Optical and Electrochemical Properties of Monolayer Protected Gold Clusters Modified with Fluorophores

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OPTICAL AND ELECTROCHEMICAL PROPERTIES OF MONOLAYER PROTECTED
GOLD CLUSTERS MODIFIED WITH FLUOROPHORES

by

Mary Sajini Devadas

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Mary Sajini Devadas

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OPTICAL AND ELECTROCHEMICAL PROPERTIES OF MONOLAYER PROTECTED GOLD CLUSTERS MODIFIED WITH FLUOROPHORES

Mary Sajini Devadas, Ph.D.
Western Michigan University, 2012

My research focused on the synthesis, characterization and the investigation of the optical and electrochemical properties of monolayer-protected quantum-sized gold clusters. Significant research attention was focused on the solution phase optical and electrochemical properties of these clusters. Highly monodisperse Au clusters with different types of ligands were successfully synthesized with sizes varying from 1 nm to 13 nm. These preformed gold clusters were modified with fluorophores and pseudo-rotaxanes for developing better nonlinear optical materials for sensing and biological imaging purposes. We have tried to focus on the interaction of the outer ligand shell, made up of \(-\text{S-Au-S-Au-S}\) bonds, with the core gold states, which alludes to changes in the optical and electrochemical footprint of these AuMPCs.

Ultrafast luminescence investigations on \(\text{Au}_{25}L_{18}\) clusters with two ligands glutathione (GS) and hexanethiol (\(C_6S\)) have revealed the nature of the higher excited states in these clusters. The growth of luminescence is independent of the ligands and is ascribed to the luminescence from the \(\text{Au}_{13}\) core states and the decay dynamics is mainly governed by its relaxation to \(\text{S-Au-S-Au-S}\) semi ring states. Temperature-dependent electronic absorption properties of the \(\text{Au}_{25}L_{18}\) and \(\text{Au}_{38}L_{24}\) clusters have been studied for the first time and are explained on the basis of electron phonon interactions.

We used pyrene and coumarin analogs as chromophores labels. It is unambiguously shown that \(\text{Au}_{25}\) clusters can work as electron donors with electrochemical and time-resolved fluorescence
upconversion and transient absorption measurements for the first time to understand the excited state dynamics. Coumarin labeled MPC was a selective turn-on fluorescent sensor for DiethylChloroPhosphate, a nevre gas mimic, in both one- and two-photon excited fluorescence. We have shown that 7-mercapto-4-methylcoumarin was used as a passivating ligand successfully and this process can be followed by common spectroscopic techniques. Two-photon measurements prove its further use as nanoparticle beacons and in the development of non-linear optical material for biological imaging. Irrespective of the size of the AuMPC, or nature of the passivating layer, electron transfer seems to be taking place, hampering the threading of the electron-rich crown ether wheel component to form a pseudo-rotaxane.
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CHAPTER I
INTRODUCTION TO QUANTUM SIZED GOLD CLUSTERS

1.1. Surge in nanochemistry research

Semiconductor and metal clusters in the nanometer [1 nanometer = 0.000000001 meter or 1/100,000,000th of a meter] size regime display many interesting optical, electronic and chemical properties due to quantum size effect.\textsuperscript{1-5} Over the last decade there has been increased interest in “nanochemistry”. A variety of supramolecular ensembles, multifunctional super molecules, carbon nanotubes, and metal and semiconductor nanoparticles have been synthesized and proposed as potential building blocks for optical and electronic devices.\textsuperscript{6-9} Metal particles are particularly interesting nanoscale systems because of the ease with which they can be synthesized and tuned chemically as per the needed application. In the 1990s, the advent of improved synthetic methods for metal nanoparticles was augmented by a report by Brust and Schiffrin.\textsuperscript{10, 11} This fueled research effort on the chemical and electronic nature of metallic nanoparticles. There are tremendous opportunities in the nanoparticle world for chemists to contribute to understand the diversity to this dimension of matter, bridging small molecules and bulk materials. Schiffrin and co-workers’ contribution was to combine classic two-phase colloid synthesis with metal-alkanethiolate self-assembled monolayer chemistry to produce nanoparticles much smaller (<5nm) than traditional colloidal materials.\textsuperscript{10, 12-15} These nanoparticles are named “monolayer protected clusters” (AuMPCs or AuNC). From the perspective of a chemist, the great importance of MPCs, in addition to their small dimension, is that, owing to the protecting monolayer, they are stable as dry chemicals. Usually nanoclusters or nanoparticles vary from 1 to 100 nm, and
nowadays the term Monolayer protected gold clusters is reserved for clusters less than 5nm in diameter.

1.2. Versatility of nanoparticles

Organized inorganic-organic nanohybrids can be developed by assembling monolayers of organic molecules containing functional groups, such as amines, thiols, isothiocyanates, and silanes, on the three-dimensional surface of metal nanoparticles. These AuMPCs, are used to build up nanoparticle arrays and superstructures as building blocks for the creation of nanocomposite materials for sensor and biomedical applications. Tailoring the optoelectronic properties of metal nanoparticles by incorporating chromophores of specific properties and functions on gold nanoparticles can yield photo responsive organic-inorganic nanohybrid materials. The organization of the densely packed photo responsive shell encapsulating the nanoparticle core offers exciting opportunities for the design of novel photon-based devices for sensing and drug delivery. Gold nanoparticles are widely used for biomolecular labeling, as immunoprobes and in light harvesting devices. So depending on the application, nanoparticles especially the gold clusters can be modified. Low toxicity of gold makes it an important trait for it to be used in biomedical applications.

1.3. Why gold clusters?

Gold clusters/nanoparticles (MPCs or AuNC) can be synthesized by making use of the hard-soft acid base principle. AuMPC resist aggregation of the metal cores when dried to a solvent-free state and can be repeatedly isolated and redissolved. This crucial feature allows subsequent monolayer chemical derivatization reactions via ligand exchange or condensation reactions, which Murray and co-workers have reported in a project aimed at developing large, polyfunctional molecules. AuMPCs have unique electrochemistry and electron transfer
chemistry. Establishing basic nanoparticle size and surface chemistry electronic function relationships in these materials is at the forefront of current nanoscale electronics research. The identification of novel electronic behavior and device applications which capitalize on quantum effects follows from fundamental structure-function determinations. One electronic behavior observed in nanoscale objects is single electron tunneling which correlates transfer of electrons one by one through the object. Single electron tunneling was first hypothesized in the early 1950s, a time when many physicists pondered how the electronic properties of a material (e.g., a metal wire) would change as material dimensions were reduced to the micron or nanometer scale. This aspect is very important for electron storage devices.16, 51-55

1.4. Forte of quantum sized gold clusters

i) Size induced changes in band gap

Quantum sized gold clusters are those with a diameter of 1nm. In this size range they lose all metallic properties, such as luster and behave like molecules. These are very difficult to synthesize. Owing to their quantum size they have a large band gap and behave as semiconductors or insulators, which opens them up to be applied to a plethora of applications. Figure 1.1 shows the difference between bulk gold and quantum sized gold clusters. The large band gap in nanoclusters, AuMPC, prevents electron (e⁻) and hole (h⁺) recombination and hence can be used for solar energy conversion and catalysis. In the case of bulk/metallic gold there is an overlap of the conduction and valence band and therefore there is rapid recombination of e⁻ and h⁺.
ii) Size induced changes in luminescence

The luminescence of quantum-sized metal clusters has enthralled the scientific community in recent years.\textsuperscript{56-59} Ultrafast excited-state dynamics of hexanethiol and glutathione protected \( \text{Au}_{25} \) clusters are investigated with time-resolved fluorescence spectroscopy. Gold clusters show a low quantum-yield visible photo-luminescence in addition to near-infrared luminescence. Femtosecond time-resolved luminescence measurements have shown unique growth in luminescence and also proved that the upper excited-states in monolayer-protected \( \text{Au}_{25} \) clusters have a finite lifetime (200 fs up to a few picoseconds) which can be utilized for applications in solar-energy harvesting and catalysis. Interesting changes have been noted with the difference in ligand structure.\textsuperscript{57} Figure 1.2 indicates the two types of luminescence in quantum sized gold
clusters: the visible and the near IR luminescence. The near IR luminescence is useful for biological imaging as it would require lower energy Infra red (IR) radiation rather than UV radiation. This also increases the range of wavelengths at which these AuMPCs can absorb light.

Figure 1.2. Cartoon diagram showing the relaxation pathways in Au$_{25}$L$_{18}$ clusters showing the gold core-states and semi-ring states and the origin of visible and near IR luminescence.

Current research efforts have focused on three aspects: (a) electronic charging of the electrical double layer of the Au core dissolved in electrolyte solutions, (b) electron transfer dynamics in AuMPC films, and (c) photoinduced electron transport between semiconductors and MPCs. More recently, the electronic properties of metal particles have been investigated within the context of decreasing electronic device size features to the nanoscopic level.

1.5. Electrochemical properties of AuMPCs

Progress in nanoparticle research has been promoted by innovative synthetic procedures and fabrication methods for single nanoelectrodes. Metal nanoparticles having dimensions less than 10 nm and the lower size ranges have seen many recent advances. In this thesis we concentrate on the behavior and characteristics of quantum sized gold clusters. Many research groups have targeted in making smaller and smaller clusters, but science becomes meaningless unless we can demonstrate the size, shape and composition of the products and the extent to which scientists can tailor its properties for potential applications.
Colloidal nanoparticles exhibit the same electrical behavior as colloidal dispersions\textsuperscript{72-74} arising due to surface charges created by adsorbed ions, surfactants and subsequent electrokinetic changes. The charge on the surface helps to prevent aggregation of these metal nanoparticles. Aggregation and instability of these metal nanoclusters can occur if their stabilizing or passivating layers interact hydrophobically in an aqueous dispersion which would result in precipitation. This phenomenon can find application in waste-water remediation and in ocean chemistry.

Metal and semiconductor colloids exhibit similar surface ionic charging and electrokinetic phenomena as other colloids but also can behave as electron donors and acceptors. Henglein\textsuperscript{75} and Gratzel\textsuperscript{76} made early breakthroughs with silver nanoparticles for electron storage during pulse radiolysis and with the photocatalytic reduction in the presence of colloidal Pt. Thereby they called these metal nanoparticles as colloidal microelectrodes. Gratzel explained the role of Pt colloids in the photoreduction of water to hydrogen.\textsuperscript{77, 78}

The improvement in nanoparticle stabilizing chemistry has paved the way for direct voltammetric observations on solutions of nanoparticles. A nanoparticle can be viewed as consisting of a core of metal atoms, surrounded by a monolayer of ligands that prevent agglomeration of the metal atom. This can be stably dissolved in a solvent containing an inert, supporting electrolyte to perform voltammetric experiments.\textsuperscript{79-84} In principle, the electron- transfer chemistry that can take place at the working electrode can be categorized into three voltammetric regimes, based on the core sizes.\textsuperscript{71} They are:

\begin{itemize}
  \item bulk-continuum,
  \item quantized double layer charging and
  \item molecule-like as described by the Murray group.
\end{itemize}
In addition there is a fourth regime wherein the ligand monolayer may contain some intrinsically
electroactive group, whose voltammetry would add to that attribute of the nanoparticle core.

1.5.1. Bulk-continuum Voltammetry

Nanoparticles with a diameter greater than 3 nm exhibit this kind of behavior. A simple but
fundamental relation that distinguishes such nanoparticles from the smaller ones is

\[ \Delta V = ze/C_{CLU} \]  (1.1)

Where \( \Delta V \) = change in electrochemical potential of a monolayer with double layer capacitance

\( z \) = electrons to/from the nanoparticle

\( C_{CLU} \) = Capacitance of an individual metal cluster

CLU indicates the capacitance of an individual metal cluster (i.e. not surface area normalized)

The \( \Delta V \) value is considered in view of the Boltzmann thermal energy distribution factor, which is

\( k_B T_{25} = 25.7 \text{ meV at room temperature.} \)

If \( \Delta V \leq \) this factor, then successive electron transfers to and from the nanoparticle will result in a
continuous –as opposed to stepwise – change in the nanoparticle’s potential. This bulk-continuum
behavior is expected from nanoparticle having a sufficiently large \( C_{CLU} \), which is > 6aF.
Therefore the room temperature experiments of nanoparticle solution should result in a smooth I-V curve.
Capacitance charging currents due to transport of nanoparticles to the working
electrode are controlled by mass transport hence can be distinguished from background currents
namely working electrode double layer charging and slower kinetically controlled faradaic
reactions. The capacitance currents for electronically charging the nanoparticle should for
example increase with the square root of the potential scan rate and of the electrode rotation rate.
in Cyclic Voltammetry and Rotating Disk Electrode experiments. In a potential step experiment in unstirred solutions, the diffusing nanoparticles would produce a current that decays inversely with the square root of time. These relations come from the Randles, Levich and Cottrell equations.  

\[ i = \frac{nFAD^{1/2}C_{Ag}}{\pi^{1/2}\tau^{1/2}} \]  

(1.2)

\[ i = \text{current, } n = \text{number of electrons transferred from working electrode to nanoparticle, } F = \text{Faraday's constant, 96,487 C/mol e}, \ A = \text{electrode surface area in cm}^2, \ D = \text{analyte diffusion coefficient (cm}^2/\text{s}), \ C_{Ag} = \text{Capacitance of any metal nanoparticle, in this instance Ag, } t = \text{time} \]

\[ i = 0.62nFAD^{1/2}\omega^{1/2}v^{1/6}C_{Ag} \]  

(1.3)

\[ i = \text{limiting current, } n = \text{number of electrons transferred from working electrode to nanoparticle, } F = \text{Faraday’s constant, 96,487 C/mol e}, \ A = \text{electrode surface area in cm}^2, \ D = \text{analyte diffusion coefficient (cm}^2/\text{s}), \ \omega = \text{angular velocity}, \ v = \text{viscosity, } C_{Ag} = \text{Capacitance of silver} \]

Mulvaney et al demonstrated the earliest example of bulk-continuum voltammetry in a 10 nm Ag nanoparticle. It has been determined that for potential of 1V there was an estimated 1600 to 1650 electrons transferred from Working electrode to nanoparticle using an RDE experiment.  

A similar value of \( n = 1790 \) electrons were estimated on applying potential to an optically transparent thin film of Ag. This proves that one can measure the reducing equivalents stored on large metal nanoparticles that have a large double layer capacitances. A nanoparticle surface area normalized value of 80\( \mu \text{F/cm}^2 \) was estimated.

According to this value Equation 1.1 predicts spacing between successive electrons transferred to the Ag of about 0.5 mV which is much smaller than the resolution thermally imposed by \( k_B T \).
The observed I-V potential responses accordingly contained no discontinuities relatable to successive single electron transfer.\textsuperscript{71}

Subsequent voltammetry of metal nanoparticles has been on very small Au nanoparticles that are stabilized by thiolates ligand monolayers.\textsuperscript{85, 90-94} This kind of nanoparticles are called monolayer protected clusters or MPCs.\textsuperscript{95} Voltammetry that resembles bulk-continuum voltammetry has been reported for Au MPCs coated with hexanethiolate ligand shells.\textsuperscript{85, 90-92, 96-99}

Figure 1.3. Chronocoulometry plots of concentration-normalized $Q_F$ and $Q_R$ against $t_{1/2}$ (upper) and $\tau$ (lower), respectively, for a 48 $\mu$M solution of C$_6$ MPC-A in methylene chloride/0.05 M Bu$_4$NClO$_4$ at a Pt electrode. $E_{\text{FINAL}} = +200$ (closed circles), +400 (open circles), +600 (closed triangles), and +800 mV (open triangles); $E_{\text{INIT}} = −200$ mV\textsuperscript{97}
1.5.2. Quantized double layer charging voltammetry

In this regime of nanoparticle voltammetry, the MPCs behave as quantum capacitors. Equation 1.1 points out that a sufficiently small nanoparticle double layer capacitance \( C_{\text{nanoparticle}} = C_{\text{MPC}} = C_{\text{CLU}} \) will cause the potential interval between successive single-electron changes in the electronic charge on the nanoparticle core to become experimentally observable. The discussion of equation 1.1 also teaches that not only must the nanoparticle be dimensionally small but it must also have a small double layer (charge/area) capacitance. In this regard, use of alkanethiolate coated nanoparticles is the choice to explore quantized double layer (QDL) charging of nanoparticles. Figure 1.4, panel B shows the first reported\(^{100} \) example of QDL charging voltammetry, done on size-fractionated octanethiolate- coated Au MPCs.

![Figure 1.4](image)

Figure 1.4. Voltammetry (CV s, 100 mV/s; DPV, * are current peaks, 20 mV/s, 25 mV pulse, top and bottom are negative and positive scans, respectively) of a 0.1 mM 28 kDa cluster solution in 2:1 toluene:acetonitrile/0.05 M Hx\(_4\)NClO\(_4\) at a 7.9 \( \times \) 10\(^{-3} \) cm\(^2\) Pt electrode, 298 K, Ag wire pseudoreference electrode\(^{71} \)

The molecular weight and number of atoms were determined by laser desorption-ionization (LDI) mass spectrometry to have cores of mass \( \sim 28 \) KDa, which means roughly 146 Au atoms/core. Panel A shows the variation of tip current with tip potential in scanning tunneling spectroscopy of a single MPC resting on a cold (83 K) surface. The current steps, spaced at regular intervals, represent a coulomb staircase of successive one electron changes in the electronic charge resident
on the MPC core. Panel B shows room temperature cyclic (CV) and differential pulse (DPV) voltammetry of a solution of the same MPCs. The current peaks are more prominent in the DPV experiment than in the CV; DPV has a differentiation like property of magnifying small current features. Theoretical analysis of the QDL charging of MPCs in electrolyte solutions was based on modeling the MPC as a concentric sphere capacitor. The inner and outer spheres have radii $r$ and $r + d$, corresponding to the MPC core radius and the core radius plus thiolate monolayer thickness $d$. The thiolate monolayer exerts an effective dielectric constant $\varepsilon$. The relation for a concentric sphere capacitor is given by

$$C_{CLU} = A_{CLU} \frac{\varepsilon_0 r d}{r + d} = 4\pi\varepsilon_0 \frac{r + d}{d}(r + d)$$

(1.4)

$C_{CLU} =$ Capacitance of an individual metal cluster, $A_{CLU} =$ Au core surface area, $\varepsilon =$ dielectric constant, $\varepsilon_0 =$ permittivity of free space, $r =$ radius of inner sphere, $r + d =$ radius of outer sphere, $d =$ core radius plus thiolate monolayer thickness. Assuming that $C_{CLU}$ is invariant with MPC core charge leads to a relation for the ratio of nanoparticles with core charge $z$ to those with core charge $z - 1$, at a macroscopic electrode interface to which the potential $E_{App}$ is applied.

$$\alpha_z = \frac{N_z}{N_{z-1}} = \exp \left\{ \frac{e}{k_B T} \left[ E_{App} - E_{PZC} - \frac{(z-1)\varepsilon}{C_{CLU}} \right] \right\}$$

(1.5)

$N_z =$ Nernstian character, $e =$ electronic charge, $k_B =$ Boltzmann constant, $T =$ Temperature, $E_{App} =$ potential applied, $E_{PZC} =$ potential of zero charge for nanoparticle core, $z =$ nanoparticle core charge state. $E_{PZC}$ is the potential of zero charge for the nanoparticle core. Equation 1.5 is Nernstian in its form, so that a QDL charging voltammetric wave shape should ideally be identical to that of a reversible one-electron redox couple, with a formal potential characteristic of the $z/z - 1$ charge state change. The Nernstian character of stored charges on MPCs was established by observing Nernstian shifts in equilibrium potentials of solutions prepared by mixing solutions of different, separately prepared, core charge states. Equation 1.5 predicts the set
of identical waves, at both positive and negative potentials, in which the formal potentials of the successive $z/z-1$ charge state changes can be expressed as

$$E^{0}_{Z,Z-1} = E_{PZC} + \frac{(z-1/2)e}{C_{CLU}}$$  \hspace{1cm} (1.6)

$Z, Z-1 = core charge states, E_{PZC} = potential of zero charge for nanoparticle core. This relation predicts a linear plot of peak potentials of a QDL voltammograms against $z$ (the nanoparticle core charge state). Such “z-plots” require assignment of the $E_{PZC}$ (see below) in order to define $z = 0$. Such assignment is not necessary to obtain average values of $C_{CLU}$ from their slopes. The $E_{PZC}$ of alkanethiolate-coated MPCs (a monolayer of them anchored to a surface) has been estimated as ca. -0.2V vs Ag/AgCl, from a double layer capacitance minimum. The capacitance minimum is the potential at which the diffuse layer of ions around the MPC is universally neutral.

Seeing QDL peaks like those in Figure 1.4 requires nanoparticle samples to be fairly monodisperse. In QDL voltammetry, it is common to see current peaks atop a substantial continuum of background current. Attaining perfect uniformity with regard to MPC size (or capacitance) is still not fully understood. This was in fact the case in the bulk-continuum-like voltammetry discussed in Figure 1.3; the hexanethiolate - coated Au MPCs samples used were sufficiently small as to potentially yield QDL current peaks but did not because of their size dispersity. Voltammetric simulations have shown that mixtures of MPCs with differing $C$ values, with one of somewhat higher population, can produce voltammetry with a few clear current peaks near $E$, that fade to a continuum of current at higher charge state potentials. Methodologies that produce high purity MPCs are essential in electrochemical studies of QDL charging. A number of further studies of quantized double layer charging voltammetry have been reported, as have been reviews. Many have concerned Au MPCs with ~29 kDa core masses and alkanethiolate monolayers of various chain lengths.
Equation 1.4 has been tested for its dependence on the alkanethiolate monolayer thickness, by varying the chain length from C4 to C16 (a 4-fold variation of d). Experimental values of $C_{\text{CLU}}$ taken from $z$-plots over $z = -2$ to +3 charge states were typically within 10% of those predicted by equation 1.4 assuming $d$ values for fully extended chains and an effective dielectric constant $\varepsilon = 3$ taken from measurements of the capacitances of alkanethiolate self-assembled monolayers on flat Au(111) surfaces.\(^{111}\)

Some additional examples of QDL voltammetry of Au\(_{140}\)MPC are shown in Figures 1.5 and 1.6. Figure 1.5 shows an experimental CV of a hexanethiolate-coated Au\(_{140}\) MPC that is compared to a simulation of an ideal, reversible CV response that included a (measured) solution resistance factor.\(^{113}\)

Figure 1.5. Cyclic voltammetry (50 mV/s, solid line) for the +4/+3, +3/+2, and +2/+1 charge state changes of annealed EtOH-soluble C\(_6\) Au\(_{140}\) MPCs\(^{112}\)
An assumption of equation 1.4 is that $C_{CLU}$ does not change with MPC charge state or with the solvent. In reality this is not true, and indeed deviations from the predicted uniform spacing between current peaks have been reported. Peak spacing often decreases and can be somewhat irregular at higher charge states. Peak spacing is sensitive to solvent and electrolyte; The sensitivity of QDL peak spacing to solvent and electrolyte is related to the permeability of the MPC monolayer. We observed similar behavior in our Au$_{225}$, Au$_{144}$ and Au$_{38}$ samples, Figure 1.7. We can see how the slope ($\Delta V$) increases as we decrease the size of the nanoparticles.
Figure 1.7. SWV and Z plots of Au$_{225}$, Au$_{144}$, Au$_{38}$
In general the stability and ease of preparation of appropriate size and purity Au nanoparticles that are protected by low dielectric ligand monolayers have produced much better defined QDL voltammetry than has been achieved when using other metals for the MPC core.\textsuperscript{119-127}

1.5.3. Voltammetry of molecule like nanoparticles

The metal-to-molecule transition is signaled by the emergence of an energy gap, which can be detected optically or electrochemically or both. The optical energy gap for a nanoparticle is the electronic band edge or absorbance spectrum onset for transitions from the HOMO (highest occupied molecular orbital) to the LUMO (lowest unoccupied molecular orbital). Optical absorbance band edges are detectable when they occur in the near-infrared region or at higher energies, i.e., above \textasciitilde 1 eV. Optical energy gaps can have energies greater than those detectable electrochemically (owing to the limitation of the electrochemical potential window). The electrochemical energy gap is a difference (which is larger than the QDL charging energy) between electrochemical potentials for the first oxidation and first reduction peak for a parent species. Electrochemical energy gaps are detectable at lower energies than are usually feasible optically, and so they are useful estimators of small HOMO-LUMO energy gaps, while accommodating correction for charging energy.\textsuperscript{128, 129} Charging energy (or Born charging energy) is the voltage increment associated with generating, in the solvent medium employed, positive and negative species from a neutral molecule or with increasing the charge on an already charged species. Its value can be expected to be roughly approximated by the spacing of potentials between QDL peaks. Optical HOMO-LUMO electronic excitation is not accompanied by a change in overall charge, and no charging energy correction is required.

In the case of Au MPCs smaller than those exhibiting quantized double layer charging voltammetry, where there is already an electrostatically based potential spacing between one-electron voltammetric peaks (equation 1.4), appearance of a HOMO-LUMO energy gap would
produce an enlarged potential spacing between the current peaks for the first one electron loss and the first one-electron gain of the parent nanoparticle. Subtracting the QDL potential spacing (as an estimate of the charging energy) gives the electrochemically estimated HOMO-LUMO gap energy, Figure 1.10.

Within the family of Au nanoparticles protected by organothiolate ligands (MPCs), electrochemical energy gaps, and/or optical HOMO-LUMO energy gaps, have been detected for MPCs designated as having core sizes of \(~\text{Au}_{75}\) (or 14 kDa), \(~\text{Au}_{55}\), \(~\text{Au}_{38}\), \(~\text{Au}_{25}\), and \(~\text{Au}_{13}\). This progress toward defining the metal-to-molecule transition (for Au nanoparticles with organothiolate ligands) is summarized in Figure 1.8 as three regimes of nanoparticle behavior, from bulk continuum on the left (\(\text{Au}_x\)), where no voltammetric features are observable, to quantized double layer charging for \(\text{Au}_{225}\) and \(\text{Au}_{140}\), to molecule-like on the right, for \(\text{Au}_{75}\), \(\text{Au}_{55}\), \(\text{Au}_{38}\), \(\text{Au}_{25}\), and \(\text{Au}_{13}\). Available gap energy data are in blue at the upper right. It is somewhat remarkable that the \(~\text{Au}_{140}\) nanoparticle, with core diameter \(\text{ca.} 1.6\) nm, exhibits no detectable HOMO-LUMO gap whereas the HOMO-LUMO gap has grown to a considerable 1.3 eV energy for the \(~\text{Au}_{38}\) and 1.6 eV for \(\text{Au}_{25}\) with a \(\text{ca.} 1\) nm diameter core. The metal to molecule transition for Au nanoparticles is very steep. This will be later on proved with transient absorption measurements as well in this chapter.
Figure 1.8. Summary of electrochemical behavior by MPC core size, showing estimated energy gaps (HOMO-LUMO gaps) and electrochemical energy gaps (which is the spacing between the first oxidation peak and the first reduction current peak for the native nanoparticle).

A variety of size-dependent properties has now been detected for metal nanoparticles, such as the QDL charging size and protecting monolayer dependence, the emergence of confirmable HOMO-LUMO gaps, and the metal-insulator transition.
Figure 1.9. (a) 25 °C and (b) −70 °C differential pulse voltammograms (DPVs) at 0.02 V/s, and (c) −70 °C cyclic voltammogram (0.1 V/s) of Au_{25}(PhC_2S)_{18} in 0.1 M Bu_4NPF_6 in degassed CH_2Cl_2 at a 0.4 mm-diameter Pt working electrode, a Ag wire quasireference (AgQRE) electrode, and a Pt wire counterelectrode. Arrows indicate solution rest potentials and * indicates the wave for incompletely removed O_2, which varied from experiment to experiment.

Thus electrochemistry helps in understanding and characterizing the as prepared AuMPC. It is a valid tool to check on the monodispersity of the sample as well as to determine the core size, Figure 1.10.

