to long relaxation times. For example, biological molecules in solution have T2 values in the tens of milliseconds and T1 values are on the order of seconds. Thermal equilibrium is mainly induced by internal interactions that fit within three categories: dipolar interactions, anisotropy of chemical shift (CSA), and electric quadrupolar interactions. The dipolar interaction is by far the largest contribution to relaxation for biological macromolecules and most important. The dipolar interaction will be considered the sole relaxation mechanism for the remainder of this discussion.

A magnetic nucleus with a gyromagnetic ratio $\gamma$ and spin $I$ is surrounded by a magnetic dipolar field $B_d$ which is given by

$$B_d = -\frac{\mu_0}{4\pi r^3} \left( I - 3 \frac{r(I \cdot r)}{r^2} \right)$$

The dipolar energy $H_d$ of a second nucleus with a gyromagnetic ratio $\gamma_2$ and spin $I_2$ in the field of the first nucleus with gyromagnetic ratio $\gamma_1$ and spin $I_1$ is then given as

$$H_d = -\mu_2 \cdot B_d = (\gamma_2 h I_2) \cdot \frac{\gamma_1 h \mu_0}{4\pi r^3} \left( I_1 - 3 \frac{r(I_1 \cdot r)}{r^2} \right)$$

$$= \frac{\gamma_1 \gamma_2 h^2 \mu_0}{4\pi r^3} \left( I_1 \cdot I_2 - 3 \frac{(I_1 \cdot r)(I_2 \cdot r)}{r^2} \right)$$

By examining the above equation we see that the dipolar field and energy has a distance vector $r$ dependency. If the two nuclei are part of the same molecule, then during thermal movement (Brownian motion) the direction of $r$ changes. If the molecule has isotropic orientation in solution then the average of the dipolar interaction energy over all angles vanishes. Yet the Brownian rotation leads to fluctuations in the dipolar field and interaction energy. These fluctuating fields can induce rotations of the spins and provide
a mechanism to exchange energy between spins-spins and spins-mechanical motion of the molecule. This leads to relaxation of the spins to their thermal equilibrium.

7.4 Relaxation and MRI signals

In order to understand how this applies to MRI contrast, it is important to understand how the relaxation time constants are generated and measured. When nuclei are exposed to an external RF energy source they realign in a high-energy state. As high-energy nuclei relax and realign they emit energy at material specific characteristic rates. T1-weighted MRI imaging employs an inversion recovery sequence. An initial 180° RF pulse inverts the spin populations. The spin system starts to relax towards equilibrium. After a time \( \tau \), a 90° pulse is applied that brings the residual longitudinal magnetization into the x-y or transverse plane where it can be detected by an RF coil and the relaxation time is acquired. T2-weighted MRI imaging relies upon local de-phasing of spins following the application of the transverse RF energy pulse. An initial 90° pulse turns the equilibrium magnetization into the xy-plane. The magnetization vectors from different nuclei then began to fan out and the signal decays. A 180° pulse is applied after time \( \tau \) which has the effect of rotating all the magnetization vectors about the y-axis. They continue to move in the same direction and after a further time \( \tau \) they are again in phase in the y-direction. Once they are aligned the signal in the receiver coil is maximal. The process of refocusing by the 180° pulse is repeated many times. The amplitudes of successive echoes decay exponentially and the true value of T2 can be found from the envelope of the echoes.
7.5 References


Chapter 8: Dual Magnetic and Plasmonic Theranostic Agent

Chapter 8 of this thesis deals with the design and implementation of a multimodal magnetic hollow gold nanoshell complex for enhanced magnetic resonance imaging and photothermal therapy applications. The magnetic properties of iron oxide are combined with the plasmonic properties of gold. While chapter 7 introduced magnetic theory and its use in magnetic resonance imaging, chapter 8 focuses on the implementation of magnetic material in the form of iron oxide nanoparticles (Fe$_3$O$_4$). We fabricated and tested our novel magnetic hollow gold nanoshell (magHGN) complex that incorporates small iron oxide nanoparticles in the hollow interior of the nanoshells. In Vivo studies prove the utility of these multimodal complexes as theranostic agents in MRI and photothermal applications. The work presented in chapter 8 was done in collaboration with Adam Y. Lin and will be published at a later date.