Figure 1.10. SWV plots of Au_{25}
1.6. Optical properties of AuMCs

The optical spectroscopic signatures serve as a key means to make a distinction between them, as well as to discuss the size evolution of their electronic properties. The use of gold nanoparticles for decorative purposes dates back to ancient times, although at that time no-one knew the material was indeed nanoscale particles due to the unavailability of nanoscale imaging tools. Scientific research on gold nanoparticles, however, did not start until the mid-19th century. In 1857, Michael Faraday successfully prepared a gold colloid through the reduction of a gold salt with (white) phosphorus in a two phase system. The brilliant ruby-red color of the gold colloid spurred Faraday, and also motivated a great deal of experimental and theoretical research on metal colloids. In 1908, the striking optical properties (including absorption and scattering) of gold nanoparticles were successfully explained by the classic work of Gustav Mie, who solved Maxwell’s equations for light interaction with a single gold nanoparticle. But the physical nature of the optical excitation of gold nanoparticles (as well as other types of metal nanoparticles) was not understood until the full establishment of the electron band theory for metals in the 1960s. By then, electronic excitation in metal nanoparticles was finally understood as a collective excitation of the conduction electrons in the particle, which is the so-called surface plasmon resonance. Since the 1990s, tremendous research efforts have been pursued in shape and size control of gold nanoparticles as well as exploration of their applications in nanoelectronics and optics, biological sensing, catalysis, and biomedicine.

The ultra-small size of these nanoclusters induces distinctive quantum confinement effects, which result in discrete electronic structure and molecular-like properties, such as HOMO–LUMO electronic transition, enhanced photoluminescence, intrinsic magnetism, and many others. This is analogous to the electrochemical measurements. This makes them fundamentally different
from those of their larger counterparts—crystalline Au nanoparticles (also called nanocrystals) in which the optical properties are dominated by plasmon excitation and possess a collective nature (as opposed to the single-electron transition in gold nanoclusters).156 Generally gold clusters can be denoted as \( \text{Au}_n(\text{SR})_m \) where, \( n \) and \( m \) are the respective number of metal atoms and thiolate ligands (\(-\text{SR}\)). The most interesting size (\( n \)) range is from about 13 atoms to a few hundred atoms (equivalent to the subnanometer to ~2 nm range).

### 1.6.1. Electronic energy quantization

To understand the fundamental properties of metal nanoparticles, the free-electron theory for metals is of particular importance, which is a quite successful model and provides intuitive insight into the electronic properties of metal nanoparticles, although it lacks a comprehensive explanation in certain aspects such as, no electron–electron or electron–ion lattice interactions.

### 1.6.2. The free electron Model

The free-electron model was first developed by Drude in 1900 (just three years after Thompson's discovery of the electron), and later refined by Sommerfeld by replacing the classical Maxwell–Boltzmann statistics with the Fermi–Dirac statistical function for electrons. Accompanying the major development of quantum physics in the 1920s, the electron band theory for the solid state started to come into being around the 1930s, but at that time the calculation of electronic band structures of metals was still formidable.157 Shortly after the establishment of the band theory of bulk metals, physicists started to think about the electronic structure of small particles. Bulk metals typically adopt cubic or hexagonal crystal structures, including fcc, bcc, and hcp. Due to the extremely high electron density (for example, \( \sim 6 \times 10^{22} \) per cm\(^3\) for 6s valence electrons \( i.e. \) conduction electrons) in bulk gold (fcc structure, unit cell edge length: 0.408 nm), the electronic states are densely spaced, forming quasi-continuous bands \( e.g. \) 6s or 6sp band and 5d...
bands). If \( N \) electrons are in a cube of volume \( a^3 \); the Schrödinger equation can be greatly simplified into a one-electron equation under the major assumptions of the free-electron model \((i.e., \) no electron–electron or electron–ion interactions),

\[
H\Psi = \left(-\frac{\hbar^2}{2m} \nabla^2 + V\right)\Psi = E\Psi
\] (1.7)

In the Cartesian coordinate system, the equation can be readily solved by separating \( x, y \) and \( z \) variables (one can refer to any quantum mechanics textbook). The as-obtained eigenvalues \( E_n \) are

\[
E_n = \frac{\pi^2 \hbar^2}{2ma^2} n^2
\] (1.8)

where, \( n^2 = n_x^2 + n_y^2 + n_z^2 \), and \( n_x, n_y \), and \( n_z \) are the principal quantum numbers and take integers such as 0, 1, 2, \( etc \). The \( E_n \sim n^2 \) relation forms a sphere with radius \( n \), and the equal energy states are distributed on the spherical surface. Thus, the number of energy states \( (N') \) with energy up to \( E_n \) can be represented by the volume of the sphere. Since \( n_x, n_y \), and \( n_z \) take positive integers only, \( N' \) is represented by the positive octant of the \( n \)-space, that is, the number of energy states is

\[
N' = \frac{1}{8} \left( \frac{4\pi}{3} \right)^{1 \over 3} n^{1 \over 3}
\] (1.9)

Substituting \( n \) with eqn (2), we obtain,

\[
N' = \frac{\pi}{6} \left( \frac{2ma^3}{\pi \hbar^2} \right)^{1 \over 3} E^{1 \over 3}
\] (1.10)

Now, the density of energy states can be obtained by differentiating eqn (4) with respect to \( E \),

\[
\frac{dN'}{dE} = \frac{\pi}{4} \left( \frac{2ma^3}{\pi \hbar^2} \right)^{1 \over 3} E^{-1 \over 3} = \frac{a^3}{4\pi^2 \hbar^2} \frac{1}{E} E^{1 \over 3}
\] (1.11)

where, \( a^3 \) is the volume of the particle. The spacing \( (d) \) of the electronic energy levels equals the reciprocal of the density of energy states, thus,
The above free-electron model works quite well for gold, silver, and copper. In the band theory, the rigorously computed s band (6s for gold) indeed closely resembles what is derived from the simple free-electron model. The free electron model can be extrapolated into the cluster size regime to obtain some useful results for metal nanoclusters.

1.6.3. The critical size for electronic energy quantization

For small particles, the number of atoms in the particle is finite, thus the spacing $\delta$ becomes appreciable and increases with decreasing cluster size. If we use the thermal energy ($k_B T$) at room temperature (~298 K) as a criterion, using equation (1.12) we can estimate the size at which the electronic energy quantization will be comparable to thermal energy, that is,

$$\delta = \frac{4\pi^2}{a^3} \left(\frac{\hbar^2}{2m}\right)^2 E - \frac{i}{a}$$  \hspace{1cm} (1.12)

By substituting all the constants (SI units) and the highest occupied energy level of gold (i.e. the Fermi level, $E_F = 5.5$ eV = 8.8 x $10^{-27}$ J), the corresponding particle volume ($a^3$) is ~5x10$^{-27}$ m$^3$ (equivalent particle size ~1.7 nm, or roughly 2 nm since the free-electron model only provides an estimation). This gives the critical particle size at which electronic energy quantization will become important. Indeed, at this size the collective Plasmon mode is no longer supported by the particles.

1.6.4. Electronic structure and optical properties of Au clusters

Prior to the X-ray structural determination of Au$_n$(SR)$_m$ clusters and theoretical calculations based upon X-ray structures, the nature of the cluster's absorption peaks and their electronic origin were not understood. The available crystal structures of Au$_{102}$(p-MBA)$_{44}$ and [Au$_{25}$(SCH$_2$CH$_3$Ph)$_{18}$]$^q$ ($q = -1, 0$) have permitted an in-depth understanding of both the electronic and optical properties.
For the Au\textsubscript{102}(p-MBA)\textsubscript{44} nanocluster, Hakkinen \textit{et al.} performed DFT calculations and found that the gold atoms in the cluster are in two distinct chemical states: the 79 core Au atoms are in a metallic (charge-neutral) state whereas the 23 Au atoms that belong to the protecting RS(AuSR), units are oxidized.\textsuperscript{158} This composition evokes the “divide and protect” structural feature proposed earlier.\textsuperscript{159} The Au\textsubscript{23}(p-MBA)\textsubscript{44} layer can further be divided into 19 RS(AuSR) units and 2 RS(AuSR)\textsubscript{2} units. The electronic structure of the Au\textsubscript{79} features a delocalized electron-shell structure. Zeng and coworkers also performed DFT calculations on a Au\textsubscript{102}(SCH\textsubscript{3})\textsubscript{44} model cluster.\textsuperscript{160} The calculated HOMO–LUMO gap is 0.54 eV, consistent with the DFT calculations by Hakkinen \textit{et al.}\textsuperscript{158} and by Li \textit{et al.}\textsuperscript{161} These results imply that the relatively high stability of the Au\textsubscript{102}(p-MBA)\textsubscript{44} nanocluster may be attributed to the electronic shell closing of the 58 valence electrons, that is, 102 (Au) − 44 (thiolate) = 58e, by assuming each thiolate consumes one 6s valence electron of gold. With respect to the optical properties of Au\textsubscript{102}(p-MBA)\textsubscript{44} clusters, no experimental data has been reported.

In the case of Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}Ph)\textsuperscript{−18}, shortly after its X-ray structure was determined, by Jin and co-workers, Schatz and Aikens, carried out DFT calculations on the basis of the crystal structure and achieved a deep understanding of the quantum confinement nature of the electronic structure and the optical absorption spectrum.\textsuperscript{162} To reduce the computational demand, Au\textsubscript{25}(SH)\textsuperscript{−18} was used as a model of Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}Ph)\textsuperscript{−18}. The calculated Kohn–Sham molecular orbitals (MOs), energies, and atomic orbital (AO) contributions are shown in Fig. 1.11A. In the electronic structure of Au\textsubscript{25}(SH)\textsuperscript{−18}, the HOMO and the lowest three LUMOs are mainly composed of 6s (or 6sp) atomic orbitals (labeled in green) of gold, thus, these orbitals constitute the sp-band, Figure1.11. The HOMO level is nearly triply degenerate while the LUMO is doubly degenerate. The HOMO-1 through HOMO-5 levels are mainly constructed from the 5d\textsuperscript{10} atomic orbitals (labeled in blue) of gold and hence constitute the d-bands, Figure 1.11. Note that both sets of HOMO and LUMO orbitals have a significant degree of the S(3p) (labeled in yellow) character,
indicating the ligand influences the electronic structure of the cluster. The computed HOMO–LUMO gap (1.3 eV) agrees well with the experimental value,\textsuperscript{162} also consistent with the theoretical calculations by Akola \textit{et al.}\textsuperscript{163}

Figure 1.11. Kohn–Sham orbital energy level diagram for a model compound $\text{Au}_{25}(\text{SH})_{18}$. Each KS orbital is drawn to indicate the relative contributions (line length with color labels) of the atomic orbitals of Au (6sp) in green, Au (5d) in blue, S (3p) in yellow, and others in grey (those unspecified AOs, each with a $<1\%$ contribution). The left column of the KS orbitals shows the orbital symmetry (g, u) and degeneracy (in parentheses); the right column shows the HOMO and LUMO sets. (B) The theoretical absorption spectrum of $\text{Au}_{25}(\text{SH})_{18}$\textsuperscript{162}
1.6.5. Optical properties of Au\textsubscript{n}(SR)\textsubscript{m} clusters and correlation with core size

The optical properties of gold clusters are one of the most interesting properties to observe and give a qualitative idea of the extent of dispersity of the sample. No experimental data on the optical properties of Au\textsubscript{102}(p-MBA)\textsubscript{44} clusters has been reported. We can use Au\textsubscript{25}(SR)\textsubscript{18} as an example to illustrate the quantum size effect on the optical absorption spectrum. A precise correlation of the Au\textsubscript{25} structure with its optical absorption properties has been obtained.\textsuperscript{162} The theoretical spectrum for Au\textsubscript{25}(SH)\textsuperscript{−18} agrees quite well with experimental findings, especially in the spectral shape (compare Figure 1.11 and 1.12). The first excited transition occurs at 1.52 eV (peak \textit{a} in Figure 1.11 and Figure 1.12) and corresponds to a LUMO$\leftarrow$HOMO transition (Figure 1.11A), which is essentially an intraband (sp$\leftarrow$sp) transition. Because only the three orbitals in the HOMO (triply degenerate) have more s character than d character, transitions arising out of the other occupied HOMO-$n$ orbitals tend to be interband (sp $\leftarrow$ d) transitions (Figure 1.12A). The peak at 2.63 eV (\textit{b} in Figure 1.11B and Figure 1.12) arises from mixed intraband (sp$\leftarrow$sp) and interband (sp$\leftarrow$d) transitions (Figure 1.12A). The peak at 2.91 eV (\textit{c} in Figure 1.11B) arises principally from an interband transition (sp$\leftarrow$d). Taken together, the optical absorption properties of Au\textsubscript{25} clusters apparently root in strong quantum confinement of electrons in the cluster. This cluster serves as a good example to illustrate the effect of quantum size on the optical properties of gold clusters. Unlike Au nanocrystals in which surface plasmons (collective excitation of conduction electrons) dominate the optical properties, ultra-small Au\textsubscript{25}(SR)\textsubscript{18} clusters manifest one-electron transitions.
Figure 1.12. The UV–vis spectrum of Au$_{25}$ clusters (single crystals redissolved in toluene)$^{162}$

An important conclusion is that in the optical absorption spectra we cannot separate the core and ligand contributions; geometric and electronic interactions between the Au$_{13}$ core and the surface are responsible for complex absorption spectra, even though in discussions (vide supra) the Au$_{25}$(SR)$_{18}$ structure were dissected into an Au$_{13}$ core and six –S–Au–S–Au–S– stapled motifs. When the optical spectra of the anion and of neutral [Au$_{25}$(SC$_2$H$_4$Ph)$_{18}$]$^q$ ($q = -1, 0$) were compared, the cluster charge shows some effects on the optical absorption spectrum.$^{164}$ The overall spectral profiles are quite similar, but the 800-nm band observed in the anion disappears in the neutral [Au$_{25}$(SR)$_{18}$]$^0$ cluster, and the 400 nm band is less pronounced in the anion but become very prominent in the neutral cluster. These fine features can serve as spectroscopic fingerprints and allow for a convenient and direct method to identify the cluster charge state in practical work.

Comparing with Au nanocrystals that are metallic and exhibit a strong plasmon absorption band (at 520 nm for spherical nanocrystals), the multi-band absorption spectrum of Au$_{25}$ clusters is quite striking. It remains to be seen how the optical properties of Au$_{n}$(SR)$_{m}$ clusters evolve with
increasing cluster size. Experimentally, previous work have shown a gradual broadening and concurrent decrease in intensity of the surface plasmon resonance at 520 nm into a nearly featureless decay spectrum observed from the 3.2 to 1.4 nm size series.\textsuperscript{165}

![Graphs showing optical absorption and extinction coefficients.]

Figure 1.13. (A) Normalized optical absorption spectra of the investigated gold clusters dissolved in toluene (B) Extinction coefficients\textsuperscript{58,59}

The X-Ray crystallographic analysis\textsuperscript{162} shows that the Au\textsubscript{25} cluster features a centered icosahedral Au\textsubscript{13} core Figure 1.14, which is further capped by a second shell comprised of the remaining twelve Au atoms. By taking into consideration the electronic structure of the cluster (vide infra), one may view the Au\textsubscript{25}(SR)\textsubscript{18} structure as an Au\textsubscript{13} icosahedral core capped by six extended staple motifs –S–Au–S–Au–S– (i.e. Au\textsubscript{3}S\textsubscript{3}) along the ±x, ±y, and ±z axes (i.e., six pairs of Au\textsubscript{2}S\textsubscript{3}).

![Atom diagrams showing Au–S framework.]

Figure 1.14. Anatomy of the Au–S framework of a [Au\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}Ph)\textsubscript{18}]\textsuperscript{−} cluster (counterion: TOA\textsuperscript{+})\textsuperscript{162}
1.7. **Nonlinear optical properties**

**Nonlinear optics** (NLO) is the branch of optics that describes the behavior of light in *nonlinear media*, that is, media in which the dielectric polarization \( \mathbf{P} \) responds nonlinearly to the electric field \( \mathbf{E} \) of the light. This nonlinearity is typically only observed at very high light intensities (values of the electric field comparable to interatomic electric fields, typically \( 10^8 \) V/m) such as those provided by pulsed lasers. In nonlinear optics, the superposition principle no longer holds. The nonlinear properties are:

1) **Two-photon Excited Fluorescence (TPA)**

The process of TPA was theoretically predicted by Maria Göppert-Mayer in 1931 in her PhD dissertation.\(^{166}\) It is interesting to note that such a prediction was made nearly thirty years before the discovery of lasers. However, the first experimental evidence for TPA was provided by Kaiser and Garrett in 1961 at Bell Labs in New Jersey.\(^{167}\) At longer wavelengths, scattering is reduced, which is in accordance with Rayleigh’s law, which can be expressed as:

\[
\text{Scattering} \propto \frac{1}{\lambda^4} \tag{1.14}
\]

Another key feature of TPA is greater penetration depth of excitation radiation into the absorbing material. This once again arises due to use of longer wavelength for excitation which causes reduced linear absorption and less scattering. These features also result in smaller focusing volumes of the material. TPA offers certain unique advantages over conventional steady state or single photon absorption. Another exciting application of TPA materials lies in Optical Power Limiting (OPL).\(^{168-172}\) OPL refers to the state when a large change in input signal produces only a small change in the output.
2) Fluorescence Upconversion

The ultimate time resolution is provided by methods that bypass the limited time-resolution of the detectors, and rely on the ps and fs pulse widths available with modern lasers. The basic idea is to pass the fluorescence signal through an upconversion crystal, and to gate the crystal with another ps or fs light pulse. One observes the shorter wavelength harmonic generated by the combined effects of the laser pulse and the emission. Upconversion provides impressive time resolution; however, the instrumentation is rather complex. For instance, even a seemingly minor change of the emission wavelength can require a major readjustment of the apparatus since the orientation of the upconversion crystal has to be adjusted according to the wavelength. Decay times of more than 1–2 ns are difficult to measure because of the use of a delay line (0.1 ns/foot). Alignment of the delay line can be quite difficult to maintain as the time delay is altered. See Chapter II for details.

With respect to the nonlinear optical properties of AuMPCs, Ramakrishna et al. demonstrated that quantum-sized gold clusters can be used as efficient two-photon absorbers. Miller et al. have performed a femtosecond spectroscopic study on the ultra-fast excited-state relaxation dynamics of Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}^-$ clusters and observed an 80 cm$^{-1}$ vibration localized to the Au$_{13}$ core; this mode reflects the strong vibronic coupling of a delocalized Au–Au bond-stretching vibration, which is analogous to the radial breathing modes of larger Au nanoparticles . The nonlinear optical properties and electron dynamics of gold thiolate clusters remain to be fully explored in future studies when more size-discrete clusters become available.

Materials with the luminescence in near-infrared region have found applications in biological imaging of breast-cancer tissue. Since small-size metal clusters such as Au$_{25}$ have decent luminescence efficiencies ($\sim 2.5 \times 10^{-4}$) in the near-infrared region, they can be made useful for two-photon imaging with infrared light. In this context, we have investigated the two-photon
emission and cross-section of Au$_{25}$ clusters with infrared light. In addition, investigations have been aimed at understanding the scaling laws of TPA cross-section as the cluster size is decreased from a gold nanoparticle (4 nm) down to Au$_{25}$ clusters (1.1 nm).

Figure 1.15. (A) Two-photon excited fluorescence spectra of Au$_{25}$ at different pump powers after excitation at 800 nm. (B) Power dependence of emission at 510 nm. (C) Absolute TPA cross-sections obtained by one and two-photon excited fluorescence upconversion measurements for different gold clusters. (D) TPA cross-section per gold atom as a function of cluster size. TPA results shown in panels C and D are obtained with 800 nm excitation. Solid lines in panels C and D are guides to the eye.

Absolute TPA cross-sections observed for the gold clusters are very high and are much larger than any of the experimentally investigated organic macromolecules (typically $\sim$1000 GM at 800 nm for some of best organics) or semiconductor nanocrystals. Such large TPA cross-sections are useful in several applications such as optical power limiting, nanolithography, and as labels for
multiphoton biological imaging. In addition, an interesting trend is observed in the plot of the TPA cross-section per gold atom as a function of cluster size shown in Figure 1.15D. δ per gold atom decreases drastically with increasing the cluster size and there is a saturation-type of behavior for large gold nanoparticles. The results can be explained by the evolution and involvement of interband transitions in smaller clusters. Even the TPA cross-section per gold atom is as high as 17,080 GM for Au$_{25}$ and taking into account the number of valence electrons in gold atom, the observed cross-sections per gold will approach the theoretical limit predicted for TPA cross-sections.$^{175}$ In our experiments we have studied the changes to the two-photon properties when these gold clusters are labeled with chromophores.

The degenerate transient absorption dynamics at 415 nm as a function of the gold cluster size has also been reported by the Goodson group. For larger size clusters exhibiting an SPR band (3 nm, 4 nm), the pump probe profile showed a rise-time feature followed by the decay. This profile was nearly the same as that of much larger 25 nm nanospheres.$^{176}$ A very different behavior in transient absorption dynamics and spectra has been observed for 2.2 nm clusters and smaller when compared to 3 nm clusters. A much faster initial decay was detected for the cluster size 2.2 nm Figure 1.16a). This decay profile is qualitatively similar to that previously observed in much smaller clusters.$^{177, 178}$ In addition, an interesting subpicosecond oscillatory feature has been detected in the transient absorption profiles for small gold clusters. For relatively large gold nanoparticles a coherently excited “breathing” vibrational mode was previously observed in the transient signal.$^{179}$ The suggested mechanism should not allow the vibrations to have a period shorter than a picosecond (which is expected for small particles) due to a relatively slow (∼1 ps) electron–phonon relaxation process which is responsible for the impulsive lattice heating.$^{179}$ Oscillatory features for particle sizes greater than 2.2 nm were not observed. Distinct oscillations with a period of 450 fs (∼75 cm$^{-1}$) in smaller clusters (less than 2.2 nm) were seen, Figure 1.16. Analysis of these oscillations$^{180}$ suggests a displacive excitation mechanism of coherent phonons,
similar to the phenomenon observed in femtosecond transient absorption signals of semiconductors and semimetals. For this mechanism the excited state electron configuration lives much longer to maintain a new equilibrium position for many periods of vibrations as it takes place in semiconductors and molecular systems. Hence, the appearance of the oscillations for small MPCs can be correlated to the emergence of an energy gap in smaller clusters.

Figure 1.16. (a) Demonstration of different transient absorption dynamics for MPCs of 2.2 and 3 nm size particles. Instrument response function profile (IRF) is also shown. (b) Decay profiles with oscillatory feature for small MPCs.

Considering these experimental findings it was proposed that a relatively abrupt optical gap opening at ~2.2 nm (~300 atoms). The existing information about a possible structural motif change in the range of sizes 100–1000 atoms is not conclusive enough to relate the observed transition to the particular structural rearrangement in the gold core.
Figure 1.17. Transient absorption mean decay time (a), visible fluorescence decay time (b), and two-photon absorption cross section (c) as a function of MPC size. Schematic illustration of the gap opening in the gold density of states is also shown.

It can be projected that an abrupt change in the optical properties (fluorescence, transient absorption, two-photon absorption) is observed once the diameter of the cluster is smaller than 2.2 nm. Displacively excited vibrations for smaller MPCs which are associated with the presence of the band gap have been detected for only particles smaller than 2.2 nm. With this information, scientists and engineers may be able to tailor the small clusters to optimize specific properties (electric or optical) for particular applications.

1.8. Chromophore interaction with gold clusters AuMPCs

Noble metals in the bulk are photoactive only to a small extent, while their nanoparticle counterparts’ exhibit increased photochemical activity owing to their high surface/volume ratio and unusual electronic properties. Photoactive molecules binding to metal nanoparticles makes these nano assemblies to exhibit enhanced photo-activity. Metals in the nanoparticle dimensions are more electronegative than the bulk material, so they can participate in an electron-transfer process. The electron transfer mechanism is normally observed for particle sizes less than 2 nm as
in this size regime the particles do not exhibit any surface plasmon band in the visible region. For particles of larger sizes, the free electron absorption develops a coherent character and partly transforms into the surface plasmon resonance absorption band. The nature of charge transfer interaction of fluorophore with a gold surface determines the pathways with which the excited-state deactivated. It has also been noted that, in addition to electron transfer from the probe molecules to the metal surface, the efficient internal electron transfer within the probe or fluorophore might also be responsible for the “super-quenching” of the molecular fluorescence over the metal surface. Due to the high surface-to-volume ratio of small clusters and sizes much smaller than the mean free electronic path (41 nm in Au), the conduction electrons are very likely to be found near the surface such that these small clusters more could accurately be described as fragments of metal surfaces, rather than fragments of bulk metal crystals.

For ultrasmall metal nanoparticles the SPR will localize at the surface of the gold nanoparticle, resulting in the formation of a localized surface plasmon (LSPR) best described by a skin-depth oscillation of the electric field. Depending on the nature of coupling between the SPR oscillation and the proximity of the dye to the metal surface, either radiative quenching or enhancement can be observed. This has been empirically described by Lakowicz via the radiating Plasmon (RP) model in which quenching arises from the absorption component in the extinction spectra, while the enhancement reflects the scattering contribution.\textsuperscript{184}

The extinction properties ($C_E$) of metal particles can be expressed as both a combination of both absorption ($C_A$) and scattering ($C_S$) factors, when the particles are spherical and have sizes comparable to the incident wavelength of light, i.e., in the Mie limit\textsuperscript{185, 186}

\[
C_E = C_A + C_S = k_1 \text{Im}(\alpha) + \frac{k_1}{6\pi} |\alpha|^2
\]

(1.15)
where \( k_1 = 2\pi n_1/\lambda_0 \) is the wavevector of the incident light in medium I and \( \alpha \) is the polarizability of a sphere with radius \( r \), \( n_1 \) is the refractive index, and \( \lambda_0 \) is the incident wavelength. The term \( |\alpha|^2 \) is square of the modulus of \( \alpha \)\textsuperscript{185, 186}

\[
\alpha = 4\pi r^3 \frac{(\varepsilon_m-\varepsilon_1)}{\varepsilon_m+2\varepsilon_1}
\]

(1.16)

where \( \varepsilon_1 \) and \( \varepsilon_m \) are the dielectric and the complex dielectric constants of the metal, respectively. The first term in Equation (1.15) represents the cross section due to absorption, \( C_A \), and the second term, the cross section due to scattering, \( C_S \). Current interpretation of metal-enhanced fluorescence\textsuperscript{187} is one underpinned by the scattering component of the metal extinction, i.e., the ability of fluorophore-coupled plasmons to radiate (plasmon scatter).\textsuperscript{188} Larger particles have wavelength distinctive scattering spectra (\( C_S \)) as compared to their absorption spectra (\( C_A \)),\textsuperscript{185, 186} facilitating plasmon coupled emission from the larger nanoparticles.

As the diameter of the nanoparticle is reduced below 40 nm, the absorption term dominates the extinction spectra, and therefore radiative quenching is supposed to be the predominant component. Thereby, energy transfer from the dye to the metal results in electron-hole pair formations (polaritons) and subsequent Ohmic losses (resistance of flow of electrons). Following from the RP theory, the rate of energy loss will reflect the consistency of the nanoparticle oscillation by influencing the ability of the plasmon to radiate following energy transfer. Increasing the nanoparticles size or changing the shape can lead to larger contributions from the scattering term and therefore enhancement of the dye oscillator rather than quenching.\textsuperscript{189}

The development of novel acceptors and donors in recent years has focused on the use of metal nanoparticles (NPs) as universal acceptors\textsuperscript{190-192} and quantum dots (QDs) as tunable donors\textsuperscript{193-196} due to their unique properties. It is also known that metal nanoparticles can act either as a radiative quencher or radiative enhancer, depending on the particle size, shape, composition, and
the distance between the donor and metal nanoparticle. The competition between enhancement and quenching relates to the magnitude of the electric field at the particle surface and the dielectric dispersion for the materials, which give rise to quenching in small sizes and radiative enhancement in larger size nanoparticles. The unique properties of metal nanoparticles can be attributed to its absorption and scattering characteristics, typically referred to as the extinction cross section, discussed earlier.