8.1 Multiple metallic materials for biological applications

In earlier chapters we discussed, in detail, that gold nanostructures possess an intense optical absorption attributed to the surface plasmon resonance phenomenon and serve as thermally stable heat transfer sources that can be utilized for photothermal therapy (PTT) [1-3]. Commonly used gold nanostructures for PTT include nanospheres, nanoshells, nanotubes, and nanorods [4]. These nanoparticles are tuned to absorb light in the NIR, which has minimum water and hemoglobin absorption and, therefore, has the deepest optical tissue penetration depth [5].

Another class of nanoparticles, iron oxide nanoparticles, have been widely used for T2 MRI for several biological applications including cancer, cardiovascular, and
neurological imaging [6-7]. IONP stability depends greatly on the surface coating [8]. Among the many surface modifications, coating the IONP with gold is one of the most promising designs [9]. Yet this approach limits the plasmon peak tunability of the complex (550 to 650 nm) when varying the gold shell thickness and IONP core diameter, rendering it unsuitable for PTT within the transparency window range of tissue. In order to produce a NIR resonance, several groups have introduced an additional silica spacer layer between the IONP core and gold shell to mimic traditional silica gold nanoshells [10]. However, structural dimensions (140 to 150 nm in diameter) which are not ideal for cell endocytosis and tumor accumulation [11-13], material composition, and difficult and time-consuming synthesis process of these NS serve as barriers for clinical applications.

8.2 Combining plasmonic and magnetic properties of nanostructures

In an effort to reduce the overall complex size and increase the magnetizability of each individual particle while maintaining a NIR resonance, we developed a NP design to combine the optical and thermal properties of hollow gold nanoshells (HGNs) with the magnetic resonance imaging (MRI) contrast capabilities of IONPs (Figure 8.1).

Different from the traditional silica-cored gold nanoshells, HGNs are readily synthesized by using a silver core as a sacrificial template and can be tuned to the desired plasmon wavelength by altering the thickness of the gold layer as detailed in chapter 6 of this thesis. With a hollow or partially hollow interior, which decreases the optical dielectric in comparison to silica cored NS, HGNs can be synthesized in the 20-60 nm size regime while still maintaining resonance in the NIR region. This size regime is optimal for cellular retention and tumor accumulation of nanoparticles [11-12]. The
smaller nanoparticle size provides higher absorption effects and greater thermal transduction efficiencies in comparison to larger nanoparticles such as silica-gold NS [14]. The synthesis process of the above mentioned HGN was used as a foundation for the design and synthesis of our dual plasmonic and magnetic nanoparticle complex.

Briefly, IONPs were conjugated onto silver cores using 3-mercaptopropyltrimethoxysilane (MPTMS). Then, a second layer of silver was formed over the IONPs. When gold salt was added to the silver complex, a thin gold layer was formed while etching away the silver in a similar fashion to traditional HGNs. This novel theranostic magnetic hollow gold nanoshell (magHGN) nanoparticle complex combines the optical and photothermal properties of HGNs with the magnetic properties of IONPs, for MRI contrast, while maintaining dimensions in the 40-80 nm size regime.

![Figure 8.1](image.png)

**Figure 8.1.** Magnetic hollow gold nanoshells synthesis schematic. A silver template core was coated with 3-mercaptopropyltrimethoxysilane (MPTMS) and conjugated with IONPs. A second layer of silver was reduced onto the complex with hydroxylamine. The silver was etched with the addition of gold salt and a gold shell was formed.
8.3 Advantage of a multi-magnetic NP design

The new magHGN complex design incorporated multiple IONPs, while maintaining the physical size similar to that of traditional HGNs (40 - 45 nm in diameter). Using multiple IONPs should cause an increase in magnetic susceptibility per particle complex by increasing the effective magnetic size when compared to particles containing a single IONP-core [6, 15-17]. To explain this phenomenon, we first investigated the size-dependent magnetization saturation of IONPs. The saturation magnetization values for IONPs of 10, 20 and 40 nm in diameter were obtained using a super-conducting quantum interference device (SQUID) magnetometer (Figure 8.2).