In the past, substantial efforts have been made to look at the enhancement effects, but the nature and mathematical understanding of energy transfer quenching of a photoluminescent dye by small gold nanoparticles has received less attention. Energy transfer leading to quenching of a dye at or near a small gold nanoparticle surface clearly occurs. However, whether the quenching relates to contributions from the interband transitions or the coupling to the LSPR is unclear. Energy transfer processes must follow the Fermi Golden Rule and therefore have constraints with respect to the separation distance, orientation, and energy overlap between the donor and acceptor wave functions. In general conceptual terms, a transition rate depends upon the strength of the coupling between the initial and final state of a system and upon the number of ways the transition can happen (i.e., the density of the final states). In many physical situations the transition probability is of the form

$$\lambda_{gf} = \frac{2\pi}{\hbar} |M_{gf}|^2 \rho_f$$  \hspace{1cm} (Fermi Golden Rule)  \hspace{1cm} (1.17)

Where, $\lambda_{gf}$ = transition probability, $M_{gf}$ = Matrix Element for the interaction, $\rho_f$ = Density of final states. The transition probability $\lambda$ is also called the decay probability and is related to the mean lifetime $t$ of the state by $\lambda = 1/t$. The general form of Fermi’s golden rule can apply to atomic transitions, nuclear decay, scattering, and a large variety of physical transitions.
A transition will proceed more rapidly if the coupling between the initial and final states is stronger. This coupling term is traditionally called the “matrix element” for the transition: this term comes from an alternative formulation of quantum mechanics in terms of matrices rather than the differential equations of the Schrodinger approach. The matrix element can be placed in the form of an integral where the interaction which causes the transition is expressed as a potential \( V \) which operates on the initial state wavefunction. The transition probability is proportional to the square of the integral of this interaction over all of the space appropriate to the problem.

\[
M_g = \int \Psi_f^* V \Psi_i dv \tag{1.18}
\]

Where, \( \Psi_f^* \) = Wave function of final state, \( V \) = Operator for the physical interaction which couples the initial and final states of the system, \( \Psi_i \) = Wave function for initial state.

Luminescence spectroscopy is a powerful technique as fluorescence measurements are very sensitive and can offer sub nanometer spatial resolution. It is a straightforward property for molecular recognition and labeling for medical, biological and analytical purposes. To ascertain the best sensitivity certain photo physical properties have to be optimized such as

- High molar absorption coefficients (\( \epsilon \))
- High quantum yield (\( \Phi \))

Hence the organization of photophysically active units such as chromophores on nanoparticle surfaces can give rise to combined effects which could be exploited to make new fluorescent material. In our project we paid attention to developing such material on AuMPC surfaces. The binding of the chromophores can take place via covalent or noncovalent bondings, such as electrostatic interactions or hydrogen bonds. Binding events can be proved using simple tools
such as UV-vis absorption. Until recently metal nanocores were reported to have only quenching effects on the surface-bound chromophores.

1.9. Quenching of fluorophores directly linked to nanoparticles

Murray et al. reported an acetamido fluorescein labeled covalently functionalized Tiopronin-AuMPC. According to this report the fluorescence of fluorescein is completely quenched. They predicted an electron transfer phenomenon to be taking place. Other chromophores such as dansyl, stilbene, porphyrin, pyrene, and cyanine were attached to Au surfaces. The energy transfer or electron transfer that takes place is found to be distance dependent. The size of the core of the nanoparticles seems to also play a role in the energetics. Different excited deactivation pathways can be controlled based on the nature of the chromophores. Most commonly, pyrene labeled clusters show excimer based luminescence due to \( \pi-\pi \) stacking. Electron transfer is the more favored deactivation pathway. Montalti et al. have also shown the possibility of a modulation of the photophysical properties of pyrene-functionalized gold nanoparticles by an accurate control of the degree of surface coverage. They have also determined that the fluorescence of pyrene is regained when it is unbound from the Au surface.

1.10. Nonquenched fluorophores directly linked to nanoparticles

Thomas and Kamat were the first to report fluorescence enhancement of a chromophore directly linked to the surface of a gold nanoparticle and within very close proximity to the gold surface. They reported that 1-Methylaminopyrene was attached on gold clusters (with a diameter ranging from 5 to 8 nm), weakly stabilized by tetraoctylammonium bromide (TOAB), via exchange reaction in THF. The emission spectrum featured the presence of both bound chromophores and TOAB moieties on the gold surfaces; in this environment the pyrene moieties are quite isolated from one another, indicated by the absence of the excimer emission. On the contrary, an intense
and red-shifted monomeric emission was detected, while the nanoparticles are nonfluorescent and the 1-methylaminopyrene in THF was weakly luminescent. Absorption, steady-state emission, and lifetime measurements showed that this can be explained by taking into account the interaction of the amino lone pair with the metal, when the pyrene is bound on its surface. This decreases its donating ability and suppresses the photoinduced electron transfer responsible for the main nonradiative decay process of the singlet excited state of the chromophore. These amine binding pyrenes, organized on gold nanoparticles, represent the first example of organic–inorganic materials of this kind that are fluorescent and open up new potentialities for these heteromaterials to be used for useful applications in photodevices or biophotonics.

1.11. Photoluminescence in gold nanoparticles

Photoluminescence from gold nanoclusters was predicted by Mooradian in 1969, who observed and investigated the light emission from noble metal surfaces.²⁰⁷ The role of roughness in the luminescence efficiency was then studied by Boyd²⁰⁸ but the phenomenon was finally correlated to the electronic structure only in 1988 by Apell et al. Excitation was then related to transitions from occupied d bands to states higher in energy than the Fermi level while emission was related to the recombination of the hole with an electron from an occupied sp band; it has to be noted that, between the two processes, electron–phonon and hole–phonon scattering take place causing a partial energy loss.

El-Sayed and Whetten proposed a schematic model of excited state levels that combines both intraband (sp) and interband (sp–d) transitions to explain the two-component emission shown by the system that they were investigating.²⁰⁹ Twenty-eight-atom gold clusters having 16 glutathione molecules absorbed on the surface (Au₂₈ SG₁₆) present a structured luminescence spectrum which
can be deconvoluted into two bands with maxima at around 800 nm (1.55 eV) and 1100 nm (1.13 eV). In the molecular-like model proposed, Figure 1.18), the excitation is represented as a singlet–singlet transition that moves an electron from the d band to an unoccupied orbital of the sp band.

a)

Figure 1.18. Solid-state model for the origin of the two luminescence bands. The high energy band is proposed to be due to radiative interband recombination between the sp and d-bands while the low energy band is thought to originate from radiative intraband transitions within the sp-band across the HOMO-LUMO gap. Note that intraband recombination has to involve prior nonradiative recombination of the hole in the d-band created after excitation with an (unexcited) electron in the sp-band. (b) Molecular model for the origin of the two luminescence bands: Excitation into higher excited states (S_n) is followed by rapid relaxation (internal conversion, IC) to the lowest excited singlet state (S_1). Radiative recombination with the ground state (S_0), fluorescence, gives rise to the high energy luminescence band. Intersystem crossing (ISC) to the lowest excited triplet state (T_1) followed by radiative relaxation to the ground state, phosphorescence, causes the luminescence band at lower energies.

Relaxation of the system can lead to the electron–hole recombination giving a “fast” (fluorescence-like) light emission. Alternatively, a nonradiative recombination of an sp electron with the hole in the d band can generate a triplet-like state that could deactivate “slowly” giving a phosphorescence-like emission. This model, even though nonrigorous, has the advantage of
giving a simple interpretation of the results obtained with luminescence time-resolved experiments. The luminescence decays recorded at different wavelengths show that at least two exponential components must be taken into account: a “fast” one with a lifetime on the order of 100 ns and a “slow” one of about 1000 ns. Moreover the contribution of the “slow” decay is larger at longer wavelengths in agreement with the proposed model. The molecular-like behavior of Au$_{28}$SG$_{16}$ was confirmed by femtosecond transient absorption studies. Similar behavior was reported by the Murray group on Au$_{38}$. Later in Chapter III we have investigated the origin of luminescence for these quantum sized gold clusters and have come up with a rational mechanism involving the core and semi-ring states.

1.12. Objectives of this thesis

In this dissertation work we have investigated the optical and electrochemical properties of Au clusters, especially the quantum-sized molecules, in light of the above discussed background to give us better insights into understanding the structure and optical footprint of these quantum sized gold clusters. The broad aim of the project is as follows:

i. Synthesize AuMPCs of several different sizes by using suitable surface modifiers such as hexanethiol, glutathione (GS), cysteine, 6-(ferrocenyl) hexanethiol (FcSH), 7-mercapto-4-methyl coumarin (MC). Measure their monodispersity using TEM and electrochemistry and UV-vis absorption (Chapter II and Appendix I)

ii. Surface modification of the synthesized AuMPCs with the fluorophores (PyNHS, PySH, MC, Coumarin) or intended drug (DOX) via ligand exchange reaction or condensation while maintaining the original core size (Chapter II)

iii. Characterize by electrochemical and optical studies (both linear and non linear methods) (Chapters II to VIII)
iv. Assess the quantum efficiency, lifetimes of each system and measure its activity as optical fluorescent sensors. (Chapters V, VI, VII)

v. Measure two-photon fluorescence values to assess their future use as biological imaging agents (Chapters V, VI, VII)

vi. Understand the unique luminescence dynamics of Au$_{25}$L$_{18}$ clusters vs nanoparticles (Chapter III)

vii. Investigate the temperature dependence of optical properties of Quantum Sized Clusters Vs nanoparticles (Chapter IV)

viii. Study the electron transfer or energy transfer and enhanced TPA cross-sections of Chromophore-functionalized Gold clusters (for use as biological imaging agents, catalysis, energy storage devices and targeted drug delivery) (Chapters V, VI, VII, XI)

ix. Use non linear optical techniques such as fluorescence upconversion, two-photon excitation fluorescence and transient absorption measurements to understand the nature of electron or energy transfer between the chromophores and Au surfaces (Chapters V, VI, VII)

x. Assemble pseudo - rotaxanes on AuMPCs and study their electrochemical property changes on binding to Au surfaces (Chapter VIII)

xi. Assess the change in electrochemical activity for the bare AuMPCs in the presence of pesticides mimics such as diethyl chlorophosphate (DCP) and Dimethyl methylphosphonate (DMMP) (Chapter VI)

xii. Assess the effectiveness of these systems in sensing organothiolate pesticides such as parathion, fenthion, malathion, and ethion as they have similar action to their organophosphorus compounds and they can act as false positives (Chapter VI)
CHAPTER II

SYNTHESIS AND EXPERIMENTAL NUANCES INVOLVED IN THE MAKING OF QUANTUM SIZED MONOLAYER PROTECTED GOLD CLUSTERS

2.1. Synthesis of monolayer protected clusters

The synthesis of monolayer protected gold clusters has been improved upon since the breakthrough achieved by Brust and Schiffrin. Over two decades there are several groups such as the Murray group at University of North Carolina and more recently the Jin group at Carnegie Mellon University, who have worked on improved syntheses and developed a protocol to generate AuMPC with almost perfect control of size and monodispersity. The tailoring of these AuMPCs according to need has found several applications in electronics, medicine and catalysis. The stabilization of these AuMPCs is usually achieved via ligand stabilizers such as carboxylates, amines, thiolates and dendrimers. We have focused on using thiolates since they form highly stable Au-S bonds, which are effective in preventing aggregation. MPCs can be derived from several noble metals such as platinum, palladium, silver and copper apart from gold. For the purpose of our research we have used gold. Apart from Au being inert and comparatively expensive, AuMPCs are well researched and thus far are photo stable and less susceptible to oxidation and aggregation. Gold MPCs have been synthesized with non-thiolate ligands, but they usually form large nanoparticles and as we were studying quantum sized clusters we made use of thiolate ligands for the major part of the project. Still the structure of these AuMPCs present a challenge, as direct determination via x-ray crystallography was not available due to the mammoth task of producing a single crystal suitable for study. Very few crystal structures have been determined; $\text{Au}_{105}$, $\text{Au}_{25}$ and $\text{Au}_{38}$ are available in literature. This brought about the elucidation of the “staple motifs”, but not until 2008.
2.2. Au cluster synthesis

A general outline for the synthesis is given in Figure 2.1. As mentioned earlier, the most prominent and commonly used procedure is the Brust-Schiffrin synthesis. Their pioneering work produced MPCs passivated by a monolayer of alkanethiol generating core diameters between 1-5nm. This straightforward procedure involves either starting with an aqueous gold salt (Au\textsuperscript{III}) precursor solution that is transferred to the organic phase using a phase transfer agent tetraoctyl ammonium bromide (Oct\textsubscript{4}NBr) or Au\textsuperscript{III} in the aqueous phase. Then a precalculated amount of alkanethiol is added which reduces Au\textsuperscript{III} to Au\textsuperscript{I} and forms gold-thiolate species. The final step is the rapid addition of a strong reducing agent, namely sodium borohydride. This results in a black solution wherein, Au\textsuperscript{I} is reduced to Au\textsuperscript{0}. These Au\textsuperscript{0} atoms nucleate to form a gold core and the thiolates in solution bind to and passivate the surface of the core. The resulting solution is a mixture of polydisperse AuMPC. By adjusting the initial feed ratio of Au:thiol and controlling temperature at the reduction stage in the protocol, a rough distribution of core-size can be achieved. Usually, larger ratios of thiols result in core sizes less than 2nm at low temperatures. Lower thiol ratios and higher temperatures resulted in greater than 2nm AuMPCs. This is not a universal truth, as we discovered during the myriad options pursued to tailor the synthetic process, that the nature of the ligand has a more pronounced role in the core size distribution. For instance, when we used glutathione as the passivating ligand, we could generate Au\textsubscript{25} both at room temperature and at lower temperatures as well as when we used a 1:4, 1:3, or a 1:1 Au: thiol ratio. This contradicts all previous hypotheses, although one must add that the core size distribution is sensitive to synthesis procedures.
Figure 2.1. Schematic of modified Brust-Schiffrin Synthesis

The synthesis protocol for these MPCs has to be further investigated in order to come to a concrete conclusion. It is very difficult to determine the exact cluster formula and attribute any meaningful properties to the nanoclusters\textsuperscript{213} if the product is a mixture. Therefore fundamentally, the main factors that control the core size distribution are:

\textit{Surfactant-thiol ratio: Micrometer-sized crystals grown in the presence of an organic additive (e.g. a surfactant) that preferentially binds to a certain set of crystalline faces will have a morphology that expresses these faces.}\textsuperscript{214} The organic additives lower the free energy of the crystalline faces to which they bind and retard the growth of those faces; these interactions control the resulting morphologies of the crystals by selecting the crystalline planes that are expressed at the surface of the crystal. The same principles may apply at the nanometer-scale, where the differential binding of surfactants to selected crystalline faces and polymorphs has been shown to influence the size, shape, and polymorphism of nanocrystals.\textsuperscript{215-218} The ratio of alkanethiol to HAuCl\textsubscript{4} controls the size of the resulting nanoparticles by adjusting the relative rates of particle nucleation and growth (higher ratios yield smaller particles).\textsuperscript{219, 220}
forming gold nanoparticles in the presence of thiols can only be used to form small (<5 nm in diameter) particles. The formation of particles with diameters >5 nm requires the use of surfactants that have a faster desorption rate than thiols (e.g. phosphines and cetyltrimethylammonium bromide (CTAB) or stabilization by electrostatic charges (citric acid synthesis)). These larger particles can be functionalized with thiols via ligand-exchange methods to displace the weakly bound surfactants.

Temperature of reaction: Formation of self assembled monolayers (SAMs) nanoparticles at temperatures above 25°C can improve the kinetics of formation of nanoparticles and it also can lead to longer nucleation processes in the reaction resulting in larger core size nanoparticles. Elevated temperatures increase the rate of desorption for adventitious materials and solvent molecules physisorbed on the surface of the substrate. It enables the system to cross activation barriers for processes such as lateral rearrangements of the adsorbates more easily than at room temperature. Uosaki and co-workers suggest that the effect of temperature is particularly relevant during the first few minutes of the formation of a SAM when most of the adsorption and reorganization of the SAM is taking place.

The syntheses essentially involve kinetic control. The nucleation mechanism remains a major challenge, and the formation of the AuI intermediate also deserves further investigation. Apart from the importance of kinetic control in nanocluster synthesis, another important factor is the thermodynamic stability of nanoclusters. Both principles were indeed critical in the successful synthesis of Au19(SC2H4Ph)13 nanoclusters as reported by Jin and co-workers. Stable nanoclusters can exist in solution after aging for a long time, while unstable nanoclusters are converted either to stable ones or to AuI(SR).

The organic phase is separated, dried over a dehydrating agent such as sodium or magnesium sulfate, rotary evaporated and taken for further purification and size evolution and separation.
There are several methods that can be used, such as size exclusion chromatography, capillary electrophoresis, gel electrophoresis, ion-exchange chromatography, ultra centrifugation etching etc.\textsuperscript{221, 229-235} But these methods result in low yield separations; since we need these MPCs for applications later we must come up with a more feasible and economically viable method. For this we employed conventional solvent fractionation techniques and or recrystallization, originally introduced by the Murray and Whetten groups. Size–selective precipitation is a common method to isolate AuMPC. This method is based on the size-dependent solubility of the MPCs. For example, when we begin with a 1:3 mole ratio of Au:hexanethiol, we get a spectrum of sizes ranging from 1 to 4 nm. The first step is to remove solvent from the crude MPC solution by rotary evaporation. Then a non-solvent, typically ethanol, is introduced in the dried as-prepared AuMPCs, in order to precipitate the larger core size (>2.0 nm). Continual addition and removal of ethanol is carried out until the supernatant is clear. This is done over a period of 12-15h. This addition of non-solvent weakens the ligand-solvent or AuMPCs-solvent interaction in the solution and thus results in precipitation of AuMPCs. The ethanol soluble portion gave smaller core sizes (<1.7nm) while what remained insoluble were the larger core sizes. In order to collect AuMPCs with a narrower core size distribution, acetone was introduced to the remaining dried off sample. Acetone works as a solvent for isolating AuMPCs from the core size range of 1.1-1.3nm. The acetone-soluble portion was then evaporated, further washed with acetonitrile and ethanol non-solvent to remove any impurities. Figure 2.2 shows a schematic outline of the work-up procedure.
Figure 2.2. Schematic of solvent fractionation method used to generate different core sizes

By using the solvent fractionation method, monodisperse 1.1, 1.7, 2.2, 3.1, 4.0, 4.9 nm core size AuMPCs were isolated from the as-prepared AuMPCs. One can see that in general the particles exhibit spherical shape as shown in Appendix I and more than 59% of the particles fall within the average core size with <10% standard deviation. The choice of solvents used to remove impurities and achieve narrow size distribution is still poorly understood. In the synthesis of nanoclusters, the purity of the product is of critical importance.
Alternatively, Hutchison and coworkers prepared alkanethiolate-protected Au$_{11}$ by replacing the triphenylphosphine ligand of a preformed Au$_{11}$. AuMPCs were also obtained by reacting “Au$_{55}$(PPh)$_{12}$Cl$_6$” with different alkanethiols. Shoawei Chen used Au$^+$ precursor to make a Au$_{11}$. Using milder reducing agents results in larger clusters as proved by the Stucky group. Mechanistic details of AuMPC synthesis are poorly understood, although researchers have made significant progress in recent years. The Au$^+$ precursor of the two-phase method was generally assumed to be a polymeric Au$^+$-thiolate complex. Such polymers could be observed by dynamic light scattering and the size uniformity of these polymers was confirmed to affect the monodispersity of resulting AuMPCs. Yet, under anhydrous conditions the precursor was determined by Goulet and Lennox to be a complex of Au$^+$ with Oct$_4$N$^+$. The thiols, upon reduction of Au$^{III}$, are in turn oxidized to disulfides. However, for one-phase methods in which polar solvents are employed, Au$^+$ thiolate complexes are in fact the precursors. Later it was discovered that the Oct$_4$N$^+$ actually forms an inverse micelle structure with the anionic gold halide complex for both the Au$^{III}$ and Au$^+$ species. It was further revealed that if water is present during thiol mixing, the resulting precursor is a mixture of a [R$_4$N$^+$$]$[AuX$_2$] complex micelles and polymeric [Au$^+_n$SR]$_n$. The amount of water present after phase-transfer is important for the composition of this mixture. As more water is introduced, the amount of [Au$^+_n$SR]$_n$ increases. Water is encapsulated inside the polar core of the inverse micelles and was found to function as a proton acceptor to remove the thiol proton, thus facilitating the formation of [Au$^+_n$SR]$_n$ polymers. This mechanistic detail also explains why [Au$^+_n$SR]$_n$ polymers are the observed precursor in the one-phase syntheses using polar solvents. After the addition of sodium borohydride, the evolution of AuMPC size is controlled by the amount of thiol in the system. It has been shown repeatedly that smaller, more monodisperse AuMPCs result from syntheses that use relatively higher thiol:Au ratios.
Water-soluble AuMPCs have been synthesized without the use of phase-transfer agents in water/methanol\textsuperscript{247, 248} mixed solvent systems with polar functionalities.\textsuperscript{245, 248-251} The procedure is similar in that it involves the direct addition of the thiol to the Au\textsuperscript{III} precursor forming the Au\textsuperscript{I}S\textsubscript{R} polymer, followed by rapid addition of reducing agent. Here again, the amount of water present determines the size of the core; larger core sizes are obtained with higher percentage of water as opposed to methanol. The resultant crude AuMPC solution is polydisperse. Solvent fractionation is carried out either using methanol or acetone to isolate the different fractions. It should be noted that the size separation has to be done before removing impurities; otherwise it will not show a regular gradation in the precipitation. The larger clusters precipitate out first, as in the case of the Au(GS) clusters, the 4 nm/2.2 nm size (subject to the amount of water in the reaction medium) is in the first fraction and the last fraction is Au\textsubscript{25}. Further cleaning of impurities are carried out using a 4:1 water:methanol mixture. For the synthesis of Au\textsubscript{38}, etching of the as-prepared MPCs was performed followed by ligand exchange with the relevant thiol, as demonstrated by the Jin group.

**Surface Modification**: Surface modification on the preformed AuMPCs are carried out either by performing a ligand exchange reaction or a condensation reaction in order to form an amide bond to tag the MPC with chromophores. Care is taken to see that the size does not change before or after the reaction.

### 2.3. Chemicals

Hydrogen tetrachloroaurate trihydrate (HAuCl\textsubscript{4}·3H\textsubscript{2}O reagent grade), 1-hexanethiol (98%), L-glutathione reduced, tetraoctylammonium bromide (Oct\textsubscript{4}NBr, 98%), sodium hydroxide and sodium borohydride (NaBH\textsubscript{4}, 99%) were used as received from Aldrich. HPLC-grade toluene, acetone, absolute ethanol, acetonitrile, dichloromethane and dimethyl sulfoxide (DMSO) were purchased from Aldrich and was used as received. Spectroscopic grade methanol, 1,2-dimethoxy
ethane (anhydrous) 99.5%, were procured from Aldrich and were used as received. Chloro(triphenylphosphine)gold(I) and Trihydro(2-methyl-2-propanamine)boron were purchased from Alfa-Aesar, 6-(ferrocenyl hexanethiol) was used as received from Aldrich. Water was purified using a Millipore Milli-Q system (18.2 MΩ cm) and all glassware used were washed with aqua regia.

2.4. Synthesis of Au$_{25}$($\text{C}_6\text{S}$)$_{18}$ MPCs

Synthesis of Au$_{25}$($\text{C}_6\text{S}$)$_{18}$ was carried out following a published procedure.$^{57, 252, 253}$ The synthesis was carried out with a 5:1 thiol/gold molar ratio, and NaBH$_4$ reduction reaction for 30 min at 0 °C. For the preparation of hexanethiolate Au$_{25}$ MPCs, to a vigorously stirred solution of 3.3 mmol of Oct$_4$NBr in toluene was added 2.5 mmol of HAuCl$_4$·3H$_2$O in 20 mL of water. The water phase was rendered colorless, when the toluene solution became dark red due to the transfer of AuCl$_4^-$.

To this 12.8 mmol of 1-hexanethiol was added and stirred at 0 °C until the solution became colorless, indicating Au$^I$- thiol polymer formation. Then an ice-cold solution of 25.6 mmol NaBH$_4$ dissolved in 20 mL of water was quickly added to the toluene solution with vigorous stirring. The solution immediately became black, indicative of MPC formation. Stirring was continued for another 30 min at 0 °C. The aqueous phase was removed and the black organic phase was washed with water and rotary evaporated to produce a black product. DMSO (200 mL) was added to the black product and allowed to stand overnight. After the removal of DMSO, acetone (10 mL) was added to the flask to extract Au$_{25}$ MPCs. The acetone solution was rotary evaporated and subsequently washed with copious amounts of acetonitrile and absolute ethanol, providing 80-100 mg of a dark-brown product (Au$_{25}$($\text{C}_6\text{S}$)$_{18}$) which was used for further reaction.
2.5. Synthesis of \( \text{Au}_{25}(C_6S)_{17}\text{PyS} \) MPCs

To the \( \text{Au}_{25}(C_6S)_{18} \) clusters (40 mg, 0.005 mmol) dissolved in 30 mL of dichloromethane was added PySH ligand (8.6 mg, 0.028 mmol). The exchange reaction was allowed to continue for 45 min at room temperature with constant stirring. This solution was then rotary evaporated and washed with 300 mL of acetonitrile to remove any unexchanged dye molecules. These particles were characterized by TEM, UV-Vis and electrochemical measurements to confirm that the size remained unchanged. For the quantitative estimation of the number of pyrene molecules, the UV-Vis spectrum of a known mass of the exchanged product was recorded. The absorption profile of \( \text{Au}_{25} \) was subtracted from the pyrene-attached gold cluster to get the absorption due to the dye alone. From this absorption values were obtained, and using the extinction coefficient of the pyrene (58577 M\(^{-1}\)cm\(^{-1}\) at 340 nm), the concentration and the number of pyrene exchanged onto the gold core were calculated. From these calculations, the pyrene-attached gold clusters were estimated to be \( \text{Au}_{25}(C_6S)_{17}\text{PyS} \).

From the \(^1\text{H}\) NMR data we can calculate the average number of dye per \( \text{Au}_{25} \) cluster to be equal to one. The peak that is upfield [0.87 ppm; integral = 0.59; equivalent to \(^3\text{H}\)] is attributed to the terminal \( \text{CH}_3 \) of the hexanethiol molecule and the peaks that are downfield [between 7.8 ppm and 8.1 ppm; integral = 0.1069; equivalent to \(^9\text{H}\)] are attributed to the 9 protons on the PySH molecule. Therefore, ONE proton from PySH = 0.0119 and ONE proton from \( C_6\text{SH} \) = 0.1967. For every PySH there are 16.53 \( C_6\text{SH} \) ligands [0.1967/0.0119]. Hence the average number of PySH per \( \text{Au}_{25} \) cluster = 18/[1+16.53] =1.03. The \(^1\text{HNMR}\) spectrum is provided in Appendix I.

2.6. Synthesis of \( \text{Au}_{25}\text{GS}_{18} \) MPCs

The synthetic method employs a 3:1 thiol/gold molar ratio based on methods available in the literature\(^{264}\), and NaBH\(_4\) reduction reaction for 30 min at room temperature. For the preparation of
Au$_{25}$-MPCs, to a vigorously stirred solution of 1.14 mmol HAuCl$_4$·3H$_2$O in 80 mL methanol was added 3.4 mmol of L-glutathione dissolved in 30 mL of water. The solution became colorless after 15 min, indicating Au$^{1+}$-thiol polymer formation. Then a solution of 11 mmol NaBH$_4$ was added with vigorous stirring. The solution immediately became black, indicative of MPC formation. Stirring was continued for another 30 min. This was then dried to produce a black product (as-prepared MPC). Pure Au$_{25}$ clusters were obtained by recrystallization. The products were washed with a 4:1 water-methanol mixture providing 30-40 mg of a light brown product (Au$_{25}$GS$_{18}$MPCs).

2.7. Synthesis of 2.2 nm and 3.0 nm Au(C$_6$S) MPCs

The 2.2 nm and 3.0 nm Au MPCs were also synthesized by using a modified Brust–Schiffrin method,$^{255}$ in which the core size was controlled by employing a varied thiol to gold precursor molar ratio of 3:1 and 2:1, respectively, at 25 °C. The reduction was carried out using NaBH$_4$ for an hour and quenched to suppress particle growth.$^{253,256-258}$ For the preparation of 2.2 nm Au MPCs, to a vigorously stirred solution of 3.60 g (6.58 mmol) of Oct$_4$NBr in 160 mL of toluene was added 2.00 g (5.08 mmol) of HAuCl$_4$·3H$_2$O in 20 mL of water. The water phase quickly became clear, and the toluene solution became dark red as AuCl$_4^-$ was transferred into it. The aqueous phase was separated, and 2.2 mL (16 mmol) of 1-hexanethiol was added and stirred until the solution became colorless, indicating Au(I)-thiol polymer formation.$^1$ 1.94 g (51.3 mmol) NaBH$_4$ that had been dissolved in 10 mL of water was quickly added to the toluene solution with vigorous stirring. The solution mixture immediately became black, indicative of MPC formation. Stirring was continued for 60 min at 25 °C. After removing the aqueous phase, the black organic phase was collected and washed four times with 200 mL of water and rotary evaporated to produce a black product (as-prepared MPC). 200 mL of ethanol was added to the as-prepared MPC and allowed to stand overnight. DCM (30 mL) was added to the flask to extract 2.2 nm Au
MPCs after removing the supernatant liquid. The DCM solution was rotary evaporated and subsequently washed with copious amounts of acetonitrile and ethanol to remove impurities.

2.8. Synthesis of Au$_{25}$(GS)$_{18}$ and 2.2 nm -Au (GS) MPCs

The synthetic method for Au$_{25}$(GS)$_{18}$ employs a 3:1 thiol/gold molar ratio based on methods available in literature$^{259}$, and NaBH$_4$ reduction reaction for 30 min at room temperature. Briefly, to a vigorously stirred solution of 1.14 mmol HAuCl$_4$·3H$_2$O in 80 mL of methanol, 3.4 mmol of L-glutathione dissolved in 30 mL of Milli-Q water was added. The solution became colorless after 15 min, indicating Au$^{1-}$-thiol polymer formation. Then a solution of 11 mmol NaBH$_4$ was added with vigorous stirring. The solution immediately became black, indicative of MPC formation. Stirring was continued for another 30 min. This was then dried to produce a black product (as-prepared MPC). The crude product was dissolved in 10 ml of water. Pure Au$_{25}$ and 2.2 nm clusters were obtained by stepwise recrystallization with methanol followed by centrifugation at 3000 rpm for 10 min. The products were washed with a 4:1 water-methanol mixture providing 30-40 mg of a light brown product (Au$_{25}$GS$_{18}$MPCs) and a dark brown product which is the 2.2 nm-Au(GS) MPC.

2.9. Synthesis of 13 nm-Au(Cit) nanoparticles

Synthesis of 13 nm-Au (citrate stabilized) nanoparticles was carried out following a well-established procedure.$^{260}$ To a boiling solution of 60 mg of HAuCl$_4$·3H$_2$O in 50 ml water, 180 mg of sodium citrate was added. The solution turned black at first followed by the formation of a ruby red solution. The mixture was refluxed for another 10 minutes, cooled to room temperature and used for immediate analysis.
2.10. Synthesis of Au\textsubscript{38} clusters

The synthesis of Au\textsubscript{38} clusters involves a two step procedure, wherein glutathione-capped AuMPs are first synthesized, and then they are subject to ligand exchange reaction with the target thiol, in this case hexanethiol to form stable Au\textsubscript{38}MPCs.

Synthesis of Au:SG clusters.

The Au:SG clusters were synthesized by a modified method. Typically, 0.3 mmol HAuCl\textsubscript{4}·3H\textsubscript{2}O was first dissolved in 20 ml methanol and 1.2 mmol GSH was dissolved in 10 ml water. The two solutions were then mixed to form a cloudy suspension, which was known to be Au(I)-SG polymers. The mixture was cooled to 0\degree C in an ice bath. NaBH\textsubscript{4} (3 mmol, dissolved in 6 ml cold nanopure water) was rapidly added to the suspension under vigorous stirring. Upon addition of NaBH\textsubscript{4}, the solution color immediately changes to black, indicating the formation of Au:SG clusters (in a mixture). The solution was allowed to react for another hour. The resulting precipitate was collected by centrifugation and washed several times with methanol.

Ligand exchange with hexanethiol to produce Au\textsubscript{38} clusters.

The Au:SG clusters synthesized by the previous step, were dissolved in 4 ml H\textsubscript{2}O. Then, 6 ml acetone and 8 ml hexanethiol (C\textsubscript{6}-SH) were added; the C\textsubscript{6}-SH phase forms the upper layer and the aqueous solution of Au:SG clusters forms the bottom layer. The solution was heated to 80\degree C and allowed to react for several hours under vigorous stirring. After 3 hrs, the Au clusters were found to completely transfer to the organic phase. During the phase transfer process, gold cluster core etching and secondary growth occurs. The isolated hexanethiol phase was washed thoroughly with excess ethanol and then with acetone to remove excess hexanethiol and by-products. The major fraction containing Au\textsubscript{38} clusters was extracted with toluene from the crude product. The purity of Au\textsubscript{38} clusters were further improved by extraction with a mixed toluene/acetone (1:5
v:v) solvent. Typically, 50-100mg pure Au	extsubscript{38}(SR)	extsubscript{24} clusters were obtained when 1g HAuCl	extsubscript{4}·3H	extsubscript{2}O was used as precursor.

2.11. Synthesis of 7-mercapto-4-methyl coumarin functionalized AuMPC [AuMC]

For AuMC synthesis the Brust Schiffrin two-phase method was followed. A 2:1 thiol:gold ratio was used and NaBH	extsubscript{4} reduction reaction for 30 min at 0 °C. For the preparation, to a vigorously stirred solution of 1.08 g, of Oct	extsubscript{4}NBr in 40mL toluene was added 0.5 g, of HAuCl	extsubscript{4}·3H	extsubscript{2}O in 10 mL of water. The water phase was rendered colorless, when the organic solution became dark red due to the transfer of AuCl	extsubscript{4}-. To this 0.26 g, mercapto coumarin in 40 mL DCM was added and stirred at 0 °C until the solution became colorless, indicating Au	extsuperscript{I}- thiol polymer formation. This was done overnight. Then an ice cold solution of 0.0855g, NaBH	extsubscript{4} dissolved in 20 mL of ice cold water was quickly added to the toluene/dichloromethane solution with vigorous stirring. The solution immediately became black, indicative of MPC formation. Stirring was continued for another 30 min at 0 °C. The aqueous phase was removed and the black organic phase was washed with water, dried over Na	extsubscript{2}SO	extsubscript{4} and rotary evaporated to produce a black product. The resulting solid was washed several times with hexane and acetone to remove excess ligand, and then stored cold for further reaction and analysis. The resulting core size was 2 nm.

2.12. Synthesis of 6-ferrocenylhexanethiol and C	extsubscript{6}S Au clusters

For synthesizing these sub-nanometer particles, a one phase procedure was followed. To 0.1g chloro(triphenylphosphine)gold(I) was added 0.2306g 6-(ferrocenyl hexanethiol) dissolved in 25 mL dichloromethane. The solution was stirred overnight for 15 hrs, until the color of the solution fades. Then added 0.27 g trihydro(2-methyl-2-propanamine) boron, (a reducing agent) or NaBH	extsubscript{4} was added to the stirred solution at -10°C, and stirring was continued for 3 hours. The resulting solution was rotary evaporated and washed with copious amounts of acetonitrile. The resulting
product was dried in a dessicator and stored in a refrigerator until further use. The resulting MPCs had a diameter of 1 nm and was predicted it to be Au_{11} or Au_{13,261} but eventually it is Au_{25} with a different crystal structure as reported by Tsukuda and Jin groups. Similar reactions were carried out with hexanethiol.

2.13. Synthesis of rotaxane-functionalized AuMPC

Ligand exchange reaction:

50 mg of 4 nm-Au(SG) in 20 mL water was mixed with 10 mg of MV^{2+}-SH for 10 hours at pH = 7. The resulting solution was rotary evaporated and washed several times with acetonitrile and methanol. The synthesis of MV^{2+}-SH is outlined later in the chapter, Figure 2.4. For the quantitative estimation of the number of MV^{2+}-SH molecules, the UV-Vis spectrum of a known mass of the exchanged product was recorded. The absorption profile of Au-SG was subtracted from the MV^{2+}-SH -attached gold cluster to get the absorption due to the MV^{2+}-SH alone. From this absorption value and using the extinction coefficient of MV^{2+}-SH, the concentration and the number of MV^{2+}-SH exchanged onto the gold core were calculated. From these calculations, MV^{2+}-SH attached to gold cluster was estimated to be 10.

2.14. Synthesis of Au_{25}(GS)_{18} PyNHS_{x} and Au_{314}(GS)_{101} PyNHS_{25} clusters

The Au clusters (30 mg) each were dissolved in 2:1 mixture of water and glyme, to this solution was added 20 parts (19 mg) of PyNHS for the Au_{25} clusters and 100 parts (11 mg) of PyNHS in the case of Au_{314} clusters. The pH of the solution was raised to 12 by adding a 0.25 M NaOH solution. The coupling reaction was allowed to continue for 12 hours at room temperature with constant stirring. This solution was then rotary evaporated and washed with 300 mL of dichloromethane to remove any excess dye molecules. These particles were characterized by TEM, and UV-Vis spectroscopy to confirm that the size remained unchanged.
2.15. Synthesis of Cysteine-AuMPCs

For the synthesis of cysteine-functionalized gold clusters a 4:1 Cysteine: Au\textsuperscript{III} or a 2:1 Cystine: Au\textsuperscript{III} is used. To 0.5 g of Au\textsuperscript{III} salt dissolved in 40 mL Methanol was added 0.6102g of Cystine in 10mL water. The pH of the solution was controlled at pH = 10. The solution was stirred for 10 min at room temperature. Then added 10 parts of NaBH\textsubscript{4} dissolved in ethanol. The temperature of the reaction mixture was maintained at 0º C. At the end of 30 minutes, the reaction was stopped and the contents were rotary evaporated. Redissolved the precipitate in 10 ml water and then reprecipitated with methanol. This process was repeated 5 times. The mixture was centrifuged and the impurities were removed.

The chromophores and rotaxanes needed for labeling the AuMPC were synthesized by our collaborators.

2.16. Synthesis of ligands


To a solution of 1-pyrenecarboxylic acid (0.7 g, 2.8 mmol) in dichloromethane (30 ml) and oxalyl chloride (0.42 g, 3.4 mmol) was added DMF (0.15 ml) dropwise. The reaction mixture was stirred for 2 hours at room temperature, and the solvent was evaporated in vacuo. The crude 1-pyrenecarbonyl chloride was used without further purification. To a solution of cysteamine hydrochloride (0.29 g, 1.27 mmol) and sodium hydroxide (0.23 g, 5.7 mmol) in distilled water was added a THF solution (20 ml) of 1-pyrenecarbonyl chloride dropwise, and the mixture was stirred for 12 hours. The yellow precipitate was filtered and washed with water. Then, the mixture
was washed with THF and filtered to remove starting materials. The resulting disulfide was treated with sodium borohydride (0.96 g, 25.4 mmol) in ethanol (100 ml) at 90 °C. After 2 hours, sodium borohydride (0.96 g, 25.4 mmol) was added and the resulting solution was stirred at 90 °C for 2 hours, and this step was repeated one more time. The mixture was cooled to room temperature and acidified with 1 N HCl solution to adjust to pH 2. After evaporation of ethanol and subsequent addition of chloroform, the organic layer was extracted, washed with water, and dried over anhydrous MgSO₄. After adding n-hexane and evaporating the organic solvent, N-(2-mercaptoethyl)pyrene-1-carboxamide was obtained in a 49 % isolated yield: ¹H NMR (400 MHz, CDCl₃) δ 8.58(d, 1H, J=9.2 Hz), 8.22(d, 2H, J=6.3 Hz), 8.15-8.00(m, 7H), 6.57(br s, 2H), 3.80(q, 2H, J=6.3 Hz), 2.92(m, 2H), 1.48(t, 1H, J=8.8 Hz) ¹³C NMR(100 MHz , CDCl₃) δ 170.2, 132.7, 131.3, 131.8, 128.9, 127.2, 126.5, 126.0, 125.9, 124.9, 124.6, 124.4, 43.1, 25.0; IR spectrum (CHCl₃) cm⁻¹; 3685, 3020, 2443, 2400, 1656, 1533, 1425, 1215, 926, 760, 659. HR-MS(FAB) Calcd. for C₁₉H₁₅NOS(M+H⁺) 306.0953 found 306.0954. Anal. Calcd for C₁₉H₁₅NOS: C, 74.72; H, 4.95; N, 4.59; found: C, 74.33; H, 4.98; N, 4.38.

N-(2-mercaptoethyl)pyrene-1-carboxamide

2.16.2. Synthesis of (E)-7-(diethylamino)-3-((4-mercaptophenylimino) methyl)-2H-chromen-2-one (Cou-SH): Mr. Fasil Adefris Abebe (cohort and fellow group member)

The synthesis was carried out in a three stage process; the formation of the 7-diethylaminocoumarin, followed by conversion to an aldehyde by means of literature procedure²⁶², this is followed by subsequent Schiff base condensation reaction between the
synthesized aldehyde and commercially available 4-aminobenzenethiol, Figure 2.3. The synthesis is outlined in brief as follows:

Stage 1: Synthesis of 7-diethylaminocoumarin

4-Diethylaminosalicylaldehyde (1.93g, 10mmol), diethylmalonate (3.2g, 20 mmol) and piperidine (1mL) were combined in absolute ethanol (30 mL) and stirred for 16hrs under reflux conditions. Ethanol was evaporated under reduced pressure, then concentrated HCl (20 mL) and glacial acetic acid (20 mL) were added to hydrolyze the reaction with stirring for another 16 hours. The solution was cooled to room temperature and poured into 100 mL ice water. NaOH solution (40%) was added dropwise to adjust the pH of the solution to 5.0, and a pale precipitate formed immediately. After stirring for 30 min., the mixture was filtered, washed with water, dried, then recrystallized with toluene to give 1 as grey powder (1.73 g, 8.0 mmol) in 80% yield. $^1$H-NMR (CDCl$_3$): δ 7.53 (d, 1H), 7.20 (d, 1H), 6.48 (d, 1H), 6.40 (s, 1H), 6.01(d, 1H), 3.40 (m, 4H), 1.20 (t, 6H).

Stage 2: Synthesis of 7-diethylaminocoumarin-3-aldehyde

Freshly distilled DMF (2 mL) was added drop wise to POCl$_3$ (2 mL) at 35°C in a N$_2$ atmosphere and stirred for 30 minutes to yield a red solution. This solution was combined with a portion of 1 (1.50 g, 6.91 mmol, dissolved in 10 mL DMF) to yield a scarlet suspension. The mixture was stirred at 60 °C for 12 hours and then poured into 100 mL of ice water. NaOH solution (20%) was added to adjust the pH of the mixture to yield a large amount of precipitate. The crude product was filtered, thoroughly washed with water, air dried and recrystallized in absolute ethanol to give the product 2 (1.20 g, 4.89 mmol) in 70.8% yield. $^1$H NMR (CDCl$_3$): δ 10.15 (s, 1H), 8.24 (s, 1H), 7.40 (d, 1H), 7.40 (d, 1H), 6.43 (d, 1H), 6.47 (s, 1H), 3.47 (m, 4H), 1.21 (t, 6H).

Stage 3: Synthesis of Cou-SH
In a 25 mL flask, 7-diethylaminocoumarin-3-aldehyde 2 (0.10 g, 1 mmol) and 4-aminobenzenethiol (0.05 g, 1 mmol) were suspended in 20 mL ethanol. The mixture was refluxed for 7 hr with stirring, during which time a gray precipitate formed. The precipitated was separated by filtration and washed with 2 x 10 mL ethanol. After drying, a gray solid in 85% yield was obtained. $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ (ppm) 8.40 (s, 1H), 7.69 (s, 1H), 7.66 (s, 1H), 7.30 (d, 2H), 7.03 (d, 2H), 6.8 (s, 1H), 6.61 (s, 1H), 6.47 (s, 1H), 3.42 (m, 4H), 3.3 (s, 1H), 1.16 (t, 6H).

![Figure 2.3. Schematic for the synthesis of Cou-SH](image)

2.16.3. Synthesis of Rotaxane: Dr. Gellert Meizi’s group; Ms. Isurika Rosini Fernando, PhD student and a South West Michigan High School Student.

Synthesis of MV$^{2+}$-SH (5)
Figure 2.4. Schematic of reaction to synthesize MV$_{2}^{2+}$SH

1-(1-butyl-4-thioacetate)-4,4’-bipyridinium bromide (2)

A solution of S-4-bromobutyl thioacetate (1) (500 mg, 2.368 mmol) in 4 mL dry acetonitrile was added to a refluxing solution of 4,4’-bipyridine (1.109 g, 7.104 mmol) in 8 mL dry acetonitrile under nitrogen. After refluxing for two days, the reaction mixture was added to 60 mL diethyl ether. The precipitate was filtered out, washed with 20 mL acetonitrile:diethyl ether (1:5) mixture and dried in vacuum to yield a brown solid (2) (653 mg, 75%). $^1$H NMR (D$_2$O, 400 MHz): $\delta$ (ppm) = 8.95 (d, J = 6.24 Hz, 2H), 8.76 (s, 2H), 8.40 (d, J = 6.20 Hz, 2H), 7.92 (d, J = 5.16 Hz, 2H), 4.67 (t, J = 7.32 Hz, 2H), 2.92 (t, J = 7.14 Hz, 2H), 2.33 (s, H), 2.10 (m, 2H), 1.67 (m, 2H).

1-(4-acetylthiolbutyl)-1’-[4-(carboethoxy)butyl]-4,4’-bipyridinium bromide (4)

Ethyl-4-bromobutyrate (3) (1.039 g, 5.332 mmol) in 3.0 mL dry acetonitrile was transferred under nitrogen into a solution of 2 (653 mg, 1.77 mmol) in 3 mL dry acetonitrile, and then refluxed for two days. After cooling to room temperature, the resulting yellow precipitate was
collected by filtration and dissolved in a minimum amount of methanol. Addition of a large volume of diethyl ether (methanol : diethyl ether ~ 1:10) lead to the precipitation of a yellow solid, which was filtered and dried in vacuum (757 mg, 76 %). $^1$H NMR (D$_2$O, 400 MHz): $\delta$ (ppm) = 9.09 (m, 4H), 8.51 (m, 4H), 4.72 (m, 4H), 4.09 (q, J = 7.21 Hz, 2H), 2.90 (t, J = 7.14 Hz, 2H), 2.53 (t, J = 7.14 Hz, 2H), 2.33 (m, 5H), 2.11 (m, 2H), 1.64 (m, 2H), 1.88 (t, J = 7.14 Hz, 3H).

1-(4-thiolbutyl)-1’-[4-(carboxy)butyl]-4,4’-bipyridinium bromide (5)

0.88 mL HBr (2 M in H$_2$O) was added to solid 4 (378 mg, 0.67 mmol). The resulting solution was heated to 100 °C, then the solvent was slowly evaporated using a mild vacuum to obtain a yellow solid (297 mg, 90 %). $^1$H NMR (D$_2$O, 400 MHz): $\delta$ (ppm) = 9.10 (t, J = 6.78 Hz, 4H), 8.52 (d, J = 3.62 Hz, 4H), 4.74 (m, 4H), 2.56 (m, 4H), 2.35 (m, 2H), 2.16 (m, 2H), 1.66 (m, 2H).

2.17. Electrochemistry

2.17.1. 3- Probe electrode fabrication

The 3-probe electrode used in the all electrochemical measurements consists of the tips of three wires exposed in an insulating plane: two Pt (0.4 mm diameter) wire electrodes for working and counter electrode, and a Ag (0.5 mm diameter) wire for quasi-reference electrode (QRE).$^{263}$ The electrode wires were connected to polymer coated copper wire with silver epoxy (Epo-Tek H2OE, Epoxy Technology Inc.). The group of three electrodes was inserted through a 1/4 in. regular polymer (PTFE) tube and potted in place with an epoxy resin (poly(bisphenol A-co-epichlorohydrin), glycidyl end-capped, MN ca. 377; Aldrich) cross-linked with 14 wt % 1,3-phenylenediamine (Fluka). The end of the assembly was polished with alumina paste (successively smaller grades down to 0.05 μm; Buehler) and cleaned electrochemically in 0.1 M H$_2$SO$_4$ solution.
Voltammetry was carried out on an electrochemical workstation (Model 660B, CH instruments) in 0.1 M Bu₄NClO₄ in CH₂Cl₂ solutions that were degassed with high-purity Ar gas. Square wave voltammetry (SWV) was carried out with potential steps of 4 mV, square wave amplitude of 25 mV, square wave frequency of 15 s⁻¹, and a quiet time of 10 s. Sublimed ferrocene (Fc) was added as an internal reference for Ag quasi-reference electrode. Hence, potentials are reported versus Fc/Fc⁺.

2.17.2. Electrochemical impedance measurements

Electrochemical impedance spectroscopy (EIS) measurements were carried out using an electrochemical sensor device, photo-lithographically fabricated on 4 in. round glass wafers, with overall device dimensions of 2 cm × 1 cm × 0.05 cm. This device consists of Au interdigitated electrodes [IDT’s], Figure 2.5 which have a spatial periodicity (λ) of 20 µm, interdigitated space of 5 µm, length of 4995 µm and Au layer thickness of 0.1 µm. The design and fabrication process of the electrochemical sensor device used in this study has been reported previously by Narakathu et al. Before use, the sensor was cleaned with acetone and distilled water, and then dried with pressurized air. An Agilent E4980A precision LCR meter, connected to the sensor via small outline integrated circuit (SOIC) test clips, was used for impedance measurements at frequency ranges between 20 Hz to 2 kHz with a 1 mV voltage excitation. All measurements were conducted at room temperature. First, 10 µL of a solution of acetonitrile/dichloromethane 1:1 mixture, then a blank solution of 1 µM AuNC were placed on the sensor to establish a reference signal. The sensor was rinsed with distilled water and dried in a stream of pressurized air. Then 10 µL of 1pM solution of DCP/AuNC was placed on the sensor. The impedance response of the sensor towards varying concentrations of 10 µL of AuNC (100 mM, 1mM, 100 µM, 1µM, 100nM, 1 nM, 100pM and1 pM) in DCP and HCl solutions were then measured. A custom-built data acquisition and analysis LABVIEW program was used for post processing of
the measured impedance data. At the end of each test, the sensor was rinsed with acetone and distilled water followed by drying in a stream of pressurized air.

Figure 2.5. Photolithographically fabricated AuIDT

2.18. Optical Methods

2.18.1. Steady-state fluorescence measurements

The steady-state fluorescence measurements were carried out on an F900 Edinburgh Spectrofluorimeter and a Hitachi F-2500 Spectrofluorimeter. For calculations of quantum yield, a standard was used, depending upon the wavelength of absorption. Usually, Coumarin 515, Bis-MSB and Exalite dyes were used. Solutions of samples with optical density of 0.1 at the excitation wavelength were used. Fluorescence spectroscopy can also be used to characterize or identify the chromophores. Quantum yield of fluorescence is a very important parameter, which is defined as the ratio of the number of fluorescence photons emitted to the total number of quanta absorbed. Experimentally, it is customary to determine the quantum yield of any unknown sample with respect to a reference whose quantum yield is known. Fluorescence quantum yield $^{265}$ ($\phi_{\text{sample}}$) of a sample can be determined with respect to the quantum yield of a reference ($\phi_{\text{reference}}$) using the equation:
\[ \phi_{\text{sample}} = \frac{A_{\text{reference}}}{F_{\text{reference}}} \times \frac{F_{\text{sample}}}{A_{\text{sample}}} \times \phi_{\text{reference}} \]  

(2.1)

where \(A_{\text{reference}}\) and \(A_{\text{sample}}\) are the absorbances of reference and sample, respectively, while \(F_{\text{reference}}\) and \(F_{\text{sample}}\) are the corresponding integrated fluorescence intensities.

2.18.2. Time-resolved fluorescence measurements

Time-resolved fluorescence measurements reveal the variation of fluorescence intensity as a function of time after the creation of the excited state. It monitors the evolution of the excited state and the mechanism by which the excited state relaxes to the ground state. A well-established technique of time-correlated single-photon counting (TCSPC) technique\(^{266, 267}\) has been used to monitor the time-resolved emission and the principle and operation can be described as follows.

The technique involves the excitation of the sample with pulses from a laser or a flash lamp and the detection system monitors the time difference between the excitation pulse and the first fluorescence photon from the sample. The prerequisite for this measurement is that only one photon is observed for a large number of excitation pulses. In other words, an extremely low count rate must be ensured such that the system operates in single photon counting mode. In such a situation, the statistics follows the Poisson distribution and a true time-resolved emission profile is obtained.

An excitation pulse (optical pulse) either from the flash lamp or a laser is split into two parts, one part is used to excite the sample kept in the sample chamber and the other part is used to generate a start pulse in the start PMT or photodiode. The optical signal at the start PMT generates an electrical START pulse, which is then routed through a constant fraction discriminator (CFD) to START the input of time to amplitude converter (TAC) to initialize the charging operation. The part of the optical pulse, which excites the sample effectively, gives rise to emission of photons.
These photons are then detected by STOP PMT (at a right angle to the direction of excitation) to generate an electrical STOP pulse. The STOP pulse is also routed to the TAC after passing through another CFD and a variable delay line. On receiving the STOP signal, TAC stops its charging operation and generates an electrical output, having an amplitude proportional to the time difference (Δt) between the START and STOP pulses reaching the TAC. The TAC output pulse is then fed to the input of a multi channel analyzer (MCA) through an analogue to digital converter (ADC). The ADC generates a numerical value corresponding to the TAC output pulse and thus selects an appropriate channel of the MCA and the count is added to the channel. The above cycle (from excitation to data storage) is repeated a large number of times and as a result a histogram of the counts versus the channel number of MCA is generated. It represents the true emission decay, when the collection rate of emission photons by the STOP pulse is very low, as illustrated by the statistical treatment.266,267

The emission decay thus observed has to be de-convoluted with the instrument response to get the actual lifetime. The instrument response function (IRF) is measured using a scatter placed in place of the sample. The width of IRF function depends on the excitation source and detection systems used. For the measurements described in the thesis, diode laser are used.

2.18.3. Fluorescence upconversion: Harmonic generation

Nonlinear optical processes such as sum- and difference frequency generation, parametric oscillation and amplification, offer the possibility of changing the central wavelength of the pulse and thus provide flexibility to the experimental demand. To obtain femtosecond pulses in the UV region, second harmonic generation (SHG) is employed. BBO crystal of 0.5 mm thickness is used for generating second harmonic of 800 nm to produce pulses at 400 nm. The second harmonic generated is polarized perpendicular to the fundamental (type I phase matching). With an input energy of approximately 100 µJ at 800 nm, focused with a lens on to the BBO crystal, 10 µJ at
400 nm is attained and is separated from the fundamental by a dichroic mirror, which reflects the 400 nm and transmits the 800 nm. The wavelength thus obtained is the central wavelength used for excitation in a majority of the investigations presented in the thesis.

The upconversion system used in our experiments was obtained from CDP Instruments, Inc., Russia. Specifically, the system used second or third harmonic generation from the mode-locked broad band Ti-sapphire laser (Spectra Physics, Tsunami, 710 to 920 nm), Figure 2.6. The present measurements were carried out with either 280 nm or 400nm excitation (fundamental of Ti:sapphire was set at 840 nm or 800nm). Polarization of the excitation beam for the magic angle fluorescence and anisotropy measurements was controlled using a Berek compensator and the sample was continuously rotated with a rotating cell of 1 mm thickness. Horizontally polarized fluorescence emitted from the sample was up-converted in a nonlinear crystal of β-barium borate using a pump beam at 800 nm, which first passed through a variable delay line. Instrument response function (IRF) was measured using the rise time of several dye molecules. Fitting the rise time of the fluorescence traces gave a sigma value of ~290 fs. Parallel and perpendicular decay traces were obtained from which the magic angle fluorescence decay was calculated. Fitting of the fluorescence decay traces were carried out with DecFit software. Spectral resolution was achieved using a double monochromator and photomultiplier tube. The excitation average power varied, but was around 10 ± 0.3 mW. No degradation of the sample was observed as the traces overlapped after each repetition.
Besides the second harmonic of the Ti-Sapphire-laser, the system can be modified with excitation using third harmonic (tunable around 266 nm) and that from fundamental beam directly (two-photon excitation mode). In order to produce the third harmonic light, the second harmonic and the fundamental beams are combined in an additional BBO crystal which is able to generate the sum frequency beam around 266 nm with intensities up to 15mW. For two-photon (fundamental) excitation mode, the second harmonic unit is disabled by either turning the BBO crystal by the appropriate amount or by simply removing it. The beam splitter which splits out the excitation beam is replaced with one that reflects 50% of the fundamental beam for excitation. The filter
rejecting visible light is installed in excitation channel to make sure that the fundamental light is the only source of excitation. The fluorescence upconversion/detection part of the system remains unchanged which allows us to accurately compare the fluorescence profiles at particular detection wavelength under one- and two photon excitation.