![Graph](image)

*Figure 8.2. Magnetization (M) vs. applied magnetic field (H) at 300 K obtained for different samples of Fe₃O₄ nanoparticles.*

As the IONP diameter increased from 10 to 40 nm the magnetization saturation value decreased. This result is in agreement with previous reports of similar magnetic responses as a function of IONP diameter [18-19].
The increase in magnetization with decrease in IONP diameter can be explained by exploring the crystal lattice structure of magnetite, which is Fe$_3$O$_4$ with an inverse spinel structure (Figure 8.3).

Figure 8.3. Schematic diagram showing the 1/4 unit cell of iron oxide (Fe$_3$O$_4$). Oxygen anions form a face-centered cubic crystalline system while Fe$^{3+}$ ions occupy the tetrahedral sites (left half) and both Fe$^{3+}$ and Fe$^{2+}$ ions occupy the octahedral sites (right half).

Previous reports have suggested that crystal lattice parameters for nanoparticles of some oxides and magnetic metals change as particle size decreases [20-21]. For Fe$_3$O$_4$, oxygen anions form a face-centered cubic crystalline system while Fe$^{3+}$ ions occupy the tetrahedral sites and both Fe$^{3+}$ and Fe$^{2+}$ ions occupy the octahedral sites (Figure 8.3). The spin moments of all the Fe$^{3+}$ ions in the octahedral position are aligned parallel to one another and opposite to the Fe$^{3+}$ ions located in the tetrahedral position (Figure 8.4).
This critical arrangement of the Fe\textsuperscript{3+} results in a cancelation of all Fe\textsuperscript{3+} spin moments and thus no Fe\textsuperscript{3+} ions contribute to the magnetization of the particles. Fe\textsuperscript{2+} ions, however, have all of their moments aligned in the same direction and are solely responsible for the net magnetization of the particles.

As the IONP size decreases, the surface to volume ratio increases which can increase the number of broken Fe\textsuperscript{2+} - O - Fe\textsuperscript{3+} bonds [18, 21]. This could give rise to an unpaired orbital at the surface, which in turn would increase the lattice constant to attain stability. Since the surface contribution increases with decreasing size, the overall lattice expansion also increases and therefore the unit cell volume increases with decreasing particle size. The increased unit cell volume could be attributed to an increase in Fe\textsuperscript{2+} content (Figure 8.3, right half). Since the saturation magnetization of a ferrimagnetic particle may be computed from the product of the net spin magnetic moment for each Fe\textsuperscript{2+} ion, an increased number of Fe\textsuperscript{2+} ions present in the particle would lead to a higher saturation value. Therefore, with the same amount of iron content, a collective of smaller
IONPs would have higher magnetization saturation value than a single larger IONP. This can provide a dramatic increase of the relaxivity value ($R_2$) and greatly enhanced the contrast in T2-weighted images. [6, 8]

Another advantage of using the multi-IONP approach is the improved spin-spin relaxation process of protons in the water molecules surrounding and within the NP complex. Under MRI, contrast comes from the signal difference between water molecules residing in different environments that are under the effect of IONPs. A grouping of smaller IONPs have a much higher combined surface to volume ratio than a larger single IONP core possessing the same amount of overall iron content. In addition, the magHGN design facilitates rapid exchange and diffusion of water molecules between the bulk phase and the adjacent layer surrounding the multi-IONP inner complex. As a result, these multi-IONP complexes gave rise to a further shortening of the T2 relaxation times.