2.18.4. UV-Vis spectroscopy

Optical absorption (ultra violet-visible, UV-Vis) spectroscopy is a widely used technique to provide the information about the ground state of the absorbing molecule (wavelength of absorption and extinction coefficient of the chromophore under investigation). UV-Visible absorption spectroscopy, being dependent on the electronic structure and the environment of the absorbing chromophore, allows the characterization and the identification of molecules. Changes in the solvent polarity often induce a shift in the absorption spectrum. This technique is the most important one to characterize the charge transfer interactions in the ground state as well as the optical properties of semiconductor nanoparticles. The absorbance ($A$) of an absorbing species (with concentration $C$) having a molar extinction coefficient $\varepsilon_{\lambda}$ at wavelength $\lambda$ is given by the equation:

$$A = \log\left(\frac{I_0}{I}\right) = \varepsilon_{\lambda}Cl$$

(2.2)

where $I_0$ and $I$ are the intensities of the incident and transmitted light, respectively and $l$ is the path length of the light beam passing through the sample in the cuvette (quartz).

Optical absorbance spectra were collected with a UV-2101PC Shimadzu UV-Vis scanning or a Perkin-Elmer spectrometer (Lambda 40). The spectra were collected over a range of 250-900 nm or 300-1200 nm in specific cases such as Au$_{38}$ spectrophotometer using a 1 cm path length quartz cuvette (STARNA cells).
For temperature dependent measurements, the optical absorbance spectra were collected with a UV-2101PC Shimadzu UV-Vis scanning spectrophotometer (Au$_{25}$) and an Olis Cary-14 spectrophotometer (Au$_{38}$), using a 1 mm path length quartz cuvette (STARNA cells). The spectra were collected over a range of 300-900 nm for Au$_{25}$ and Au-GS-4nm and 350-1200nm for the Au$_{38}$. MPC solutions were prepared in a mixture of 1:1 methylcyclohexane/methylcyclopentane for Au$_{25}$(C$_6$S)$_{18}$, Au$_{38}$(PhC$_2$S)$_{24}$ and a 3:2 glycerol/water mixture for Au(GS)-4nm. In order to control the temperature, we used an Oxford Optistat DN cryostat coupled to an external temperature controller (ITC 601). The cryostat was purged with high purity nitrogen before and during the experiment. This is absolutely essential to maintain an oxygen-free environment in order to form a clear glass at lower temperatures. The temperature within the cryostat was lowered gradually to 78 K using liquid nitrogen, under high vacuum using a molecular ion pump, Turbotronik NT10 equipped with a Varian Ion Gauge in tandem with a roughing pump, Welch Duo Seal (1405). The measurements were taken at periodic temperature intervals of 10 K/15 K after equilibration for ten minutes at each temperature. The layout is seen in Figure 2.7.

Figure 2.7. Set-up for temperature dependent UV-vis measurements
2.18.5. Two-photon measurements

Two-photon sensing and the two-photon absorption cross sections (δ) measurements were carried out using the two-photon excited fluorescence (TPEF) method described elsewhere. A 10⁻⁴ M Coumarin-485 solution in methanol was used as the reference over a wavelength range of 800–900 nm whose cross sections have been reported earlier. A broad-band Ti:Sapphire oscillator (Tsunami, Spectra Physics) was utilized for the investigations. A neutral density filter was used to change the power to measure the power dependence. The other weaker filters might heat up and develop spots on their coatings. Polarizers used for varying input intensity might result in white light generation which is found to give spurious signals. Prior to the ND filter, the beam is passed through an iris to ensure a uniform and circular beam. In case of the Ti: Sapphire oscillator, the beam is focused on the sample cell, which contains a solution of the molecule to be investigated. It is important to deliver the beam closer to the edge of the sample cell towards the direction of collection rather than center. This reduces the path length that the fluorescence has to travel prior to exiting the cell and being collected, thereby minimizing self-absorption of fluorescence. The resulting TPEF is collected in a direction perpendicular to the incident beam in order to avoid noise due to excitation light itself. Typically, in TPEF method a reference with known TPA cross section values over a series of wavelengths is used. However, modifications of this method that eliminate the need for the reference have also been reported. Typically, Coumarin 307, Rhodamine B and Fluorescein are used as standards over 700-950 nm range, p-bis (o-methyl-styryl) benzene is typically used as the reference below 700 nm and certain porphyrin dyes are used as standards above 1000 nm. The advantages of TPEF are it is relatively easy to perform and there are no spurious effects due to excited state absorption, thermal lensing etc. that are associated with some of the other techniques. The disadvantage is that the material has to
exhibit fluorescence. Hence, this method cannot be applied to certain inorganic, metallic and organometallic materials. For most of our experiments Coumarin-485 was used as the standard.

Table 2.1. Data obtained for C-485 at 760 nm excitation

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Figure 2.8. Logarithmic plot showing quadratic dependence between TPEF intensity and input intensity for Coumarin 485

Table 2.2. Data obtained for AuMC at 760 nm excitation

<table>
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<th>log(Counts)</th>
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<tr>
<td>720</td>
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<td>6.00E+00</td>
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<td>0</td>
<td>5.70E+01</td>
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</table>
Figure 2.9. Logarithmic plot showing quadratic dependence between TPEF intensity and input intensity for AuMC

Table 2.3. Calculation of two-photon cross-sections

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>R-Kαηδ</th>
<th>Φ</th>
<th>S-c</th>
<th>S-n</th>
<th>S-int</th>
<th>S-ηδ</th>
<th>S-h</th>
<th>S-δ (GM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cou Me 710</td>
<td>2.78E-24</td>
<td>3.22E+29</td>
<td>7.24E-05</td>
<td>1.3284</td>
<td>5.952</td>
<td>3.4</td>
<td>0.1700</td>
<td>20</td>
</tr>
<tr>
<td>MC DCM 710</td>
<td>2.24E-24</td>
<td>6.02E+27</td>
<td>5.46E-05</td>
<td>1.4241</td>
<td>4.130</td>
<td>3.40E+00</td>
<td>0.0400</td>
<td>85</td>
</tr>
<tr>
<td>AuMC1 DCM 710</td>
<td>4.47E-24</td>
<td>4.88E+27</td>
<td>1.09E-04</td>
<td>1.4241</td>
<td>4.339</td>
<td>3.40E+00</td>
<td>0.0020</td>
<td>1700</td>
</tr>
</tbody>
</table>

The fluorescence of Cou-SH (10⁻⁴) and Coumarin-Au (1μM) hybrid measured in dichloromethane rendered a slope of two indicating that it is quadratically dependent on the input intensity and hence is a two-photon absorption event. The experimental setup is seen in Figure 2.10.
2.18.6. Transmission electron microscopy (TEM)

TEM images of MPC samples were obtained with a JEOL transmission electron microscope (JEM-1230), Figure 2.11. Because the wavelength of electrons is much smaller than that of light, the resolution attainable for TEM images is orders of magnitude finer than that from a light microscope. Magnification (steps): MAG mode x1,000-600,000  Low MAG mode x50-1,000 IOS mode x2,500-30,000 (±90°) MPC samples were prepared by dipping a Formvar/carbon-coated copper grid (400C-FC, EMS) in 1 mg/mL MPC in CH₂Cl₂ and drying in air for 1 h before imaging. Three typical regions of each sample were imaged at 600 K magnification. Core-size histograms were read from digitized photographic images using ImageJ software [http://rsb.info.nih.gov/ij/]. Values were confirmed by manual reading.
2.18.7. Nuclear magnetic resonance

NMR spectra were recorded using a 400 MHz Jeol Eclipse nuclear magnetic resonance instrument.

2.18.8. Transient absorption measurements

As the central theme of the thesis is to investigate the phenomenon of fast and ultrafast interfacial electron transfer, high time resolution is an absolute necessity. The prominent experimental technique that has been used in this thesis is transient pump-probe spectrometry. In such an experiment, a strong pump pulse is used to initiate a reaction and the reaction dynamics is followed by recording the changes in absorbance of a weak monitoring pulse, as a function of the time delay between the pump and the monitoring pulse. In the measurement, the monitoring pulse is divided into probe and reference pulses. The probe pulse is spatially overlapped with the pump
pulse in the sample, while the reference pulse passes through a region of the sample which is unaffected by the pump. The experimental signal, i.e. the change in absorbance ($\Delta A$), is obtained as the negative logarithm of the ratio of the intensity of the probe and reference pulses as given by the equation:

$$\Delta A = \log \left( \frac{I_0}{I} \right)$$

(2.3)

Solution phase absorption and emission spectra usually contain broad bands, which make it essential to examine the temporal behavior of the photoinduced reaction over a wide range of probe wavelengths. In the experiments described here, both the probe and the reference pulses are made up of white light continuum (WLC). The WLC is generated by the non-linear phenomenon of self-phase modulation of an intense laser pulse propagating through a dense but transparent medium.\textsuperscript{272, 273} Under proper experimental conditions, a WLC can be made to extend from the near-UV to near-infrared and it can be detected by either integrating photodiodes or a spectrograph together with a charge-coupled device. In these experiments, $\Delta A (\lambda, \Delta t)$ is obtained as a function of the probe wavelength $\lambda$ and the delay time $\Delta t$ between the pump and the probe pulses.
Figure 2.12. Scheme of the states involved in a pump probe experiment in a dye solution. (A) Excited state absorption, (B) bleaching, and (C) stimulated emission

In the experiments described above, there can be three different contributions to the transient absorption signal (see Figure 2.12). First, a strong pump pulse excites the molecules from the ground state ($S_0$) to a higher excited state $S_1$ or $S_n$. Then, the molecules in the excited state absorb probe light, termed as excited state absorption (process A in Figure 2.12). This is seen as decrement in the probe light and hence there is an increase in $\Delta A$. The probe pulse may also excite the molecules from the ground state to the same excited state as the pump pulse, which will result in bleaching of the ground state, process B in Figure 2.12. This will be seen as an increase in the detected probe intensity since less probe photons are absorbed relative to the reference. An increase in probe intensity is the same as negative $\Delta A$. Finally there may be stimulated emission from the molecule, which can be seen as process C in Figure 2.12 and this will be detected as a decrease in the $\Delta A$. The time resolution of such an experiment does not depend on the response of
the detector rather on the width of the pump and probe pulses. This method is usually followed for shorter times of sub-nanosecond duration where fast detectors are not readily available.

Femtosecond transient absorption investigations were carried out at the laboratory of Prof. Theodore Goodson III at the University of Michigan, Ann Arbor and using ultrafast pump-probe spectrometer detecting in the visible region described elsewhere. Briefly, 1 mJ, 100 fs pulses at 800 nm with a repetition rate of 1 KHz were obtained from Nd:YLF (Empower) pumped Ti:Sapphire regenerative amplifier (Spectra-Physics) with the input from Millennia pumped Ti:Sapphire oscillator (Spectraphysics, Tsunami). The output of laser beam was split to generate pump and probe beam pulses with a beam splitter (85% and 15%). The pump beam was produced by an optical parametric amplifier (OPA-800C). The pump beams used in the present investigation, i.e., 345 nm, was obtained from the fourth harmonic of the signal beam. They were focused onto the sample cuvette. The probe beam was delayed with a computer controlled motion controller and then focused into a 2 mm sapphire plate to generate white light continuum. The white light was then overlapped with the pump beam in a 2 mm quartz cuvette containing the sample and the change in absorbance for the signal was collected by a CCD detector (Ocean optics). Data acquisition was controlled by the software from Ultrafast Systems. Typical power of the probe beam was around 10 µJ/cm² while the pump beam was around 1000 µJ/cm². Magic angle polarization was maintained between the pump and probe using a wave plate. The pulse duration was obtained from the non-resonant fitting of the solvent response, which was around 130 fs. The sample was stirred by a rotating magnetic stirrer and little degradation of the sample was observed during the experiments. Schematic is in Figure 2.13.
Figure 2.13 Schematic of transient absorption set-up
CHAPTER III

ULTRAFAST VISIBLE LUMINESCENCE IN MONOLAYER-PROTECTED Au_{25} CLUSTERS

3.1. Introduction

Monolayer-protected gold clusters (MPCs) have captivated the imagination of chemists for their exciting optical, magnetic and catalytic properties. When the diameter of a metal cluster is reduced to the order of the metal’s Fermi wavelength, the quantization of the energy levels takes place which leads to notable consequences for optical, electronic, charging, and transport phenomena. At sufficiently smaller cluster sizes, the continuous optical spectra, including the broad surface plasmon resonance, cede to discrete electronic transitions among quantized levels, each associated with a delocalized orbital. The MPCs with core-diameter below 2.2 nm have shown behavior pertaining to quantum size optical and electrochemical properties, of which, Au\textsubscript{25}L\textsubscript{18} clusters are the central focus of research because of their stability and structure in the electronic spectra. Exciting optical properties are observed for stable Au\textsubscript{25}L\textsubscript{18} clusters whose crystal structure is comprised of icosahedral 13 gold core atoms protected by S-Au-S-Au-S linkages (staple motifs or semi-ring motif). Density functional theory calculations are consistent with the distinct electronic excited states observed in the optical absorption spectra and also has shown that the absorption in the visible region arises out of Au\textsubscript{13} core states. Furthermore, recent ultrafast time-resolved measurements have shown excited state characteristics consistent with the relaxation of Au core states and Au-semi ring states. The degenerate pump-probe combined with femtosecond time-resolved measurements has shown
coherent oscillations and the opening of the band gap for MPCs less than 2.2 nm. The frequency of the coherent oscillations is around 2 THz and is ascribed to the Au-Au core vibrations.\textsuperscript{180} Using time-resolved grating and absorption experiments, Moran and co-workers\textsuperscript{283, 285} demonstrated the relaxation of Au core states to Au semi-ring states, present not only in Au\textsubscript{25}L\textsubscript{18} but also in Au\textsubscript{24}PdL\textsubscript{18} clusters. On the other hand near infrared (NIR) luminescence is observed in monolayer-protected Au\textsubscript{25}L\textsubscript{18} clusters, the origin of which has been under intense research.\textsuperscript{286-292} The relationship between the new time-resolved revelations, the NIR luminescence and their ligand dependence are not well understood. The intra band-gap luminescence dynamics can prove the involvement of higher excited states and the consequent relaxation of the higher excited states to the NIR luminescent states. Also, accurate lifetimes of the higher excited-states in Au\textsubscript{25}L\textsubscript{18} clusters will enable their use in solar energy conversion and photo-catalytic applications.

In this chapter, efforts are focused on studying the dynamics of higher excited state relaxation dynamics in Au\textsubscript{25}L\textsubscript{18} clusters and its ligand dependence. Time-resolved fluorescence upconversion investigations in the visible region were utilized to probe the dynamics of intra band-gap states and the relaxation to NIR luminescent states. Comparative fluorescence measurements were carried out on gold clusters of varying sizes (2.2 nm, 3 nm and 13 nm) and in different environments (DCM and water). The results are explained on the basis of the relaxation of core Au states to semi-ring states.

Gold clusters of different sizes which are soluble in organic phase (DCM) and aqueous phase are synthesized and characterized as descibed in Chapter II (Transmission electron microscopy images are provided in Appendix I). Synthesized Au\textsubscript{25}L\textsubscript{18} clusters have a core diameter of 1.1 ± 0.2 nm and the electrochemical band gap measurements of Au\textsubscript{25}(C\textsubscript{6}S)\textsubscript{18} are consistent with literature reports.\textsuperscript{85, 293}
3.2. Optical absorption and steady-state luminescence measurements

The electronic absorption spectra of $\text{Au}_{25}(\text{C}_6\text{S})_{18}$ dissolved in DCM and $\text{Au}_{25}(\text{GS})_{18}$ in water are shown in Figure 3.1A. Both gold clusters in different media show the absorption spectra with a maximum of 675 nm and shoulders in the visible to ultraviolet region that match quite well with earlier investigations. Interesting steady-state photo-luminescence features are observed for $\text{Au}_{25}(\text{C}_6\text{S})_{18}$ and $\text{Au}_{25}(\text{GS})_{18}$ with luminescence in two different frequency regions (Figure 3.1B): one strong luminescence in the near infrared (NIR) region and a weaker luminescence in the visible region. Most literature reports on the luminescence of $\text{Au}_{25}\text{L}_{18}$ clusters focus on the luminescence in the NIR region since it has higher quantum yield. The NIR luminescence dynamics has been investigated for $\text{Au}_{25}\text{L}_{18}$ clusters by Link et al. and the time constants varied from a few nanoseconds to tens of nanoseconds and were ascribed to the luminescence arising out of the singlet states and triplet states of the gold clusters. The time-correlated single photon counting measurements were carried out in the present investigation to probe the luminescence dynamics of $\text{Au}_{25}(\text{GS})_{18}$ in water at the luminescence maximum of 710 nm. Unfortunately, the luminescence did not decay within the 200 ns (maximum repetition rate of 5 MHz of the diode, lifetimes of 276 ns and 1.2 microsecond are reported for the $\text{Au}_{25}(\text{GS})_{18}$) time delay of the instrument. The results suggest that the NIR luminescence is long-lived and probably arises out of the complexes formed from the ligands attached to the surface gold atoms. A similar luminescence is observed for the Au(GS) polymer which is a precursor to $\text{Au}_{25}(\text{GS})_{18}$ clusters. In recent work, Jin and co-workers have shown that the NIR luminescence in $\text{Au}_{25}$ clusters is highly influenced by the passivating ligand and the charge state of the cluster. The optical and ligand-exchange studies support the interpretation that the luminescence occurs from
the excited-states localized on the complexes of the ligands with surface gold atoms\textsuperscript{288, 289, 293} and can be ascribed to the luminescence from the Au semi-ring states.

In addition to the NIR luminescence, a significantly lower quantum-yield luminescence is observed in the present investigation for \text{Au}_{25}(\text{C}_6\text{S})_{18} in the visible region (Figure 3.1B). The luminescence has a maximum centered around 500 nm and the quantum yield is in the range of $10^{-6}$ to $10^{-7}$ (Figure 3.1B). Also, visible luminescence is observed for \text{Au}_{25}(\text{GS})_{18} clusters though its intensity is quite weak (Figure 3.1B). In previous work\textsuperscript{280, 288, 294}, visible luminescence was shown for gold clusters of different sizes from 1.1 nm to 4 nm and also for larger gold nanoparticles possessing the surface plasmon absorption. The visible luminescence in larger nanoparticles was ascribed to the luminescence arising out of inter-band gap states (d$\rightarrow$sp transitions) and Auger recombination.\textsuperscript{295, 296} The lifetime of this luminescence was found to be less than 100 fs (instrument response limited). Along similar lines, the dynamics of the low quantum-yield visible luminescence can provide insight into the excited-state dynamics of the \text{Au}_{25} clusters.

![Figure 3.1](image_url)

Figure 3.1. (A) Optical absorbance and (B) luminescence spectra of \text{Au}_{25} clusters capped with \text{C}_6\text{S} and GS in DCM and water respectively
3.3. Fluorescence upconversion measurements

Visible luminescence comparison for different sized clusters

Fluorescence upconversion measurements are utilized to probe the visible luminescence from the MPCs. A comparative investigation of the dynamics of different sized gold clusters is carried out and the corresponding time-resolved decay traces at the luminescence wavelength of 520 nm are shown in parts A and B of Figure 3.2. Time-resolved fluorescence measurements of the monolayer-protected gold clusters were studied using the newly set femtosecond fluorescence up-conversion spectroscopic technique. The upconversion system used in our experiments was obtained from CDP Instruments, Inc., Russia. In the present work, investigations were carried out with second harmonic (400 nm) of the fundamental Ti:Sapphire laser at 800 nm as the excitation source. Horizontally polarized fluorescence emitted from the sample was up-converted in a nonlinear crystal of $\beta$-barium borate using a pump beam at 800 nm, which first passed through a variable delay line. Instrument response function (IRF) was measured using the rise time of several dye molecules. Fitting the rise time of the fluorescence traces gave a sigma value of ~290 fs. Parallel and perpendicular decay traces were obtained from which the magic angle fluorescence decay was calculated from these traces. Anisotropy of the fluorescence measurements was calculated and the anisotropy was close to zero for the fluorescence from the gold clusters. Spectral resolution was achieved using a double monochromator and photomultiplier tube. The excitation average power was around 10 ± 0.3 mW and an average collection time of 3 sec was used for most wavelengths. No degradation of the sample was observed as the traces overlapped after each repetition and the optical absorption measurements did not show any change before and after the measurements. The fluorescence upconversion measurements for C$_6$S-capped clusters are measured in DCM while GS and citrate functionalized gold clusters are measured in water. The 13 nm, 3 nm and 2.2 nm gold clusters are chosen for
comparative investigations since the 13 nm and 3 nm gold nanoparticles represent metallic nature with surface plasmon absorption while the 2.2 nm clusters are close to the gold clusters without surface plasmon absorption but exhibits quantum-size effects.\textsuperscript{282} It can be observed from Figure 3.2 that all the luminescence traces decay very fast and the decay is complete within 1.2 ps. Since the growth and decay rates are comparable to the instrument time resolution for the investigated systems, the time constants are evaluated by convolution fitting. The decay trace \((F(t))\) was fitted by a convolution of the instrument response \(g(t)\) and a fitting function \((f(t))\) comprising an exponential function with ultrafast growth and decay components followed by a slow decaying component.\textsuperscript{297, 298}

\[
F(t) = \int_{-\infty}^{t} f(t - t')g(t')dt'
\]

Where \(f(t) = a_1 \left( \exp \left( \frac{-t}{\tau_g} \right) + \exp \left( \frac{-t}{\tau_1} \right) \right) + a_2 \exp \left( \frac{-t}{\tau_2} \right)\)

\(\tau_g, \tau_1\) and \(\tau_2\) represent the growth and decay constants and \(a_1, a_2\) are the respective amplitudes. Average lifetimes are determined to have a comparison of the decay time constants.

\[
\tau_{av} = \frac{\sum a_i \tau_i}{\sum a_i}
\]

From Figure 3.2A and 3.2B, it is observed that the luminescence decay traces of \(\text{Au}_{25}\text{L}_{18}\) clusters are entirely different from that of either 2.2 nm gold cluster or gold nanoparticles with surface plasmon absorption. There is a growth in the luminescence only for \(\text{Au}_{25}\) clusters and absent in other clusters suggesting that \(\text{Au}_{25}\) clusters are indeed very unique and the dynamics can give deeper insight into the relaxation processes that occur in these superatom complexes.\textsuperscript{274, 283, 299}

The decay time constant for 3nm-\(\text{Au}(\text{C}_6\text{S})\) is closer to the instrument response function (lifetime of < 100 fs) which is in accordance with previously observed time-resolved luminescence measurements.\textsuperscript{295, 296} However, 2.2 nm-\(\text{Au}(\text{C}_6\text{S})\) shows an instrument response limited growth followed by a slower 250 fs decay. Contrarily, \(\text{Au}_{25}(\text{C}_6\text{S})_{18}\) clusters have shown slower growth
(180 fs) followed by a decay (240 fs) suggesting that the dynamics in Au$_{25}$ clusters is quite unique when compared to other gold clusters. Similar luminescence growth is also observed for Au$_{25}$(GS)$_{18}$ clusters which is different from 2.2 nm-Au(GS) and 13 nm-Au(Cit) [citrate stabilized] nanoparticles (Figure 2B).

Wavelength dependence of luminescence in Au$_{25}$L$_{18}$ clusters

As the luminescence dynamics of Au$_{25}$ MPCs is quite distinctive, wavelength dependent luminescence decays were monitored and corresponding luminescence traces are shown in Figures 3.3A and 3.3B for Au$_{25}$(C$_{6}$S)$_{18}$ in DCM and Au$_{25}$(GS)$_{18}$ in water, respectively. Similar wavelength-dependent luminescence traces were also monitored for 2.2 nm-Au(GS) and 13 nm-Au(Cit) clusters in water, and corresponding luminescence decay traces are provided in Figures 3.3C and 3.3D. It can be noted that the luminescence decay traces for 2.2 nm-Au(GS) and 13 nm-Au(Cit) are wavelength independent while that of Au$_{25}$L$_{18}$ clusters are wavelength dependent. The luminescence decay traces are modeled with an exponential growth and decay time constants using convolution fitting (equation 1) and the lifetimes are given in the Supporting Information.
Interesting trends emerged from the wavelength dependent luminescence analyses. Both the growth and decay time constants for Au$_{25}$L$_{18}$ clusters slowed down with an increase in monitoring luminescence wavelength. At a wavelength of 520 nm (Figure 3.3A), the luminescence of Au$_{25}$(C$_6$S)$_{18}$ has a growth time constant of 180 ± 30 fs followed by a 200 ± 40 fs decay. As the wavelength is increased to 610 nm, growth of the luminescence is increased to 235 ± 40 fs and a decay of 420 ± 60 fs. At 670 nm, luminescence grows with a time constant of around 300 fs and the average decay constant increased to 980 ± 150 fs. Both the growth and decay time constants increased as a function of wavelength and the decay of luminescence did not match the growth of the luminescence from the next state, thereby ruling out the possibility of a two-state relaxation.

The changes in growth and decay time constants suggest that the excitation relaxes through a manifold of electronic states to the lowest-unoccupied molecular orbital (LUMO) and gets trapped in the Au-semi ring states. Identical changes in luminescence traces are observed for Au$_{25}$(GS)$_{18}$ in water suggesting that the dynamics is indeed independent of the passivating ligands.
Figure 3.3. Luminescence decay traces as a function of wavelengths for (A) \( \text{Au}_{25} (\text{C}_6 \text{S})_{18} \) in DCM, (B) \( \text{Au}_{25} (\text{GS})_{18} \) in water, (C) 2.2 nm-\( \text{Au}(\text{GS}) \) in water and (D) 13 nm-\( \text{Au}(\text{Cit}) \) in water after excitation at 400 nm

Table 3.1. Lifetimes of \( \text{Au}_{25} \) clusters capped with hexanethiol and glutathione at different emission wavelengths after excitation at 400 nm

<table>
<thead>
<tr>
<th>Sample/wavelength</th>
<th>( \tau_0 )</th>
<th>( \tau_1 )</th>
<th>( \tau_2 )</th>
<th>( \tau_{d, \text{av}} ) (fs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Au}_{25}(\text{C}<em>6 \text{S})</em>{18}/500 \text{ nm} )</td>
<td>180 fs</td>
<td>200 fs (99.8%)</td>
<td>1.65 ps (0.2%)</td>
<td>200</td>
</tr>
<tr>
<td>( \text{Au}_{25}(\text{C}<em>6 \text{S})</em>{18}/520 \text{ nm} )</td>
<td>200 fs</td>
<td>230 fs (99.8%)</td>
<td>1.05 ps (0.2%)</td>
<td>230</td>
</tr>
<tr>
<td>( \text{Au}_{25}(\text{C}<em>6 \text{S})</em>{18}/550 \text{ nm} )</td>
<td>220 fs</td>
<td>280 fs (99.4%)</td>
<td>1.65 ps (0.6%)</td>
<td>290</td>
</tr>
<tr>
<td>( \text{Au}_{25}(\text{C}<em>6 \text{S})</em>{18}/580 \text{ nm} )</td>
<td>230 fs</td>
<td>310 fs (98.6%)</td>
<td>1.65 ps (1.4%)</td>
<td>330</td>
</tr>
<tr>
<td>( \text{Au}_{25}(\text{C}<em>6 \text{S})</em>{18}/610 \text{ nm} )</td>
<td>235 fs</td>
<td>350 fs (96.1%)</td>
<td>2.2 ps (3.9%)</td>
<td>420</td>
</tr>
<tr>
<td>( \text{Au}_{25}(\text{C}<em>6 \text{S})</em>{18}/640 \text{ nm} )</td>
<td>245 fs</td>
<td>420 fs (97.3%)</td>
<td>1.5 ps (2.7%)</td>
<td>450</td>
</tr>
<tr>
<td>( \text{Au}<em>{25}(\text{GS})</em>{18}/480 \text{ nm} )</td>
<td>140 fs</td>
<td>185 fs (99.9%)</td>
<td>1.6 ps (0.1%)</td>
<td>185</td>
</tr>
<tr>
<td>( \text{Au}<em>{25}(\text{GS})</em>{18}/500 \text{ nm} )</td>
<td>180 fs</td>
<td>230 fs (99.5%)</td>
<td>1.6 ps (0.5%)</td>
<td>240</td>
</tr>
<tr>
<td>( \text{Au}<em>{25}(\text{GS})</em>{18}/520 \text{ nm} )</td>
<td>210 fs</td>
<td>270 fs (99.3%)</td>
<td>1.6 ps (0.7%)</td>
<td>280</td>
</tr>
<tr>
<td>( \text{Au}<em>{25}(\text{GS})</em>{18}/550 \text{ nm} )</td>
<td>220 fs</td>
<td>290 fs (97.6%)</td>
<td>1.65 ps (2.4%)</td>
<td>320</td>
</tr>
<tr>
<td>( \text{Au}<em>{25}(\text{GS})</em>{18}/580 \text{ nm} )</td>
<td>220 fs</td>
<td>340 fs (95.9%)</td>
<td>2.8 ps (4.1%)</td>
<td>440</td>
</tr>
<tr>
<td>( \text{Au}<em>{25}(\text{GS})</em>{18}/610 \text{ nm} )</td>
<td>235 fs</td>
<td>330 fs (90.1%)</td>
<td>2.1 ps (9.9%)</td>
<td>505</td>
</tr>
<tr>
<td>( \text{Au}<em>{25}(\text{GS})</em>{18}/640 \text{ nm} )</td>
<td>250 fs</td>
<td>410 fs (84.2%)</td>
<td>4.8 ps (15.8%)</td>
<td>1100</td>
</tr>
</tbody>
</table>
Ligand dependence of the visible luminescence

Luminescence decay traces at similar wavelengths for Au\textsubscript{25} clusters with ligands (C\textsubscript{6}S and GS) were compared at different luminescence wavelengths, as seen in Figure 3.4 and the luminescence growth is same for both the ligands though the average decay time constants varied slightly. The obtained growth and decay constants are plotted as a function of wavelength and shown in Figure 3.5. Wavelength-dependent growth and decay time constants are also plotted for 2.2 nm-Au(GS) and 13 nm-Au(Cit) (citrate stabilized) clusters in Figure 3.6.
Figure 3.4. Comparative fluorescence decay traces for $\text{Au}_{25}(\text{C}_6\text{S})_{18}$ in DCM and $\text{Au}_{25}(\text{GS})_{18}$ in water at the monitoring wavelengths of (A) 500 nm, (B) 520 nm, (C) 550 nm, (D) 610 nm, (E) 640 nm and (F) 670 nm after excitation at 400 nm.