8.4 **BEM modeling of magHGN complex**

A critical consideration when choosing the optimal IONP diameter to incorporate into the magHGN design was the IONP size contribution to the overall size of the complex. The boundary element method (BEM) was employed to theoretically study the quasi-static size regime [22-23]. Based on BEM results, if the IONPs were sufficiently small they could be incorporated into the matrix of a HGN while maintaining NIR resonance with the overall particle complex in the 60 to 70 nm size regime (Figure 8.5).
In contrast, if the IONPs were large in diameter the overall magHGN complex diameter would have to significantly increase in order to obtain an NIR resonance. Consequently, sub-10 nm IONPs in the range of 6 to 8 nm in diameter were chosen to optimize the magHGN particle design based on the aforementioned magnetic size effect studies and theoretical results.

Several sub-10 nm IONPs were embedded in the hollow section of the HGNs in accordance with the synthesis mentioned in Figure 8.1. The hysteresis loop obtained for the nanoparticle complex measured at room temperature demonstrated superparamagnetic properties (Figure 8.6).
The saturation magnetism was 59.45 emu/g with a coercivity value of 165 Oe. The non-zero coercivity value of the magHGNs can be attributed to the IONPs operating in the single domain region along with surface interactions. As previously mentioned, there is a relative increase in surface to volume ratio with the decrease in particle size. Due to the large number of broken bonds and surface stress the surface anisotropy term could become large. Furthermore, in smaller IONPs in the single domain region, there is a strong interaction between the core and surface. These interactions cause an overall increase of anisotropy resulting in an increase in coercivity [21]. The resultant data indicated that the magHGN complex exhibited favorable magnetic properties, making them suitable for use as MRI contrast agents in biomedical applications.
In addition to providing MRI contrast, the functional magnetic properties of the magHGN complex allowed them to be manipulated with smaller external magnetic fields and used in magnetic separation devices. Magnets (1T) were able to isolate the magHGNs and move them to the side of the cuvette (Figure 8.7). Therefore, magHGNs can be used with standard magnetic cell sorting devices such as MACS (Miltenyi Biotec, CA).

### 8.5 Synthesis of magHGN complex

Silver seed particles were first synthesized and subsequently grown to larger cores sizes with the addition of hydroxylamine (NH$_2$OH) and silver nitrate (AgNO$_3$) [24]. The diameter of the Ag cores was ~35 nm as measured by TEM. After synthesis of the Ag core particles MPTMS dissolved in water with 1% Tween 20 was injected into the solution. The MPTMS formed a self-assembled monolayer on the Ag cores by Ag-S dative bonds and is illustrated in Figure 8.8.
Once excess MPTMS was removed, IONPs that were first washed with tetramethylammonium hydroxide solution were added to the MPTMS functionalized Ag cores. The IONPs formed stable covalent bonds to the silane groups on the MPTMS-Ag cores under heated conditions (50-55°C) after 24 hours. Next, the IONPs-Ag complexes were collected via centrifugation steps to separate excess IONPs from the IONPs-Ag complexes. As illustrated in the TEM images (Figure 8.9A and 8.9B), small IONPs decorate the surface of the Ag cores showing successful conjugation.
Energy dispersive spectroscopy (EDS) was used to confirm the presence of IONPs on the surface of the Ag cores. EDS data (Figure 8.10) obtained by scanning the green region highlighted in Figure 8.9A shows the presence of Si, O and Fe atoms. The presence of Si can be attributed to the silane groups present in MPTMS.

The O signal is attributed both to the presence of MPTMS and IONPs on the surface of the Ag cores. To further confirm the conjugation of IONPs to the surface of the Ag cores.
EDS measurements (Figure 8.11) was carried out on the extremities of the Ag cores as indicated by the red highlighted box in Figure 8.9A. The resultant EDS spectrum shows the presence of Fe and lack of Si atoms. This confirms the aforementioned synthesis and conjugation steps.

![Figure 8.11. EDS spectrum of IONP-Ag core complex region highlighted by the red box in "Figure 8.9A".](image)

Next, NH₂OH and AgNO₃ were used to grow a second layer of silver around the IONPs; thus, encapsulating the IONPs in a larger silver structure. Various amounts of gold salt (25mM) were then added to the complexes to form magHGNs. Similar to normal HGN synthesis, the process of adding gold salt to the Ag complex solution initiated a galvanic exchange between gold and silver, etching the silver while depositing gold onto the surface. Figure 8.12 shows the formation of a gold shell encompassing the IONP-Ag core complex. The space between the gold shell and the core suggested successful etching of the second layer of silver. The patchiness of the core implied that the etching reached the IONP layer.
A range of 0.25-1.64 mM HAuCl₄ was added to the Ag-IONPs-Ag complex solutions. The absorbance spectra of the final magHGN particles were obtained with an UV-Vis-NIR spectrophotometer (Figure 8.13). The plasmon peak position red-shifted as a function of Au salt addition. There is also an increase in absorbance value as a function of Au salt addition (Figure 8.14).
Figure 8.13. UV-vis-NIR spectrum of gold salt titrated (0.25-1.64 mM HAuCl₄) Ag-IONP-Ag complexes. As the Au-Ag galvanic reaction proceeds the gold shell forms and shifts the spectrum towards the NIR.

Figure 8.14. Plot of the magHGN plasmon peak position and absorbance intensity as a function of gold salt concentration. The plot follows a linear progression and is in line with what others observed during the growth and formation of a gold shell.
The spectra shown in Figure 8.13 are broad and represent a polydisperse particle set. The magHGN particles synthesized with 1.640 mM Au salt produced a large peak width. Yet after washing via centrifugation the spectral peak width narrowed representing a monodispersed and removal of smaller non-NIR resonant particles (Figure 8.15).

![Graph showing UV-vis-NIR spectrum of magHGNs after washing and centrifugation.](image)

**Figure 8.15.** UV-vis-NIR spectrum of magHGNs after washing and centrifugation. The washing steps reduces the overall polydispersity of the sample concentrating the nanoparticles to a particular size regime that in turn narrows the peak width.

Further addition of gold contributed to a thicker and more complete gold layer (Figure 8.16), which can electronically screened the inner core complex.
Figure 8.16. TEM micrograph of magHGNs between 60-70 nm diameters. The inset shows the electronic screening effect on the core as the gold shell increases in thickness. Au salt concentration of 1.64 mM was used for this particular magHGN set.

Figure 8.17. Normalized experimental absorbance spectra of as synthesized magHGNs with corresponding theoretical BEM spectrum. The experimental peak is broader mostly due to particle size distributions and defect driven surface phase retardation effects with a smaller contribution from the latter.

The optimum Au salt concentration was found to be 1.3 mM, at which the resultant magHGNs measured approximately 60 nm in diameter and had a peak absorbance at ∼800 nm. The experimental spectra for the magHGNs synthesized with 1.3 mM Au salt
matched theoretical results fitted to the same physical size layer parameters (Figure 8.17). The peak corresponding to the experimental spectra is broader than the theoretical spectra due to particle size distribution and defect generated phase retardation effects not accounted for in the theoretical model.

### 8.6 MRI capabilities of magHGN complex

After confirming successful synthesis of sub-100 nm NIR resonating magHGNs, we then evaluated magHGNs’ capabilities to reduce T2 relaxation time in tissue phantoms and in vivo melanoma tumors. For better solubility of the magHGNs and to protect the gold surface, thiolated methyl polyethylene glycol (PEG) chains (MW 5000 Da) were self-assembled onto the magHGN surface. To calculate the relaxivity (R₂), magHGNs were encapsulated in phantoms (Figure 8.18) and the T2 relaxation time was measured with a MRI. Multi Slice Multi Echo images were acquired by using a 7.0T, Bruker Pharmascan, 22-mm to center-bore horizontal scanner.

![Figure 8.18. MRI image of magHGNs encapsulated in phantom tissue at a concentration of $10^{11}$ and $10^8$ particles per mL.](image)
The relaxation time vs. particle concentration for the magHGNs in phantom tissue is shown in Figure 8.19. The T2 relaxation curve was created utilizing Paravison 4.0 software (Bruker, Billerica, MA).