Figure 3.5. Plot of growth lifetimes for different gold clusters as a function of wavelength. Inset shows the average decay lifetimes of the clusters as a function of wavelength.

For the larger gold clusters, the growth and decay kinetics did not vary with the change in luminescence wavelength while pronounced changes are observed for $\text{Au}_{25}$ clusters. It is evident from these observations that the growth time constants are much slower than the instrument response and are identical for the $\text{Au}_{25}$ clusters with different protecting ligands. The growth time
constants also increase while going down in energy pointing towards a cascade relaxation through various electronic energy levels. The ligand and luminescence wavelength independence suggest that the dynamics arises from the Au$_{13}$ core states where ligands have little or no influence. It is worth noting that even larger gold clusters and gold nanoparticles have luminescence in this wavelength region. The luminescence decays of larger gold clusters did not change with the monitoring wavelength.

Comparative fluorescence decays as a function of wavelength are followed for 13.5nm-Au(Cit) (citrate stabilized) and Au 2.2 nm clusters after excitation at 400 nm and the corresponding traces are shown in Figures 3.6A and 3.6B. It can be observed that there is no change in both the growth and decay kinetics as a function of wavelength. On the other hand, Au$_{25}$L$_{18}$ clusters have shown wavelength dependent growth and decay kinetics.

Fluorescence traces obtained for gold clusters, Au$_{25}$(C$_6$S)$_{18}$ in DCM and Au$_{25}$(GS)$_{18}$ in water at different emission wavelengths are shown in parts A, B, C, D, E and F of Figure 3.4. The growth of the emission is independent of the ligand while the decay of fluorescence is varied with passivating ligand. Slower decay is observed for the GS capping ligand over C$_6$S.

Figure 3.6. Fluorescence decay traces as a function of wavelength for (A) 13.5nm-Au(Cit) (citrate stabilized) in water and (B) 2.2nm-Au(GS) in water
On the contrary, the average decay time constants for Au$_{25}$L$_{18}$ clusters increase with increase in monitoring luminescence wavelength. Note from Figure 3.5 inset that the decay lifetimes of Au$_{25}$L$_{18}$ clusters are dependent on the passivating ligand. Comparatively slower decay constants are observed for Au$_{25}$(GS)$_{18}$ clusters over Au$_{25}$(C$_6$S)$_{18}$ clusters. Thus, the slower decay time-constant is ascribed to the relaxation of Au$_{25}$ core states to the S-Au-S-Au-S semi-ring states and are influenced by the protecting ligand. It is already mentioned that the luminescence in NIR region is long-lived and dependent on the passivating ligands and possibly arising out of the excitation localized on the LUMO and the semi-ring states.$^{287, 288, 290}$

3.4. Mechanism of the excited state relaxation in Au$_{25}$L$_{18}$ clusters

On photo-excitation of molecules to higher excited-states, relaxation to the LUMO or S$_1$ state occurs almost instantaneously or within hundreds of femtoseconds by the process of internal conversion in accordance to Kasha’s rule. The rule states that “photon emission [fluorescence or phosphorescence] occurs in appreciable yield only from the lowest excited state of a given multiplicity”. In contrast, it is interesting to see that the higher energy states in Au$_{25}$ clusters are longer lived and the average lifetimes of the states varied from a couple of 100 fs to less than a picosecond depending on the monitoring wavelength. The result suggests that the quantum-sized Au$_{25}$ clusters are remotely linked to the molecular type character and have their own behavior which is entirely different from either a molecule or a semiconductor. The phenomenon of superatom complexes suggested by Whetten and co-workers$^{274, 297}$ and others for Au$_{25}$ MPCs can be aptly used to explain the observed excited-state dynamics. The presence of core Au (Au$_{13}$ core) followed by an outer complex of S-Au-S-Au-S semi-rings are revealed in the present luminescence dynamics investigations which supports the concept of “superatom complex” in Au$_{25}$ clusters. Mechanistically, the observed luminescence in the Au$_{25}$ clusters can be depicted in Figure 3.7. The NIR luminescence arises from the complex of the semi-rings and the visible luminescence is from the Au core states (Au$_{13}$ core); the decay of visible luminescence
corresponds to the relaxation of core Au states to the semi-ring states. Also, longer lived higher excited states are observed for the investigated Au$_{25}$L$_{18}$ clusters which can be used in applications such as solar energy harvesting, catalysis and nonlinear optical properties. The longer lived Au$_{13}$ core states in Au$_{25}$L$_{18}$ clusters can be attributed to the structural differences of the core gold and semi-ring gold atoms which contribute to the slower relaxation of core gold states to semi-ring states.

![Diagram of relaxation pathways in Au$_{25}$L$_{18}$ clusters](image)

Figure 3.7. Cartoon diagram showing the relaxation pathways in Au$_{25}$L$_{18}$ clusters showing the gold core-states and semi-ring states

### 3.5. Conclusions

In summary, ultrafast luminescence investigations on Au$_{25}$L$_{18}$ clusters with two different ligands have revealed the nature of the higher excited states in these clusters. A low quantum-yield visible luminescence is observed in Au$_{25}$ clusters in addition to the NIR luminescence which is used as a probe to understand the excited state relaxation. The dynamics of the luminescence from Au$_{25}$ clusters have distinct wavelength dependent growth and decay kinetics which is absent
in larger gold nanoclusters and nanoparticles. The growth of luminescence is independent of the ligands and is ascribed to the luminescence from the Au$_{13}$ core states and the decay dynamics is mainly governed by its relaxation to S-Au-S-Au-S semi ring states. Relaxation processes happens over several hundreds of femtoseconds and proceeds through a multitude of electronic energy levels. Investigations have shown that the higher excited-states are longer lived unlike in a simple molecular excited state picture. This can be harnessed for electron transfer from hot states and can be utilized in solar energy harvesting and catalysis. Thus the electronic properties of Au$_{25}$L$_{18}$ clusters suggest that they belong neither to the class of large nanoparticles nor do they bear any semblance to molecular behavior; they are best understood as a standalone “superatom complex” system. The luminescence of quantum-sized metal clusters has enthralled the scientific community in recent years. In this study, ultrafast luminescence dynamics of hexanethiol (C$_6$S) and glutathione (GS) protected Au$_{25}$ clusters are investigated with time-resolved luminescence spectroscopy. The focus of the present investigation is to understand the dynamics of higher excited-states and also the relaxation of core-Au states to Au-semi ring states in Au$_{25}$L$_{18}$ (‘L’ is the protecting ligand) clusters. Comparative luminescence measurements on larger monolayer-protected gold clusters (2.2 nm Au(C$_6$S) and 2.2 nm Au(GS)) and gold nanoparticles (3 nm Au(C$_6$S) and 13 nm Au(citrate stabilized)) were also carried out. The excited state dynamics in Au$_{25}$L$_{18}$ clusters is not a typical two-state relaxation from core to semi-ring states, but rather proceeds through a manifold of electronic states as the luminescence traces show wavelength-dependent growth and decay kinetics. Also, femtosecond time-resolved luminescence measurements of Au$_{25}$L$_{18}$ have proved that the higher excited-states in monolayer-protected Au$_{25}$ clusters decay with a finite lifetime (200 femtoseconds up to a few picoseconds) that can be utilized for applications in solar-energy harvesting and catalysis.
CHAPTER IV
TEMPERATURE-DEPENDENT OPTICAL ABSORPTION PROPERTIES OF MONOLAYER-PROTECTED Au$_{25}$ AND Au$_{38}$ CLUSTERS

4.1. Introduction

Quantum-sized monolayer protected gold clusters (MPCs) have received enormous research attention because of their exciting optical, electrochemical and catalytic properties.\textsuperscript{107, 228, 279, 280, 300-306} MPCs with a size less than 2.2 nm\textsuperscript{282, 307} show prominent quantum confinement effects with an energy gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). The advent of efficient synthetic methodologies to prepare and characterize mono-dispersed gold clusters has enabled researchers to probe their unique physicochemical properties.\textsuperscript{228, 301, 304} These measurements can provide in-depth understanding of the evolution of molecular gold to nanoparticle gold electronic transitions. Among several MPCs investigated, Au$_{25}$L$_{18}$ and Au$_{38}$L$_{24}$ clusters are under the spotlight of research interest for both theoreticians and experimentalists as these clusters show discrete electronic absorption and electrochemical properties.\textsuperscript{60, 87, 212, 283-285, 307-320} Recently, the crystal structures of two thiol-protected clusters have been determined.\textsuperscript{87, 212, 284, 310} Both structures feature a gold core-shell system, Figure 4.1. The core is (Au$_{13}$ for Au$_{25}$L$_{18}$ clusters\textsuperscript{87, 212, 284} and Au$_{23}$ for Au$_{38}$L$_{24}$ clusters\textsuperscript{309}) passivated by a semi-ring gold shell (consisting of six dimeric staple motifs (-S-Au-S-Au-S) for Au$_{25}$L$_{18}$ and six dimeric plus three monomeric staples for Au$_{38}$L$_{24}$). DFT calculations based on the structures have successfully modeled the experimentally observed absorption spectra of Au$_{25}$L$_{18}$ and Au$_{38}$L$_{24}$ at room temperature.\textsuperscript{307, 311-315} Ultrafast optical
measurements of MPCs demonstrate the existence of the gold-core and semi-ring gold states and possible relaxation between them. In a recent study, we have shown unique ultrafast visible luminescence from Au_{25}L_{18} clusters and a step-wise relaxation from gold core-states to semi-ring states.

Although several investigations have focused on the optical properties of the MPCs, its understanding is far from complete. Several questions remain to be answered, such as the nature of excitons (Wannier or Frenkel) in these MPCs, the electron-phonon interactions and coupling of the gold core states with semi-ring gold states. Temperature-dependent optical measurements can answer some of these questions as they are often used to understand the electron-phonon and exciton-phonon interactions in semiconductor structures. Although there have been some steady state and time-resolved advanced optical measurements on the MPCs, the temperature dependence is sparsely investigated. Miles and Murray have reported on the temperature dependence of electrochemical properties in quantum-size clusters and showed that the energy gap increases with a decrease in temperature. Pradeep and co-workers have measured the temperature dependence of luminescence in MPCs and have shown that the intensity of near infrared luminescence decreases with decreasing temperature. Apart from these studies, very few theoretical and experimental studies have focused on the temperature-dependent optical properties of MPCs.

4.2. Temperature dependent absorption measurements

In the present investigation, systematic temperature-dependent optical absorption measurements were carried out on Au_{25}L_{18} and Au_{38}L_{24} clusters. The measurements were performed to understand the variation in the energy gap, electron-phonon and exciton-photon interactions, and
the coupling of gold core-states with semi-ring states as a function of temperature. A comparative study was also carried out with the gold nanoparticles showing surface plasmon absorption. The measurements have shown interesting trends of optical absorption as a function of temperature in quantum-sized clusters (as opposed to plasmonic nanoparticles), such as shift to higher energies, appearance of fine structure and increased oscillator strengths with decreasing temperature. The observed temperature-dependent electronic absorption is modeled with the electron-phonon and exciton-phonon interactions where the core-gold electrons couple with semi-ring gold phonons. The average energy of phonon modes, the 0 K energy maximum and exciton-phonon coupling strengths are obtained from the analysis.

Figure 4.1. Crystal structure of Au25L18 cluster (magenta: Au, yellow: S) and corresponding cartoon diagram depicting core states and semi-ring states

Electronic absorption spectra of the Au25L18 and Au38L24 clusters dissolved in methyl cyclohexane: methyl cyclopentane (1:1 v/v forms a clear glass at low temperature) are shown in Figure 4.2. At room temperature Au25L18 shows absorption centered at 675 nm with additional peaks in the visible region. Similar optical absorption features are observed for Au25L18 in other solvents at room temperature, hence are essentially solvent independent. UV scans were carried out at the following temperatures for Au25 78 K, 93 K, 113 K, 133 K, 153 K, 173 K, 193 K, 213

The transitions are ascribed to anionic Au$_{25}$L$_{18}$ clusters and the electrochemical measurements concur with it. For Au$_{38}$L$_{24}$ clusters, the electronic absorption spectra were taken in a wider wavelength window, from 350 nm to 1200 nm. A broad absorption maximum at 1055 nm is observed along with other spectral features in the visible region. The samples were of molecular purity and the absorption spectra match well with earlier results. Interestingly, the optical absorption at low temperature for Au$_{25}$L$_{18}$ clusters shows two clearly resolved low-energy absorption peaks with maxima around 658 nm and 730 nm, together with other sharp features in the 400 to 480 nm region. The spectrum for Au$_{25}$L$_{18}$ at 78 K is very striking and systematic temperature absorption studies were performed to probe further. Similarly, the Au$_{38}$L$_{24}$ clusters have shown unique features at 90 K when compared to the room temperature absorption spectrum. Three features can be noticed with decreasing temperatures: (i) the absorption features became sharper and new peaks emerged, (ii) the absorption maximum shifted to higher energies, and (iii) the oscillator strengths increased significantly.

![Absorption spectra](image)

Figure 4.2. Optical absorption of (A) Au$_{25}$(C$_6$S)$_{18}$ and (B) Au$_{38}$(PhC$_2$)$_{24}$ clusters dissolved in methyl cyclohexane:methyl cyclopentane mixture at two different temperatures
Since the absorption features are quite broad for \( \text{Au}_{25}\text{L}_{18} \) and \( \text{Au}_{38}\text{L}_{24} \) clusters at higher temperatures, absorption intensity (\( A*\lambda^2 \)) analysis was performed.\(^{303}\) Figures 4.2A and 4.2B show the plots of absorption intensity versus energy at different temperatures for \( \text{Au}_{25}\text{L}_{18} \) and \( \text{Au}_{38}\text{L}_{24} \), respectively. The absorption intensity plots show well-resolved peaks which are used to analyze the temperature dependence of the spectral energies and oscillator strengths. The temperature-dependent absorption analysis (Figure 4.3) provides important information on the trends of absorption maxima, vibronic features and corresponding oscillator strengths. Firstly, the absorption maxima are shifted to higher energies with a decrease in temperature (for example, for \( \text{Au}_{25}\text{L}_{18} \) cluster, the low-energy maximum of 1.81 eV is shifted to 1.90 eV and the 2.79 eV peak shifts to 2.87 eV). Similar shifts to higher energy are observed in the \( \text{Au}_{38}\text{L}_{24} \) clusters. The temperature-dependent absorption shift to higher energy correlates well with the increase in the electro-chemical energy gap with decreasing temperature.\(^{320}\) It should be noted that the gold nanoparticles with surface plasmon absorption did not show significant change in the absorption maximum with temperature (see supporting information). Secondly, the absorption features became very sharp and new vibronic features are observed with decreasing temperature. For example, the 1.81 eV (HOMO \( \rightarrow \) LUMO) peak of \( \text{Au}_{25}\text{L}_{18} \) clusters resolves into two peaks with maxima around 1.67 eV and 1.90 eV, respectively. The observed 1.67 eV peak is assigned to the \( \text{P}_z \rightarrow \text{LUMO} \) transition\(^ {315} \) which was predicted theoretically and had been observed as a shoulder in room temperature experiments. Similar sharp vibronic features are resolved for \( \text{Au}_{38}\text{L}_{24} \) clusters. The sharp electronic transitions and new vibronic features observed in MPCs are analyzed by incorporating electron-phonon interactions (\textit{vide infra}). Finally, an interesting trend of increased absorbance or absorption intensity (i.e. enhanced oscillator strength) is observed for the investigated MPCs with a decrease in the temperature, which is quite unusual for either semiconductors or organic molecules.
The most notable result of the temperature-dependent electronic absorption spectra is the shift of the absorption maximum to higher energies with decreasing temperature. This result is the first report of the variation of optical absorption maximum with temperature for metal clusters. The understanding of temperature-dependent optical properties of metal clusters is quite limited compared to that of their magnetic and electrical properties.\textsuperscript{324, 325} Kreibig previously reported the temperature dependence of the surface plasmon resonance of small silver and gold nanoparticles embedded in a glass matrix and found that the spectrum decreases in intensity and broadens as temperature rose from 1.5 to 300 K.\textsuperscript{326-328} The results were explained based on the electron-phonon scattering and thermal lattice contraction with decreasing temperature.\textsuperscript{326} Doremus investigated the surface plasmon absorption in 12-nm gold nanoparticles embedded in a glass matrix and varied the temperature from 469 to 767 K.\textsuperscript{329} He observed broadening of the surface plasmon absorption and a decrease in intensity with a red shift of absorption, attributed to the decreased electron concentration with increasing temperature.\textsuperscript{329} It should be stressed that in both cases, the decrease in surface plasmon absorption intensity was less than 10% over a wider range. More recently, Link and El-Sayed\textsuperscript{330} have shown a small temperature dependence of the surface plasmon absorption of 22-nm particles in water (in the range 291 K to 345 K), and negligible

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**Figure 4.3.** Intensity spectra at different temperatures for (A) Au\textsubscript{25}(C\textsubscript{6}S)\textsubscript{18} and (B) Au\textsubscript{38}(PhC\textsubscript{2})\textsubscript{24} clusters\textsuperscript{37}
temperature dependence of the plasmon absorption band for other sizes. They correlated the small temperature dependence to electron-electron scattering rather than electron-phonon interactions which can lead to significant temperature dependence. The assignment is consistent with the lower Debye temperature of bulk gold (170 K), suggesting that the contribution of phonons to the absorption spectra of plasmonic metal particles is quite limited. However, Kreibig has shown that size quantization of metal particles can have significant impact on the electron-phonon interactions. He has shown that gold nanoparticles with diameters less than 5 nm do not show any temperature dependence and attributed this to the quenching of electron-phonon scattering by the level quantization. In this scenario, electronic absorption spectra for the investigated quantum-size MPCs should be temperature-independent as the electron-phonon interaction is negligible due to the level quantization. In contrast, our results on MPCs show drastic temperature-dependent electronic absorption. However unlike the small metallic particles studied by Kreibig, our systems involve the additional S-Au-S-Au-S shell on the core-gold, which can alter the electron-phonon interactions and possibly lead to additional scattering.

The temperature-dependent shift of the energy gaps is a well-established phenomenon for semiconductor structures. The shift in the semiconductor band gaps with temperature is often attributed to electron-phonon and lattice expansion interactions. They are modeled with empirical relationships (Varshini relationship and Bose-Einstein equation) which relate the phonon occupation number with temperature and satisfactory fits can be obtained. As the quantum-sized MPCs possess discrete electronic absorption and behave more like molecules, we invoke electron-phonon interactions to model the temperature dependence of band gaps observed in quantum-sized MPCs. We use a modified version of the Bose-Einstein relationship that was developed by O’Donnel and Chen to model the temperature-dependent absorption maximum of
the MPCs. The O’Donnel-Chen equation relates the energy \( E(T) \) to temperature by the following expression\(^{333}\):

\[
E(T) = E(0) - \langle C \rangle \langle hu \rangle \left[ \coth \left( \frac{\langle hu \rangle}{2kT} \right) - 1 \right]
\]  

(4.1)

where, \( \langle C \rangle \) is a coupling constant, \( E(0) \) is the energy gap at 0 K, and \( \langle hu \rangle \) is the average phonon mode responsible for electron-phonon interaction. The magnitude of \( \left[ \coth \left( \frac{\langle hu \rangle}{2kT} \right) - 1 \right] \) represents the effective number of available phonons. Shown in Figure 4.4A is a plot of the energy maximum as a function of the temperature for \( \text{Au}_{25}\text{L}_{18} \) clusters. We have used the peak with a maximum at 1.89 eV in the plot as the peak at 1.67 eV is not well-resolved at higher temperatures. An average electron-phonon energy of 43±6 meV is obtained with a 0 K energy of 1.893±0.003 eV and \( \langle C \rangle \) of 4.9±0.6. Similar analysis of the lower energy maximum for \( \text{Au}_{38}\text{L}_{24} \) clusters was carried out (Figure 4.4B), which yielded 54±7 meV phonon energy and 0 K band-gap of 1.675±0.001 eV. The parameters obtained from the fit are provided in Table 4.1. The \( \langle C \rangle \) value obtained for \( \text{Au}_{25} \) clusters is significantly larger than that for \( \text{Au}_{38} \) suggesting stronger electron-phonon interaction. The observed \( \langle C \rangle \) values for the investigated MPCs are comparable to the values reported for conventional semiconductors (for example, \( \langle C \rangle = 1.47 \) for Si and \( \langle C \rangle = 2.94 \) for GaAs),\(^{333}\) indicating the effectiveness of electron-phonon interaction. Also, the average energy of phonon modes for MPCs (~50 meV) is significantly larger than the Debye temperature of bulk gold (15 meV),\(^{331}\) suggesting that the phonons responsible for the electron-phonon interactions are not from the gold core (\( \text{Au}_{13} \) for \( \text{Au}_{25}\text{L}_{18} \) or \( \text{Au}_{23} \) for \( \text{Au}_{38}\text{L}_{24} \)), but rather from the shell-gold composed of staples.
Figure 4.4. Variation of the absorption maximum with temperature for (A) Au$_{25}$(C$_6$S)$_{18}$ and (B) Au$_{38}$(PhC$_2$)$_{24}$. The data is fit with O’Donnel-Chen relationship (equation 4.1)

As noted earlier, the increase in the oscillator strength with a decrease in temperature is one of the surprising results of our study. Within the Condon approximation, the oscillator strength of any electronic transition is independent of the temperature.$^{339, 340}$ This is true for most complex organic molecules. However, temperature-dependent oscillator strength is observed for some organic molecules which were ascribed to the presence or absence of Hertzberg-Teller vibronic coupling or conformational relaxation or weak hydrogen bonding interactions.$^{340-342}$ However, such parameters cannot explain the observed temperature-dependent oscillator strength of the MPCs. It was also shown in the case of semiconductors that the oscillator strengths of the semiconductor transitions are invariant with temperature. In most cases, increases in the band
widths are observed, but the oscillator strength remains constant. However, we observe a significant increase in the oscillator strength (close to 300%), apart from the bandwidth changes, with decreasing temperature. This striking result cannot be explained based on a simple increase in the solvent density or change in the refractive index, which can only account for a maximum 10% increase in the oscillator strength. One major difference between the gold nanoparticles possessing surface plasmon and the quantum-size MPCs in this work is the discrete absorption spectra for the MPCs; the latter indicates the presence of excitonic states. Thus, we use exciton-phonon interactions to account for the changes in oscillator strengths.\textsuperscript{337, 338} The temperature-dependent oscillator strengths ($f$) of the electronic transitions are modeled using the Debye-Waller expression\textsuperscript{338, 343} that correlates the oscillator strength with exciton-phonon interactions.

$$f \approx f_0 \exp \left( -\langle S \rangle \coth \left( \frac{h\nu}{2kT} \right) \right)$$  \hspace{2cm} (4.2)$$

where, $\langle S \rangle$ is a Hugh-Rhyns factor which describes the coupling strength between the exciton and average phonon mode ($h\nu$),\textsuperscript{343} and $f_0$ is the oscillator strength at 0 K. The oscillator strengths of the transitions are obtained from the electronic absorption spectrum using the expression $f_{abs} \approx 4.3 \times 10^{-9} \int e(\nu)d\nu$. The oscillator strengths of the Au$_{25}$L$_{18}$ and Au$_{38}$L$_{24}$ are obtained from the analysis, and are plotted as a function of temperature. The fits are performed using equation 2 to obtain exciton-phonon coupling strength ($\langle S \rangle$) and 0 K oscillator strengths. For the fitting, the average phonon mode obtained from the energy gap analysis was used. The corresponding fit parameters are provided in Table 4.1. Similar analysis was performed by Zhang et al.\textsuperscript{337, 338} to fit the temperature-dependent oscillator strengths in InGaAs/GaAs quantum well structures. It should be noted (Table 4.1) that $\langle S \rangle$ values, which signify the exciton-phonon coupling strength, are almost ten times larger when compared to the semiconductor quantum well structures. The discrepancy can be ascribed to the nature of exciton-phonon interactions in the semiconductor quantum wells and that of quantum-size MPCs. The phonons involved in semiconductor quantum
well structures correspond to either InGaAs or GaAs while the phonons in MPCs are of the semi- 
ring gold and might possess significant ligand contribution.

![Graph A](image1)
![Graph B](image2)

Figure 4.5. Plots of oscillator strength versus temperature for (A) Au$_{25}$(C$_6$S)$_{18}$ (1.9 eV peak) and 
(B) Au$_{38}$(PhC$_2$)$_{24}$ (1.67 eV) clusters

Table 4.1. Parameters obtained from the fitting of temperature-dependent absorption spectra

<table>
<thead>
<tr>
<th>Sample</th>
<th>$E_g(0)$ (eV)</th>
<th>$\langle h\nu \rangle$ (meV)</th>
<th>$\langle C \rangle$</th>
<th>$\langle S \rangle$</th>
<th>$f_0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au$_{25}$(C$<em>6$S)$</em>{18}$</td>
<td>1.893±0.003</td>
<td>43±6</td>
<td>3.6±0.4</td>
<td>2.53±0.08</td>
<td>0.93±0.08</td>
</tr>
<tr>
<td>Au$_{38}$(PhC$<em>2$)$</em>{24}$</td>
<td>1.675±0.001</td>
<td>54±7</td>
<td>1.8±0.3</td>
<td>3.2±0.2</td>
<td>1.1±0.2</td>
</tr>
</tbody>
</table>

Three distinct features emerge from the electronic absorption measurements with a decrease in 
the temperature, viz (i) shift of optical transitions to higher energies, (ii) sharper absorption peaks 
with the emergence of new vibronic structure, and (iii) ~3-fold increase in the oscillator strength. 
The first two features are explained on the basis of decreased electron-phonon interactions at 
lower temperatures. The emergence of vibronic features can be explained on the basis of 
decreased electron-phonon interactions which sharpen the absorption peaks and resolve the 
vibronic features. The temperature-dependent energy maxima were fitted with the electron-
phonon interactions yielding average phonon energies of close to 400 cm$^{-1}$ for the MPCs. So, 
which phonons are responsible for the observed stronger electron-phonon or exciton-phonon 
interactions? The Au-Au core gold vibrations$^{344}$ occur at 80-90 cm$^{-1}$ and ultrafast coherent
vibrations of similar frequency were observed for MPCs.\textsuperscript{305, 317} Hence, core Au-Au vibrations are probably not responsible for the phonon interactions. Recent far-infrared experiments carried out on gold clusters\textsuperscript{344, 345} and MPCs have shown that the Au-S stretching frequencies fall in the range of 250 cm\textsuperscript{-1}. However, multiple vibrations and broader features were observed for MPCs with staple motifs that can shift the vibrations up to 400 cm\textsuperscript{-1}.\textsuperscript{344} Thus, we attribute the phonons to the semi-ring gold–ligand staple motif vibrations. This assignment is supported by crystal structures\textsuperscript{87, 212, 284, 309} and ultrafast time-resolved measurements\textsuperscript{60, 283, 285, 316, 317} which show the presence of core-gold states and semi-ring states corresponding to staple motifs. It has to be pointed out that the influence of Au-Au core gold phonons (80 cm\textsuperscript{-1}) might have some contribution to the exciton-phonon interactions at much lower temperatures. However, current measurements show that the shell phonons are responsible for the observed temperature-dependent absorption. It is shown both theoretically and experimentally that the electronic transitions are dominated by the gold core states absorption, suggesting that the excitons primarily arise from the core-gold. The semi-ring gold states do influence the core-gold electronic transitions at higher temperatures by borrowing their oscillator strengths, similar to the Hertzberger-Teller vibronic coupling which disappears at lower temperature. From the results, it is found that the phonons of semi-rings dictate the temperature-dependent electronic absorption spectra in quantum-size MPCs, and therefore theoretical calculations must include additional electron-phonon interactions while addressing the electronic transitions in gold clusters with a definite core.

Shown in Figure 4.6 are the temperature-dependent optical absorption spectra of Au(GS) – 4nm nanoparticles at different temperatures, and the corresponding absorption versus temperature plot is provided in Figure 4.6B. The data shows little to no temperature effect on the optical absorption spectra for the gold nanoparticles with surface plasmon absorption.
Figure 4.6. Optical absorption of 4nm-Au(GS) clusters dissolved in water:glycerol (40:60) mixture at different temperatures

Similar experiments have been performed on \( \text{Au}_{38}(\text{C}_6\text{S})_{24} \), \( \text{Au}_{144}(\text{C}_6\text{S})_{60} \), \( \text{Au}_{25}(\text{GS})_{18} \), \( [\text{Au}_{25}(\text{PPh}_3)_{10}(\text{FeS})_3\text{Cl}]^{2+} \). The absorption changes are given below. From these experiments it is evident that the quantum sized clusters have different behavior when compared to larger clusters and nanoparticles. To rule out the influence of the polymer, \([\text{S-Au-S-Au-S}]_n\) formed in the first stage of the synthesis, absorption changes were monitored, Figure 4.8.

Figure 4.7. Optical absorption of \( \text{Au}_{25}(\text{GS})_{18} \), \( \text{Au}_{38}(\text{C}_6\text{S})_{24} \)
Figure 4.8. Optical absorption of $[\text{Au}_{25}(\text{PPh}_3)_{10}(\text{C}_6\text{S})_5\text{Cl}_2]^{2+}$, $4\text{nm}$-$\text{Au-C}_6\text{S}$, $\text{Au}_{25}(\text{PPh}_3)_{10}(\text{FcS})_5\text{Cl}_2]^{2+}$ and $\text{Au-S-polymer}$
Figure 4.9. Temperature dependent optical absorption of Au cluster with a size of 1.5 nm. We attribute this size of cluster to that of Au$_{140}$(C$_6$)$_x$. This cluster size is readjusted to Au$_{144}$.
4.3. Temperature dependent optical absorption measurements of $\text{Au}_{144}(\text{PhC}_2)_x$ in toluene (177 K to 308 K)

As toluene does not form good glass, the temperature dependent absorption measurements were performed until 177 K. (Freezing point of toluene is 180 K). The solution did not freeze until 177 K due to the depression in freezing point.

Figure 4.10. Temperature dependent optical absorption of $\text{Au}_{144}(\text{PhC}_2)_x$
4.4. Conclusions

In summary, temperature-dependent electronic absorption properties of the Au$_{25}$L$_{18}$ and Au$_{38}$L$_{24}$ clusters are reported. Interesting absorption features such as the shift of the absorption maxima to higher energies, sharper absorption peaks along with additional vibronic peaks, and increased oscillator strengths of the electronic transitions, are observed with decreasing temperatures. The results are explained on the basis of electron-phonon interactions involving core-gold electrons or excitons with semi-ring gold phonons. The temperature-dependent absorption maxima and oscillator strengths are fitted with models that describe the energy gaps in semiconductor quantum dots and quantum wells. Significantly larger phonon energies of ~400 cm$^{-1}$ are observed for the MPCs suggesting that they are arising out of the semi-ring gold. Also, increased exciton-phonon coupling strengths are observed for MPCs which reduces the oscillator strength of electronic transitions at higher temperatures. The unique property of decreasing oscillator strength with increasing temperature is modeled on the Debye-Waller equation which relates oscillator strength to the exciton-phonon interaction.
CHAPTER V

ELECTRON TRANSFER IN CHROMOPHORE LABELED QUANTUM Sized Au\textsubscript{25} CLUSTERS: Au\textsubscript{25} AS ELECTRON DONOR

5.1. Introduction

Highly stable quantum-sized monolayer protected (MPCs) Au\textsubscript{25} clusters have been in the forefront of research in recent years.\textsuperscript{87, 278, 284, 346-349} Atomically precise MPCs with exciting electrochemical, magnetic, optical and catalytic properties\textsuperscript{69, 85, 92, 130, 212, 278-280, 282, 293, 308, 348-355} are being synthesized and characterized. Quantum confinement, molecule-like electronic properties of Au\textsubscript{25} MPCs and their functionalized counterparts are actively being explored for photo-induced electron and energy transfer properties which can lead to potential applications in solar energy conversion, catalysis, sensors and other optical applications.\textsuperscript{107, 200, 202, 356, 357} The interfacial electron transfer phenomena in molecule-nanoparticle hybrids have been extensively researched, especially for the molecule-semiconductor nanoparticle interface, as it is the fundamental event in well-investigated dye-sensitized solar cells.\textsuperscript{358-360} Comparatively fewer investigations have been carried out on the molecule-metal-nanoparticle interface. Many studies on the interface of molecule-metal nanoparticles have shown the evidence of energy transfer from molecule to metal or vice versa.\textsuperscript{107, 200, 202, 356, 357, 361-367} For example, pyrene-functionalized gold clusters were investigated by Montalti \textit{et al}\textsuperscript{365} and the results show energy transfer from pyrene to the gold core together with interesting odd-even effects.\textsuperscript{364} A few studies have revealed that electron transfer is another viable deactivation process that occurs in the molecule-metal interface. In pyrene-attached gold nanoparticles, Kamat and co-workers\textsuperscript{361-363} reported that electron transfer takes place from photoexcited pyrene to the gold core. However, to the best of our knowledge, there are no reports of quantum sized MPCs acting as electron donors which can be exploited in the applications of catalysis, solar energy harvesting and sensors. This report provides the first results
supporting the directional electron flow in a pyrene-Au$_{25}$ cluster system where the quantum sized Au$_{25}$ MPCs with a large energy gap$^{291}$ act as electron donors.

The present study is aimed at probing the interfacial electron transfer pathways in Au$_{25}$-pyrene clusters. The system chosen has on an average only one dye molecule covalently bonded to the surface of Au$_{25}$ nanocluster, so that intramolecular energy transfer and excimer formation would not interfere with the interfacial charge or energy transfer processes. Electrochemical and ultrafast time-resolved fluorescence and absorption investigations were carried out to probe the interfacial processes. Pyrene labeled Au$_{25}$ clusters were synthesized by the exchange of thiolated pyrene (PySH) onto hexanethiolate capped Au$_{25}$ clusters and characterized as Au$_{25}$(C$_6$S)$_{17}$PyS using TEM, UV-Vis and voltammetric measurements. Details of syntheses$^{349}$ and characterization are provided in Chapter II.

5.2. Optical absorption and steady state measurements

Shown in Figure 5.1A are the optical absorption spectra of PySH, Au$_{25}$(C$_6$S)$_{18}$ and Au$_{25}$(C$_6$S)$_{17}$PyS. As seen in the figure, the optical properties of Au$_{25}$ clusters and PySH are unaltered in the hybrid PyS-Au$_{25}$ cluster system. This suggests that pyrene and Au$_{25}$ do not have strong ground state interactions that can alter the absorption spectrum of either pyrene or Au$_{25}$. Interestingly, the fluorescence of PySH is quenched dramatically in the PyS-Au$_{25}$ hybrid, indicating stronger excited-state interactions (Figure 5.1B). The fluorescence quantum yield of PySH was determined to be 0.54 and this decreased to 0.008 in the hybrid system, indicating strong electronic interactions which can be either due to electron or energy transfer processes. Care has been taken while measuring the fluorescence quantum yield of Au$_{25}$(C$_6$S)$_{17}$PyS, where the absorption by gold clusters are factored in calculation and the error bars for measured quantum yields are about 15%.
5.3. Electrochemical measurements

Square wave voltammetry of \( \text{Au}_{25}(\text{C}_6\text{S})_{18} \) and \( \text{Au}_{25}(\text{C}_6\text{S})_{17}\text{PyS} \) was carried out to determine whether the addition of the dye molecule alters the redox behavior of \( \text{Au}_{25} \) clusters. As seen in Figure 5. 2, the oxidation potentials of the \( \text{Au}_{25} \) clusters are substantially shifted to more positive values; for example, the first oxidation potential (ox1) of \( \text{Au}_{25} \) cluster is shifted from -0.71 V to -0.48 V (vs. Fc/Fc\(^+\)) when PyS is attached. The reduction peak of \( \text{Au}_{25}(\text{C}_6\text{S})_{17}\text{PyS} \) at \(-2.30 \text{ V}\) is attributed to pyrene and is consistent with the reduction potential (-2.54 V) of pyrene reported earlier.\(^{368}\) The reduction peak of \( \text{Au}_{25} \) at -2.36 V is not well-resolved for \( \text{Au}_{25}(\text{C}_6\text{S})_{17}\text{PyS} \) due to the overlap with the reduction peak of pyrene. The changes in oxidation potentials of \( \text{Au}_{25} \) can be understood by the electronic polarization effects induced by the interaction between \( \text{Au}_{25} \) and pyrene. When pyrene is attached to the \( \text{Au}_{25} \) cluster, charge redistribution occurs, resulting in a change in the redox potential of \( \text{Au}_{25} \). In a voltammetric study on ligand exchanged gold clusters,\(^{348}\) Guo and Murray reported that electron withdrawing substituent groups on the ligand induced substantial changes in the HOMO, making the oxidation process of \( \text{Au}_{25} \) more
difficult. In the present $\text{Au}_{25}(\text{C}_6\text{S})_{17}\text{PyS}$ clusters, the charge transfer between $\text{Au}_{25}$ and pyrene shows similar behavior making it difficult to oxidize the $\text{Au}_{25}$ cluster when pyrene is attached. Thus, the present changes in oxidation potentials reflect interesting electronic interactions between $\text{Au}_{25}$ and pyrene, demonstrating the directional electron flow from the $\text{Au}_{25}$ cluster to pyrene. Thermodynamic feasibility of different electron transfer pathways are discussed later in the chapter. While there are substantial changes found in the oxidation potentials of $\text{Au}_{25}$, the absorption spectrum in Figure 5.1A show essentially no change in the absorbance above 400 nm when pyrene is attached. This reflects the fact that the optical transitions in $\text{Au}_{25}$ are rather insensitive to the ligand exchange process occurring in the exterior -S-Au-S-Au-S- semi-ring. In a correlation study of $\text{Au}_{25}$ structure and its optical absorption by density functional theory calculation, it was found that the absorption at 670 nm is viewed as the HOMO-LUMO transition and is due entirely to the $\text{Au}_{13}$ core. Thus one would expect very different optical and electrochemical responses to the exchanged pyrene and ensuing charge transfer process occurring in the exterior semi-ring.

![Graph](image)

Figure 5.2. Square wave voltammograms of 0.5 mM $\text{Au}_{25}(\text{C}_6\text{S})_{18}$ and 0.2 mM $\text{Au}_{25}(\text{C}_6\text{S})_{17}\text{PyS}$ in 0.1 M $\text{Bu}_4\text{NClO}_4$ in $\text{CH}_2\text{Cl}_2$. 

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5.4. Fluorescence upconversion

To probe the mechanism behind the excited state deactivation pathways when pyrene is attached to Au$_{25}$ clusters, ultrafast dynamics investigations were carried out with fluorescence upconversion and transient absorption. Comparative fluorescence measurements were carried out for PySH and Au$_{25}$(C$_6$S)$_{17}$PyS dissolved in dichloromethane (DCM). The corresponding emission decay traces are shown in Figure 5.3. It can be observed here that PySH decays with an ultrafast decay component (75%) with a lifetime of 420 fs. This ultra fast decay is ascribed to internal conversion from S$_2 \rightarrow$ S$_1$ and consequent thermalization (details of the dynamics at different emission wavelengths are presented in supporting information) and a long lived decay consistent with singlet state lifetime. However, the average decay became much faster in Au$_{25}$(C$_6$S)$_{17}$PyS which was fitted with two exponentials (490 fs (93%) and >20 ps (7%)). Average lifetime of pyrene-Au$_{25}$ cluster is reduced when compared to free PySH and is consistent with the fluorescence quantum yield measurements. The presence of such ultrafast decay can be either due to interfacial electron or energy transfer processes. The origin of faster decay of pyrene-Au$_{25}$ cluster is further probed with femtosecond transient absorption measurements.
5.5. Transient absorption measurements

Comparative transient absorption measurements were carried out on PySH, Au\textsubscript{25}(C\textsubscript{6}S)\textsubscript{18} and Au\textsubscript{25}(C\textsubscript{6}S)\textsubscript{17}PyS dissolved in DCM after excitation at 345 nm. Shown in parts A and B of Figure 5.4 are the transient absorption spectra at different time delays and kinetics at representative wavelengths for PySH. Immediately after excitation at 345 nm (at 100 fs time delay, Figure 5.4A), an excited state absorption (ESA) spectrum centered around 580 nm is observed which decays very rapidly to give rise to the ESA centered around 530 nm, which in turn decays with a long lifetime. The ESA at 580 nm is ascribed to the S\textsubscript{2} \rightarrow S\textsubscript{n} singlet-singlet absorption while that of 527 nm is attributed to the S\textsubscript{1} \rightarrow S\textsubscript{n} absorption of PySH. Similar S\textsubscript{2} and S\textsubscript{1} states were observed in the transient absorption of pyrene derivatives.\textsuperscript{369} Kinetics at 580 nm and 510 nm corroborate the decay of S\textsubscript{2} and the growth of S\textsubscript{1} state with lifetimes of <100 fs and 1.6 ps. The growth of S\textsubscript{1} is longer than the decay of the S\textsubscript{2} state which can be ascribed to the thermalization of singlet state. Transient absorption results show both the internal conversion and thermalization time constants as they have different excited state absorption spectra as well as sufficient time resolution (instrument response function of 130 fs). However, fluorescence upconversion measurements of
the free dye have shown the contributions from both the internal conversion and thermalization; thus we have observed different time constants for the decay of fluorescence than that of the transient absorption. The dynamics observed in free dye, PySH is the simple photophysics of internal conversion between singlet states and it will be interesting to see how the spectral features will be altered when pyrene is attached to Au$_{25}$ clusters.

![Graph 1](image1.png)

Figure 5.4. (A) Transient absorption spectra at different time delays from 100 fs to 25 ps of PySH in DCM (B) kinetic decay traces at two ESA peaks, 580 nm and 510 nm

Before analyzing the dynamics of pyrene labeled Au$_{25}$ clusters, the transient absorption features of Au$_{25}$(C$_6$S)$_{18}$ dissolved in DCM were probed and corresponding results are shown in Figure 5.5A and 5.5B. The transient absorption characteristics were investigated previously for gold
nanoparticles \textsuperscript{370} and clusters \textsuperscript{178, 285, 371} including investigations on Au\textsubscript{25}. The results for the present Au\textsubscript{25}(C\textsubscript{6}S)\textsubscript{18} suggest that the dynamics is entirely different from what is observed for larger gold clusters which show surface plasmon resonance.\textsuperscript{370} Immediately after excitation, broad ESA features spanning the entire visible region are observed with maxima around 530 and 570 nm which decay as a function of time. This ESA can be ascribed to the singlet-singlet absorption which decays with time. This absorption decayed with different lifetimes, having 750 fs and >20 ps time constants (Figure 5.5B). It is to be mentioned here that the ESA features observed in the present investigation are consistent with what has been reported for other quantum sized gold clusters.\textsuperscript{178, 285, 371}

Figure 5.5. (A) Transient absorption spectra at different time delays from 200 fs to 25 ps of Au\textsubscript{25}(C\textsubscript{6}S)\textsubscript{18} (B) kinetic decay traces at two ESA peaks at 565 nm and 530 nm
Very interesting ESA features are observed when pyrene is attached to gold clusters. Shown in Figure 5.6A and 5.6B are the ESA features of Au$_{25}$(C$_6$S)$_{17}$PyS at short and intermediate time delays. There is a growth of ESA at very short time scales up to 600 fs giving rise to an ESA with a maximum around 503 nm while the same feature decays as the time delay is increased to 20 ps. It is clear from the transient absorption features that the ESA features of pyrene-labeled Au$_{25}$ clusters are completely altered from those of free pyrene and free Au$_{25}$ clusters. It is quite interesting to note that the absorption at 345 nm excites both Au$_{25}$ clusters and pyrene chromophore with roughly 50/50 probability. However, the transient features do not show any feature corresponding to Au$_{25}$ clusters which can be ascribed to lower excited state extinction coefficients for Au$_{25}$ clusters when compared to by pyrene and the extinction coefficients of its radical. Very small negative absorption around 570 nm for the hybrid system can be ascribed to the bleaching of Au$_{25}$(C$_6$S)$_{17}$PyS. The results suggest that additional excited-state reactions are taking place in the hybrid Pyrene-Au$_{25}$ system which can be due to either electron or energy transfer processes. Energy transfer is ruled out since the ESA of the pyrene-Au$_{25}$ is completely different from that of the bare Au$_{25}$ or pyrene itself. Electron transfer from pyrene to Au$_{25}$ is also ruled out as the cation radical of the pyrene has its maximum absorption from 425 nm to 450 nm$^{361}$ while the transients observed in the present system has a maximum at 503 nm. It is interesting to note that the present ESA with a maximum at 503 nm clearly matches the absorption spectrum of pyrene anion radical observed earlier with pulse radiolysis and transient absorption measurements.$^{369, 372}$ Thus, present dynamics and spectral features are ascribed to electron transfer from Au$_{25}$ clusters to the excited pyrene. Growth in intensity of the anion radical and decay of the ESA gives the charge separation and charge recombination rate constants. Figure 5.6C shows the growth of ESA at 480 nm while decay is observed in the longer wavelength regions of 660 nm. As the kinetics is quite complex, global fit analysis of all the
kinetics at different wavelength regions is performed with single value decomposition (Figure 5.6D). Interesting spectral features are obtained from this analysis. There are three main features in the dynamics, the first being a charge separation time constant of 580 fs, which matches with the ESA of pyrene singlet state with a maximum of 530 nm.\textsuperscript{368} The spectrum associated with 8.2 ps time constant has a maximum absorption of 504 nm, matching with the absorption spectrum of pyrene anion radical.\textsuperscript{369, 372} The negative feature observed at 570 nm is ascribed to the recombination of the charged species to give rise to the long-lived ESA which resembles that of the Au\textsubscript{25} ESA. These results confirm that the forward electron transfer from Au\textsubscript{25} clusters to pyrene is around 580 fs while the recombination is multi-exponential with a major contribution from 8.2 ps time constant.
Figure 5.6. Transient absorption spectra at different time delays (A) from 100 fs to 550 fs and (B) from 600 fs to 20 ps of $\text{Au}_{25}(\text{C}_6\text{S})_{17}\text{PyS}$ in DCM after excitation at 345 nm. (C) Kinetic decay traces of two ESA peaks at 660 nm and 480 nm and (D) species-associated spectra obtained from global fit analysis with predominantly three lifetime components.

5.6. Dynamics of PySH in DCM at different excitation wavelengths

We have carried out the fluorescence upconversion measurements for free dye at different emission wavelengths to understand the mechanism of the excited state deactivation. Fluorescence decay traces of free PySH with third harmonic excitation of fundamental Ti:sapphire at different emission wavelengths are shown in Figure 5.7A. It can be observed here that there is a faster fluorescence decay which is observed for both the 380 nm emission...
wavelength as well as that of 440 nm. Thus, the observed faster decay is ascribed to the S₂ to S₁ internal conversion and consequent thermalization of the singlet state. Similar S₂ to S₁ internal conversion and thermalization was shown to occur in the dynamics of pyrene derivatives.\textsuperscript{368, 369, 373}

To further understand the same process, we have also carried out upconversion measurements after excitation at 360 nm (second harmonic of 720 nm from Ti:Sapphire oscillator) and corresponding fluorescence decay traces at 420 nm, 440 nm and 460 nm are shown in Figure 5.7B. It can be observed here that there is a slight decay at 420 nm emission while there is a growth at 460 nm, which is ascribed to the thermalization of the singlet state. However, we did not see any significant faster decay components as observed with 266 nm excitation. Thus, the faster decay observed with 266 nm is ascribed to S₂ to S₁ internal conversion and consequent thermalization.

Figure 5.7. (A) Fluorescence upconversion decay traces of PySH in DCM at 380 nm and 440 nm emission wavelengths after excitation at 266 nm. (B) Fluorescence upconversion decay traces of PySH in DCM at 420 nm, 440 nm and 460 nm after excitation at 360 nm

Comparative transient absorption spectral analyses will give a better picture of the photo-induced electron transfer pathways. Figure 5.8A shows the ESA spectra of Au\textsubscript{25}(C\textsubscript{6}S)\textsubscript{18}, PySH and Au\textsubscript{25}(C\textsubscript{6}S)\textsubscript{17}PyS at a time delay of 1 ps after excitation at 345 nm. It is evident from Figure 5.8A
that the ESA spectra at 1 ps for the Au\textsubscript{25}(C\textsubscript{6}S\textsubscript{18} and PySH are entirely different from that of the Au\textsubscript{25}(C\textsubscript{6}S\textsubscript{17}PyS. The fact that the transient absorption spectrum of the hybrid pyrene-Au\textsubscript{25} does not match either Au\textsubscript{25} or PySH rules out the possibility of energy transfer being the excitation deactivation pathway. Several literature reports of molecule-gold clusters have suggested energy transfer mechanisms\textsuperscript{107, 200, 202, 356, 357} from the molecule-gold nanocluster, which have been ruled out here. The transient absorption analysis of Au\textsubscript{25}(C\textsubscript{6}S\textsubscript{17}PyS has shown an ESA spectrum with a maximum at ~503 nm. The maximum at 503 nm matches very well with the anion radical of pyrene as reported in the literature.\textsuperscript{369, 372} Thus, transient absorption results provide direct evidence of directional electron transfer from Au\textsubscript{25} to pyrene, giving rise to the anion radical of the dye molecule. Our results explicitly show that Au\textsubscript{25} clusters can act as electron donors, opening new avenues for potential applications in solar energy harvesting, catalysis and sensors.

Finally, it is interesting to know why Au\textsubscript{25} acts as electron donor in the Au\textsubscript{25}(C\textsubscript{6}S\textsubscript{18}PyS cluster system. For photoexcited pyrene-Au\textsubscript{25} clusters, the electron transfer can occur via (i) electron transfer from Au\textsubscript{25} to the excited pyrene or (ii) electron transfer from the excited pyrene to Au\textsubscript{25}. The pathway (ii) has been reported for a pyrene-functionalized gold nanoparticle system.\textsuperscript{361-363} To examine the thermodynamic feasibility of these pathways, we have estimated the free energy of electron transfer by using a simplified Rehm-Weller equation:\textsuperscript{374}

\[
\Delta G_{ET} = e[E_{D+/D} - E_{A/A-}] - E_{00} + w_p \tag{5.1}
\]

where \(E_{D+/D}\) and \(E_{A/A-}\) represents the oxidation and reduction potentials of donor and acceptor, \(E_{00}\) is the excitation energy of the photoexcited species, and \(w_p\) the coulombic interaction term. Generally, in polar media the magnitude of the coulombic term is small\textsuperscript{48} and is neglected here. For pathway (i), \(E_{D+/D} (\text{Au}_{25}) = -0.48\ \text{V},\ E_{A/A-} (\text{pyrene}) = -2.54\ \text{V},\ E_{00} (\text{pyrene}) = 3.43\ \text{eV};\) for pathway (ii), \(E_{D+/D} (\text{pyrene}) = 0.91\ \text{V},\ E_{A/A-} (\text{Au}_{25}) = -2.36\ \text{V},\ E_{00} (\text{pyrene}) = 3.43\ \text{eV};\) for pathway (iii), \(E_{D+/D} (\text{Au}_{25}) = -0.48\ \text{V},\ E_{A/A-} (\text{pyrene}) = -2.54\ \text{V},\ E_{00} (\text{Au}_{25}) = 1.33\ \text{eV}.\textsuperscript{293}
The free energies of electron transfer estimated\(^9\) for the pathways (i) and (ii) are \(-1.37\) eV and \(-0.16\) eV, respectively. The vast difference in the driving forces for electron transfers between pathways (i) and (ii) can explain the directional electron transfer observed here. In addition, with excitation at 345 nm, both \(\text{Au}_{25}\) and pyrene are photoexcited and the electron transfer quenching can occur via (iii) electron transfer from the excited \(\text{Au}_{25}\) to pyrene. The driving force estimated for this scenario is, however, 0.73 eV, which is thermodynamically unfavorable. Thus, as depicted in Figure 5.8B, the electron transfer quenching in pyrene-\(\text{Au}_{25}\) clusters occurs via electron transfer from the HOMO of \(\text{Au}_{25}\) to the HOMO of photoexcited pyrene. These thermodynamic considerations form the basis for the observed directional electron transfer. The large HOMO-LUMO gap in the quantum sized \(\text{Au}_{25}\) clusters facilitates the directional electron transfer from \(\text{Au}_{25}\) to excited pyrene.

To illustrate further the electron donor property of \(\text{Au}_{25}\), PySH loading was varied. Table 5.1 summarizes the changes in oxidation potential. It clearly proves that with increased pyrene loading the oxidation potential became more positive. The increased pyrene loading caused the formation
of eximers due to π-π stacking. The absorption and emission spectra, TEM and NMR spectra are included in Appendix I. The UV-vis and emission profiles are in Figure 5.9 and 5.10.