![Figure 8.19. MRI T2 relaxation time vs. magHGN particle concentration in phantom tissue.](image)

The slope of the linear regression of 1/T2 relaxation time (s⁻¹) versus the amount of contrast agent used (mM of Fe) represented the R² values of magHGNs (Figure 8.20). The magHGNs had a two-phase linear regression similar to that of IONP-silica-gold nanoshells complexes [10]. At lower concentrations (2 x 10⁹ – 2 x 10¹⁰ particles/ml), the R² was calculated to be 2203.2 mM⁻¹s⁻¹ and at higher concentrations (2 x 10¹⁰ – 10¹¹ particles/ml), the R² was 548.1 mM⁻¹s⁻¹. The overall relaxivity of magHGNs was 853.6 mM⁻¹s⁻¹, which was higher than the IONP-silica-gold nanoshells (11.4-396 mM⁻¹s⁻¹) and the clinically used ferucarbotran (185.8 mM⁻¹s⁻¹) [cite]. The substantially increased relaxivity of our magHGNs vs. other competing technologies can be attributed to the ability of the magHGNs to increases the relaxation of the surrounding water proton spins.
Because of the porous outer shell of the magHGN complex, there is an increase in water molecule exposure to the inner sphere magnetic complex.

Figure 8.20. R2 relaxation rate diagram showing the slope of the linear regression of 1/T2 relaxation time (s\(^{-1}\)) versus the amount of contrast agent used (mM of Fe). The inset shows the concentration dependent two-phase linear regression.

For a more clinically relevant model, C57BL/6 mice were implanted subcutaneously with B16-F10 melanoma tumors and injected with or without magHGNs intravenously (IV) (10\(^{12}\) particles in 200 μl). The MRI image of the tumor bearing mice showed a visibly darker tumor when treated with magHGNs (Figure 8.21A). The T2 relaxation time of the magHGN untreated tumors were 53.5 ± 1.4 ms (Figure 8.21B) and the IV magHGNs treated group had tumor T2 relaxation time of 29.4 ± 4.9 ms, which was similar to values reported by Larson and colleagues who injected 10\(^{13}\) 40 nm PEGylated-IONP intravenously (10 times more particles vs. the concentration used in this study) [10]. Overall, these results show that magHGNs are suitable T2 MRI contrast agents.
Figure 8.21. (A) MRI image showing tumor bearing mice untreated and treated with magHGNs. Tumor location is represented by the red "T". (B) Bar graph showing the T2 relaxation times of the treated and untreated tumors. 53.5 ± 1.4 ms and 29.4 ± 4.9 ms respectively.

8.7 PTT capability of magHGN complex

Next, we examined the photothermal properties of the magHGNs. B16-F10 melanoma tumors were again implanted on the right flank in C57BL/6 mice (Figure 8.22B).

Figure 8.22. MRI images of B16-F10 melanoma tumor bearing mice. (A) Mouse injected with BPS. (B) Mouse intravenously injected with magHGNs.

The mice were randomly assigned to three different groups: light only control group, intravenous (IV) injection of magHGNs (10^{12} particles in 100 μl) group, and intratumor
(IT) injection of magHGNs \((10^{11} \text{ particles in } 10 \mu \text{l})\) group. Tumors in all groups were exposed to a continuous 808nm laser at \(4\text{W/cm}^2\) for 30 seconds. Over time, tumor growth in the light only control group was not affected by the laser exposure while the IV magHGN group had stunted tumor growth \((p < 0.05 \text{ by day 15})\), and the IT magHGN group had complete elimination of the tumor (Figure 8.23).

![Figure 8.23](image.png)

**Figure 8.23.** (A) Tumor growth measured in days post implantation. The arrow denoted the PTT treatment with intravenous or intratumor injections of magHGNs. \((* \ p < 0.05 \text{ in comparison to the control})\). (B) Survival percentage of the light only control group and the IV magHGNs PTT group measured in days after PTT treatment.

We noticed that the IV magHGN PTT treated tumors had the cratered centers and the edges were the only region that continued to grow outwards (Figure 8.24). Traditionally, tumor area was measured on a two-dimensional plane, longitudinal and width; however, the tumor depth in the IV magHGN group did not increase proportionally to the 2D area, which was not reflected in the results. Therefore, the treatment results were more substantial than that shown in Figure 8.23A. Furthermore, the IV magHGN PTT treated group had significantly improved survival compared to the light only control group \((p <\)
Therefore, from the results in study, we conclude that the magHGN particles cannot only be used as MRI T2 contrast agents, but also as effective PTT agents.

![Example of donut shaped tumors after photothermal therapy with intravenous injections of magHGNs (day 21).](image)

**Figure 8.24.** Example of donut shaped tumors after photothermal therapy with intravenous injections of magHGNs (day 21).

### 8.8 Summary

In this chapter we presented the design of a novel multimodal theranostic agent. We fabricated and tested a novel magnetic hollow gold nanoshell complexes that incorporates small IONPs in the hollow interior of the nanoshells. This design has two advantages. First, it incorporates several smaller IONP instead using a single larger IONP. The combined effect of the smaller IONPs improved the overall magnetic properties of the design and thus improved the particle’s capability as a MRI contrast agent. Second, the overall magHGN complex could be synthesized in the range of 60-80 nm in diameter while still having a plasmonic peak in the near infrared (NIR) region of the spectrum.
Tunability in the NIR region allows for use as a plasmonically driven photothermal agent. Several factors were optimized in the magnetic HGN synthesis process increases stability and magnetic capability. Our magHGNs complex performed well as MRI T2 contrast agent and were able to debulk tumors and improve survival with PTT treated mice.

8.9 References


Chapter 9: Summary

In the previous chapters, new synthesis methods, nanostructure complex designs and therapeutic approaches were presented, which will be briefly summarized here.

In chapter 3 we fabricated AuNPs in the form of solid gold nano-cores by using carbon monoxide gas as a reducing agent. The size and monodispersity of the AuNPs were tunable by controlling variables such as HAuCl₄ concentration and gas flow during synthesis. The CO reduction method offered excellent tunability over a broad range of sizes while maintaining a high level of monodispersity. Chapter 3 also served as brief introduction into some of the mechanisms involved in nanoparticle formation and illustrates the intricacies and sensitivity of NP formation to the synthesis environment. In chapter 4 we discussed a new method to delivery therapeutic agents in vivo. We showed that the use of AuNPs and T cells together combines the photothermal therapy and imaging advantages of AuNPs with the immunotherapy and biodistribution advantages of T cells leading to a novel theranostic approach. AuNP loading of T cells had no adverse affect on T cell function and viability.

In chapter 5 we used chalcogenides as substrates for nanocomplexes. We were able to use spherical metal-based chalcogenides as substrates for Au islet growth. The au islet hybridization effects caused near-infrared and short wave infrared optical responses. In chapter 6 we exploited the photoluminescent properties of chalcogenides, in the form of quantum nanocrystals, by combing them with the plasmonic capabilities of HGNs. In essence, we created a novel photoluminescent and thermally responsive plasmonic nanoparticle complex that serves as an active triple theranostic agent. The NP complex
serves as a triple theranostic agent by affording detection, treatment, and conformational functionality.

In chapter 8 we further expanded our use of complexed materials by combining magnetic materials with optically responsive materials. We designed and implemented a multimodal magnetic hollow gold nanoshell complex for enhanced magnetic resonance imaging and photothermal therapy applications. Our magHGNs complex performed well as MRI T2 contrast agent and were able to debulk tumors and improve survival with PTT treated mice.

A critical challenge in design of a theranostic agent revolves around the separation between diagnosis, treatment and subsequent imaging to confirm therapeutic effect. We have explored and combined the functionality of unique nanostructures and materials to develop new theranostic agents. These theranostic agents could address the many challenges associated with diagnosis, treatment and confirmation.