Table 5.1. Changes in oxidation potential with increase in PySH on Au$_{25}$

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Quantum Yield</th>
<th>No. of PySH from UV</th>
<th>No. of PySH from NMR</th>
<th>Size from TEM</th>
<th>Ox$_1$ in V vs Ag/Ag$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PySH</td>
<td>0.43</td>
<td>-----</td>
<td>1.1</td>
<td>1.2 ± 0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>45 min</td>
<td>0.04</td>
<td>1</td>
<td>1.1</td>
<td>1.2 ± 0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>2hrs</td>
<td>0.34</td>
<td>4.3</td>
<td>4</td>
<td>1.2 ± 0.1</td>
<td>0.17</td>
</tr>
<tr>
<td>5hrs</td>
<td>0.24</td>
<td>2.7</td>
<td>3.3</td>
<td>1.0 ± 0.3</td>
<td>0.15</td>
</tr>
<tr>
<td>10 hrs</td>
<td>0.084</td>
<td>1.3</td>
<td>2.1</td>
<td>1.1 ± 0.3</td>
<td>0.12</td>
</tr>
<tr>
<td>15 hrs</td>
<td>0.084</td>
<td>1.3</td>
<td>2.2</td>
<td>1.2 ± 0.3</td>
<td>0.15</td>
</tr>
<tr>
<td>25 hrs</td>
<td>0.097</td>
<td>1.4</td>
<td>2</td>
<td>1.2 ± 0.2</td>
<td>0.17</td>
</tr>
<tr>
<td>Au$_{25}$(C$<em>6$S)$</em>{18}$</td>
<td>0.004</td>
<td>0</td>
<td>0</td>
<td>1.1 ± 0.2</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Figure 5.9. UV-vis spectra of PyS loaded Au$_{25}$ clusters
Figure 5.10. Emission spectra of PyS loaded Au$_{25}$ clusters

5.7. Conclusions

In summary, precisely formulated Au$_{25}$(C$_6$S)$_{17}$PyS clusters have been synthesized with one pyrene species attached to each Au$_{25}$ cluster and its electrochemical and optical properties have been investigated. It is unambiguously shown that Au$_{25}$ clusters can work as electron donors using electrochemical and optical measurements. Ultrafast electron transfer from Au$_{25}$ cluster to pyrene was demonstrated with fluorescence upconversion and transient measurements. The interesting electron transfer ability of Au$_{25}$ clusters can be utilized in making better sensors, catalysts and light harvesting systems. Novel Au$_{25}$(C$_6$S)$_{17}$PyS clusters (pyrene functionalized Au$_{25}$ clusters) showing interesting electrochemical and optical properties were synthesized and characterized. Significant fluorescence quenching was observed for pyrene attached to Au$_{25}$ clusters, suggesting strong excited state interactions. Time-resolved fluorescence upconversion and transient absorption measurements are utilized to understand the excited state dynamics and possible interfacial electron and energy transfer pathways. Electrochemical investigations suggest
the possibility of electron transfer from Au$_{25}$ clusters to the attached pyrene. Fluorescence upconversion measurements have shown faster luminescence decay for the case of pyrene attached to Au$_{25}$ clusters pointing toward ultrafast photoinduced electron/energy transfer pathways. Femtosecond transient absorption measurements have revealed the presence of the anion radical of pyrene in the excited state absorption, suggesting the directional electron transfer from Au$_{25}$ clusters to pyrene. The rate of forward electron transfer from Au$_{25}$ cluster to pyrene is ultrafast (~580 fs) as observed with femtosecond fluorescence upconversion and transient absorption.
CHAPTER VI
SENSING OF ORGANOPHOSPHONATES USING COUMARIN LABELED GOLD CLUSTERS

6.1. Introduction

Semiconductor and metal clusters in the nanometer size regime display many interesting optical, electronic and chemical properties due to quantum size effects. For example, electrochemical and optical properties of gold clusters smaller than 1.5 nm are completely different from those of clusters larger than 1.6 nm and their bulk counterparts. Hence, different core-sized nanomaterial can be employed to produce advanced sensors, light harvesting devices and “green” high efficiency catalysts. Nerve gas Agents (NA) are used in chemical warfare. Our major goal was to apply gold cluster synthesis to design new sensors for NA detection. As mimics for the NAs we employ the less toxic organophosphorus pesticides which are sulfur analogs of the NAs. These mimics have the same chemical action as the NAs on the neurotransmitter AcetylCholineEsterase (AChE). Herein we report optical and electrochemical detection techniques used to assess the use of AuNC [1.3nm AuC₆S modified with Cou-SH] as sensors for DiethylChloroPhosphate (DCP). According to the Organization for the Prohibition of Chemical Weapons and the Chemical Weapons Convention, substances are considered chemical warfare agents (CWA) if they, through a “chemical effect on living processes, cause death, temporary loss of performance, or permanent injury to people or animals”. They were used in both World Wars and in the Cold War and as early as 431 BC in the Spartan wars. They are classified into several groups based on their mode of action as nerve agents (NA): asphyxiants/blood agents, vesicant agents, choking/pulmonary agents, lachrymatory agents, blistering agents, vomit agents, tear gases and cytotoxic proteins. Most CWA are highly
toxic and lethal even at low levels. A typical CWA will not lose its activity for a long time even on heating, or chemical reaction with water; although hydrolysis will deactivate them, they hydrolyze very slowly under normal conditions with air and water in the atmosphere. Organophosphorus compounds (nerve gases) and their structural simulants were developed as the most dangerous CWA during and after World War II. Organophosphate nerve agents inhibit the activity of AChE by covalently binding to the serine hydroxyl group in the catalytic site (esteratic site) of acetylcholine esterase to form a phosphate ester bond. This bonding blocks the enzyme from interaction with its normal substrate ACh and causes accumulation of ACh rather than the normal breakdown. The inhibition of AChE by nerve gases leads to headache, nausea, vomiting, diarrhea, respiratory failure and eventually paralysis and death depending on the dose. Current detection methods for nerve agents are based on different techniques, which include surface acoustic wave detectors, enzymatic assays, mass spectrometry, interferometry and colorimetric detectors. These detection methods have limitations such as weak response, limited selectivity, false positives, low sensitivity, cost, and low real-time recognition. Optical chemosensors and electrochemical sensors are simple and inexpensive and can overcome all the above mentioned limitations. Sometimes detection can even be performed with the naked eye by seeing a visible change in color.

The engineering of nanomaterials with photoactive species has been investigated over the years. The ability of surface binding with thiol ligands has made monolayer-protected gold clusters an ideal platforms for optoelectronic applications such as fluorescence patterning. The changes happening due to surface binding can be monitored easily using fluorescence spectroscopy, as it is a sensitive technique. Successful assembly of fluorophores without aggregation is vital and this was achieved via ligand exchange reaction and as exhibited by absence of a shift in the infra red region. In this system, the changes resulting on surface binding was monitored both electrochemically and fluorometrically. This is coupled with
Electrochemical Impedance Spectroscopy (EIS), to assess the efficiency of the molecule toward sensing activity. EIS is highly efficient over chronoamperometry and cyclic voltammetry as the changes in currents due to diffusion, double layer charging and solvent resistance are more prominent. This is because the sinusoidal perturbation used on the sample covers a wide range of frequencies which allows for detailed inquiry into the multiple electrochemical reactions that take place at various rates. It is important to investigate the gold-chromophore assemblies due to the changes that are brought about by the photo-excited electrons, and the numerous deactivation pathways that are possible.

6.2. Synthesis and characterization of AuNC

The new Au-Coumarin hybrid (AuNC) was synthesized via a ligand exchange reaction, (Figure 6.1 gives the schematic) with the goal of developing highly sensitive detectors for NA based on gold nanoclusters. These AuNC could be used as both an optical and an electrochemical sensor. The synthesis of the AuNC was carried out using a modified Brust-Schiffrin\textsuperscript{11} synthesis published by us elsewhere (details in chapter II).

![Figure 6.1. Schematic of ligand exchange reaction](image)

Shown in Figure 6.2A are the optical absorption spectra of Cou-SH, 1.3nm-Au(C\textsubscript{6}S) and 1.3nm-AuNC, wherein Au-NC is Cou-SH attached to 1.3nm-Au(C\textsubscript{6}S). As seen in the figure, the optical properties of Cou-SH and 1.3nm-Au(C\textsubscript{6}S) are unaltered in the hybrid Cou-S-Au cluster system. 1.3nm-Au(C\textsubscript{6}S) exhibits an exponential curve, due to the lowering of the number of conduction
electrons which contribute to intraband transitions in gold clusters smaller than 2 nm, which can be explained based on the Drude term.\textsuperscript{421, 422} Interestingly, the fluorescence of Cou-S is quenched in the Cou-S-Au hybrid, indicating stronger excited state interactions (Figure 6.2B). The gold-cluster, 1.3nm-Au(C\textsubscript{6}S) is itself non-luminescent, so on binding the now coumarin hybrid (AuNC) is luminescent. The origin of the emission at 480 nm was evaluated by recording the excitation spectrum (Figure 6.2C). The fluorescence quantum yield of Cou-SH was determined to be 0.59 and it decreased to 0.19 in the hybrid system, indicating strong electronic interactions, which can be due to either electron or energy transfer processes.\textsuperscript{198, 306, 361, 364, 365, 423-428}

Figure 6.2. (A) Absorbance and (B) emission spectra of coumarin and coumarin labeled Au clusters. (C) Extinction coefficient of Cou-SH. (D) Extinction Coefficient of Cou-SH. (D) Fluorescence Excitation and Emission of Cou-SH and 1.3nm-AuNC [466 to 462 nm]
Square wave voltammetry of 1.3nm-Au(C₆S) and AuNC was carried out to determine whether the addition of the dye molecule alters the redox behavior of 1.3 nmAu clusters as well as for Cou-S, Figure 6.3. The oxidation potentials of the 1.3nm-Au clusters are shifted to more negative values; for example, the first oxidation potential (ox1) of 1.3 nm-Au cluster is shifted from -0.26 V to +0.21 V when the Cou-S is attached. By contrast, the reduction potential of coumarin is shifted from -1.44 V to -1.35 V upon binding to Au. The changes in redox potentials reflect interesting electronic interactions between Au(C₆S) and coumarin. It indicates a possible electron flow from coumarin to Au.

Figure 6.3. Square wave voltammograms of 0.5 mM 1.3nm-Au-(C₆S), 0.2 mM 1.3nm-Au(C₆S)(Cou-S)₅ and Cou-SH in 0.1 M Bu₄NClO₄ in CH₂Cl₂

6.3. Steady state optical properties

Nerve agents are very toxic; therefore diethyl chlorophosphate (DCP) and dimethyl methylphosphonate (DMMP) were employed as model nerve agents for this purpose. These systems were tested with thion pesticides such as parathion, fenthion, malathion, and ethion; they
have similar activity. These sensors are designed to undergo changes in absorption/emission and in electrochemical signals in presence of the target analyte. For this a 1µM solution of 1.3 nm AuNC was used. As seen in Figure 6.4 A, the addition of 34 µM solution of DCP elicits a ten-fold increase in fluorescence emission, resulting in a pink solution from an initial brown color. To test the validity of the sensor, measurements were carried out with similar concentrations of the thions. To assess the role of Cl⁻ leaving group, fluorescence changes with HCl and DMMP were also measured. In order to determine the effectiveness of AuNC binding with the target analyte, a systematic fluorescence titration was conducted. The binding constant was calculated to be 2.6 x10³ M⁻¹ using the Benesi-Hildebrand equation⁴²⁹,⁴³⁰, Figure 6.4B.
Figure 6.4. A) Fluorescence changes on adding DCP and thions to 1 µM AuNC. B) Fluorescence titration of 1 µM AuNC with 10 µM DCP two-photon fluorescence excitation changes comparison

6.4. Two-photon excitation fluorescence

To elicit the sensing efficiency of the system, two-photon fluorescence was conducted. The investigated sensor showed good sensitivity toward DCP on one–photon excitation. The
enhancement in the two-photon fluorescence of the system further substantiates evidence for the coumarin hybrid to be a detector for DCP. The two-photon measurements were carried out with an excitation at 840nm. Comparative enhancements observed for one- and two-photon excitation are shown in Figure 6.5A. The large enhancement in two-photon excitation fluorescence could be due to enhanced electron transfer upon the binding event.

![Figure 6.5](image)

Figure 6.5. A) Comparative fluorescence histograms of 1µM AuNC with OP and thions B) schematic of electron transfer
6.5. Mechanism of Sensing

The fluorescence quantum yield of the coumarin hybrid, namely AuNC, is small when compared to Cou-SH due to electron transfer from the coumarin dye to gold cluster. While all other analytes studied do not influence the dye, DCP interacts with AuNC complex probably through the ring oxygen or the imine nitrogen and making the charge transfer to gold cluster less probable and enhancing the fluorescence. Because on exciting the conduction electrons, the cluster can be multiply ionized, enabling charge transfer. Altering charge transfer processes can explain both turn-on fluorescence behavior as well as the observed electrochemical sensing. Another explanation is that DCP interacts with gold cluster thereby altering the gold cluster's energy levels and the electron transfer from the dye to gold cluster is not possible. This can be explained based on the SWV measurements (Figure 6.3), wherein we see a partial ground state electron transfer happening from coumarin to the gold core, which is reminiscent of our previous work\textsuperscript{66}. We observed a change of $9 \text{ meV} [-1.44 \text{ V to } -1.35 \text{ V}]$. This change can be attributed to the changes in the dielectric medium caused by the nature of the ligand. This observation is in good agreement with the transient absorption measurements (Figure 6.6). The formation of coumarin cation at 575 nm within 600 fs, indicates the electron transfer phenomenon from Coumarin to Au. The free Cou-SH shows a de-excitation pathway due to stimulated emission or the degradation/breaking of the components of the Schiff base due to incandescence.
Figure 6.6. A) Normalized Transient Absorption of AuNC, 1.3nm-Au(C6S) and Cou-SH B) transient absorption spectra of AuNC at different time delays after excitation at 400nm

6.6. Electrochemical impedance measurements

Since our aim is to build and integrate the system to perform dual detection, we conducted EIS measurements using an electrochemical sensor device. EIS is one of the most powerful tools to investigate interfacial electron transfer mechanisms. To establish the system as a good detector, HCl was tested as it is a common false positive for NA. Figure 6.7A shows the change in impedance response of the electrochemical sensor device towards varying concentration of DCP. A better signal to noise ratio was achieved from 20 Hz to 90 Hz compared to higher frequencies
of beyond 120 Hz. As a representative example, at the operating frequency of 20 Hz the impedance response decreased from 300 KΩ when 1 nM DCP was added. Further investigations are under way to investigate the lowest signal that can be generated by the sensor. Figure 6.7B shows the percentage comparative impedance changes when 1 pM HCl and DCP were added. In the case of DCP there was a 50% change in impedance and 17% change in the presence of HCl at 20 Hz frequency.
AuNC are synthesized, by achieving organized binding of coumarin on gold clusters, confirmed by electrochemistry and fluorescence. They are highly selective and sensitive turn-on fluorescent sensors for DCP. Enhancements in both one- and two-photon excited fluorescence is observed with DCP and suggests their application in biological imaging and photo ablation therapy. Two-photon fluorescence enhancement is greater than that of one-photon excitation, which is ascribed to the greater two-photon cross sections upon complexation and lower interference from DCP absorption. Mechanistically, AuNC has greater association constants with DCP as they complex with the imine nitrogen, thereby assisting in electron transfer, which gives rise to the luminescence. The free ligand is unstable when not bound to the gold core. The present architecture of AuNC can be further explored to obtain highly selective and sensitive sensors and develop optical limiting materials.
CHAPTER VII

LINEAR AND NON LINEAR OPTICAL PROPERTIES OF COUMARIN PASSIVATED
GOLD CLUSTERS

7.1. Introduction

Monolayer protected gold clusters (MPC) belong to a group of matter lying in between molecules and metals. Their transition from metallic $\rightarrow$ semiconductor $\rightarrow$ molecular sizes can be controlled by synthetic procedures and the nature of the ligands. This leads to distinct electronic behavior which is now being utilized to build molecular devices, sensors and efficient catalysts. The interaction between the metallic gold core and the mixed ligand outer shell is being tailored to enhance the optical, magnetic and electronic properties. Jin and coworkers have recently reported the ligand’s role in varying the quantum yield of gold clusters depending on the electron withdrawing/donating ability of groups/hetero atoms attached to the thiol ligand being used for surface passivation. It is clearly understood that the onset of size quantization leads to shifts in absorption to higher energies.

Noble metals, especially gold is modified with fluorophores to form hybrid inorganic-organic assemblies to promote uses in catalysis and light harvesting. Fluorescence spectroscopy is an essential tool to understand the mechanistic details of a plethora of deactivation pathways of the photoexcited molecules, namely electron transfer and energy transfer. Modification of gold nanoparticles with fluorophores is important for the development of biological tracers, optoelectronic devices, efficient light-energy conversion systems, data storage and sensors. Seldom have we seen nanoparticles synthesized with chromophores as passivating ligands. In
these systems, the excited states of the adsorbate i.e. the fluorophore could be involved in surface photochemical processes, photon- and electron-stimulated desorption, resonance photoemission, and surface enhanced Raman scattering. The ability to have large chromophore loading and the ability to form highly luminescent centers that can be useful for imaging and sensing applications was the motivation for this work.

7.2. Characterization and steady state optical properties

Since we had worked considerably on ligand exchanged products of chromophores on preformed AuMPCs we decided to channel some time towards the synthesis of AuMPCs with chromophores as the surface passivating ligand. There are very few reports based on this type of systems. The synthesis has been described in Chapter II. We used commercially available 7-mercapto-4-methyl coumarin (MC) to generate gold cluster, AuMC. Successful organization of the dyes on the Au core without aggregation was achieved. The resulting nanoparticles exhibited interesting optical properties. The free dye MC, has a $\lambda_{\text{max}}$ of 320 nm, Figure 7.1. When MC was attached to gold, it exhibits a new band at $\lambda_{\text{max}}$ of 380 nm in the UV spectrum Figure 7.1. This is not an intramolecular charge-transfer state absorption (ICT). It is mostly a $\pi-\pi^*$ absorption. If this is an ICT state, one should observe the absorption maximum around 360 nm. Apart from these two bands, the nanoparticles have a surface plasmon band shoulder at $\lambda_{\text{max}}$ of 520 nm, indicating the overlap of the electronic states, and intuitively indicating a large size nanocluster. The position and shape of the plasmon absorption of metal nanoclusters are strongly dependent on the particle size, dielectric medium, and surface-adsorbed species.
Figure 7.1. Absorption profile of unbound MC and bound MC (AuMC)

The red-shift in the emission peaks parallels the shift in absorption bands (see Figure 7.1 and 7.2). These new electronic transitions of the coumarin chromophore become allowed as the -SH group binds strongly to the gold particle. The peak at 380 nm is more pronounced than the peak at 320 nm, Figure 7.1. At this stage the changes can be predicted to be due to either inter or intramolecular energy or electron transfer. Excitation wavelength of 320 nm and 380 nm was used in order to measure the steady state fluorescence of MC and AuMC respectively. The free dye has an emission maximum at 380 nm, while the nanoparticle-bound MC, has an emission maximum at 480 nm, Figure 7.2. We observed a good Stoke’s shift, giving insight into the innate extended conjugation in this system.
Figure 7.2. Emission profile of unbound MC and bound MC (AuMC)

The square wave voltammograms were measured using an in-house fabricated 3-electrode system (details are in Chapter II). The oxidation and reduction potentials are determined from these measurements. The reduction potential of the dye has shifted to a small extent toward a negative direction and the first oxidation peak of the dye is not observed once the dye is bound to the nanoparticles. Also from figure 7.3A we observe that the nanoparticles so formed do not have molecule-like behavior nor a bulk continuum voltammetric feature, instead they display quantized double layer charging.\textsuperscript{96, 100, 101, 103-106, 436, 437} A few clear current peaks are observed near the \(E_{\text{PZC}}\), the rest potential.\textsuperscript{107, 108} There could be a partial electron transfer happening in the ground state.\textsuperscript{438}
7.3. Fluorescence upconversion

From fluorescence upconversion measurements we see that the free dye has two-component decay; a very fast component and a longer component. The nanoparticle, AuMC has longer lifetimes, which is different from what we normally observe, Figure 7.4. This could be due to energy transfer taking place. Anisotropy becomes zero both in the case of dye and nanoparticles, but at longer time scales in the case of AuMC. This anomalous trend made us probe further.
To rule out the effect of the reducing agent sodium borohydride and to glean information regarding the pH effect of NaBH₄, we studied the interaction of MC with the same. The UV-vis and emission profiles were recorded, Figure 7.5. The UV spectrum showed the same λ<sub>max</sub> at 380 nm and an emission peak at 470 nm, as observed earlier in the case of AuMC. We also recorded the fluorescence upconversion profiles. The fast lifetimes did confirm the expected results. This is one indication that the peak present in AuMC @ 380 nm originates from the binding event happening between MC and gold and not due to any pH effect. Fluorescence upconversion was also done with a base such as triethylamine (TEA). The lifetimes were longer than for MC_NaBH₄ but shorter than for AuMC. Also the peak at 380 nm diminished in intensity with time. This was not seen with respect to AuMC. Furthermore, AuMC is stabilized by TOABr, the surfactant used during the synthesis, which renders stability to the system. Its presence is confirmed by NMR results, Appendix I.

Inadvertently, we found single crystal formation in the solution used for measuring NaBH₄-MC interactions the previous day. The single crystal structure is in Appendix III [analysis by Ms. Carla Sue Eribal, group member]. So we continued our optical evaluations for this system too.
The UV-spectrum had a $\lambda_{\text{max}}$ of 330 nm and an emission $\lambda_{\text{max}}$ of 420 nm that ruled out that MC_CH2_MC was the molecule interacting in situ forming Au-S bonds.

![UV-vis spectrum of MC_CH2_MC and emission spectrum of MC_CH2_MC](image)

Figure 7.6. A) UV-vis spectrum of MC_CH2_MC B) emission spectrum of MC_CH2_MC

The chemical structure of A) MC and B) MC_CH2_MC are given below.

![Chemical structures](image)

Hence it can be concluded that there exists equilibrium between the resonance forms of MC.
From the two-photon enhancements recorded we can see that there is an energy transfer taking place from the Au core to the dye on the surface. There is an 10-fold enhancement per dye molecule absorbed. Coumarin 425 was used as a standard. To prove that MC_NaBH$_4$ or MC_CH$_3$MC is not involved, their two-photon cross-sections were also determined. They did not show any enhancement, reiterating that the nanoparticle surface enhances the charge or energy transfer processes.
7.4. Transient absorption spectra of MC

To understand the one- and two-photon optical properties of coumarin-passivated gold clusters, it is necessary to investigate the photophysical properties of MC in neat solvents. Shown in Figure 7.9 are the excited state absorption (ESA) spectra at different time delays for MC in DCM after excitation at 400 nm. The ESA at a time delay of 300 fs shows a maximum around 640 nm which is assigned to singlet-singlet absorption spectrum. However, the transients decay quite rapidly with time constants of 800 fs, 43 ps and >100 ps. The fast component is similar to what has been observed with fluorescence upconversion measurements and is ascribed to a fast non-radiative relaxation arising out of the free thiol group in the dye. The excited state dynamics of free MC reveals that the ESA is dominated by singlet-singlet state absorption which decays back to ground state both radiatively and non-radiatively with time constants of 800 fs, 43 fs and >100 ps.

Figure 7.9. (A) The excited state absorption spectra at different time delays for MC in dichloromethane. The results show that the excite state of the dye has as fast component in its decay followed by the singlet state lifetime. (B) excited state decay of MC in DCM monitored at 640 nm. The decay trace is fitted with time constants of 650 fs, 43 ps and >100 ps time constants.
7.5. Transient absorption spectral analysis of MC after the addition of NaBH₄

On the other hand, MC alone cannot be used as a reference system for AuMC as the synthesis of AuMC involves addition of NaBH₄. The chromophore investigated had a distinctly different absorption spectrum in the presence of NaBH₄ and as the gold cluster medium consisted of NaBH₄, we have used this system as a reference to compare the linear and nonlinear optical properties of coumarin-passivated gold clusters. Shown in parts A and B of Figure 7.10 are the excited state absorption spectra at different time delays in a short time (300 fs to 8 ps) and a long time window (8 ps to 260 ps). It can be observed from Figure 7.10A that the ESA instantly after excitation has bleach centered on 480 nm and a broad featureless absorption above 600 nm. The bleach is ascribed to the stimulated emission from the dye which matched quite well with the fluorescence spectrum of the coumarin in the presence of NaBH₄. The featureless ESA is attributed to the singlet-singlet absorption spectrum. As the time delay is increased, the bleach shifts to longer wavelengths while the singlet-singlet ESA is shifted to blue wavelengths which follow closely with the solvation of the S1 state. However, there is decay in the stimulated emission and a growth of ESA which suggests that there is a presence of another state which is forming while the singlet state is solvated. Such features are more evident from Figure 7.10B. It can be observed that at longer time scales, both the stimulated emission bleach and the broad ESA collude to give broad ESA with a maximum around 630 nm. This ESA is attributed to the long-lived singlet state which is not allowed with photo-excitation. This state is quite long lived when compared to the singlet state. This observation also explains the fast fluorescence decay observed in fluorescence upconversion measurements. From the global fit analysis, the transients are fitted with three main time constants of 2.2 ps, 11 ps and long-lived component. The 2.2 ps is
assigned to the solvation time and the 11 ps time constant is ascribed to the conversion of the intramolecular charge-transfer state to a less emitting long-lived state (ICT') state.
Figure 7.10. Excited state absorption spectra at different time delays for MC after the addition of NaBH₄ (A) short time and (B) long time window

7.6. Transient absorption measurements of AuMC

As the focus of the present study is to understand the two-photon optical properties of coumarin passivated gold clusters and probe the mechanism behind the changes in the optical properties, we have measured the transient characteristics of AuMC after excitation at 420 nm. Parts A, B and C of Figure 7.11 show short, intermediate and long time interval transient absorption spectra for AuMC. Two main features are observed at short time delays: Firstly, a prominent bleach at 525 nm and two ESA spectral features on either side of the bleach. Such features are normally associated with the transient absorption spectra of gold clusters which possess surface Plasmon absorption. The gold clusters that are synthesized possessed surface plasmon absorption in the UV/Visible absorption spectra, this has to be kept in mind while analyzing the spectrum. Thus, the transient spectra are dominated by the surface plasmon bleach which recovers with time, and a broad ESA centered at 600 nm is formed (Figure 7.11B). This broad ESA is not recovered until 1 ns (Figure 7.11C). All transient absorption traces are fitted globally with four time constants of 1.6 ps, 16 ps, 120 ps and > 1 ns. The 1.6 ps time constant matched very well the electron-phonon
relaxation in gold clusters. However, 16 ps and 120 ps time constants are ascribed to the energy transfer from gold cluster to give rise to ICT’ state of MC. As the ICT’ state of the dye is attached to gold clusters, it will not decay fast to ground state and thus possess long-lived excited state.

Figure 7.11. ESA spectra of AuMC in acetonitrile after excitation at (A) short (B) intermediate and (C) long time windows. Also shown is the (D) species associated spectra obtained from global fitting analysis

7.7. Comparison of transient absorption traces

For complete analysis of ESA features, it is important to compare the observed transient spectra and the decay traces of AuMC with its reference of MC_NaBH₄. Such comparative ESA spectra at different time delays are shown in Figure 7.12A, B and C. It has to be mentioned here that 420 nm excitation for AuMC excites not only the gold clusters but also a significant component of MC. However, at a time delay of 300 fs, the transient is dominated by the bleach arising out of
the surface Plasmon resonance of gold clusters. This can be attributed to the large extinction coefficient for the surface Plasmon and also the transient bleach of MC is influenced significantly in the presence of gold clusters. However, as the time delay is increased to 3 ps, the bleach of MC is shifted to red wavelengths showing the effects of solvation while the transient bleach of AuMC is shifted to blue wavelengths pointing to the decay of the surface Plasmon bleach and eventually giving rise to the bleach of the MC. The results suggest that there is significant energy transfer from the gold cluster to the attached MC. However, one cannot expect similar ESA features for both MC\(_{NaBH_4}\) and AuMC and the dye is entirely in different environments. Also, at longer time delays of 80 ps, there is a featureless absorption for both MC\(_{NaBH_4}\) and AuMC. But, the maxima are different which can be attributed to the different environments of MC. The comparative transient absorption spectra suggest that the main deactivation mechanism for AuMC is an energy transfer from the gold clusters to the adsorbed dye leading to long-lived ESA of MC.
Figure 7.12. Comparative ESA spectra of MC_NaBH₄ and AuMC at (A) 300 fs, (B) 3 ps and (C) 80 ps time delays

7.8. Kinetic decay trace comparison

The kinetic decay traces for AuMC and MC_NaBH₄ at two different wavelengths are shown in Figure 7.13A and 7.13B. The growth of ICT’ state or recovery of bleach is faster in the case of AuMC suggesting the involvement of energy transfer from the gold cluster to the attached MC. It is more evident from Figure 7.13B that the longer growth components are more or less similar except for the short component.
Figure 7.13. Kinetic decay traces for MC\textsubscript{NaBH\textsubscript{4}} and AuMC at (A) 500 nm and (B) 600 nm

Figure 7.14 A explains the possible pathways that the energy transfer is taking; we suggest that there is a combination of intermolecular energy transfer between dyes on the gold surface and energy transfer from Au core to fluorophores on the surface. But the fluorescence upconversion and transient absorption results point towards energy transfer from Au core to dye. Figure 7.14 B and C shows realtime transient absorption spectra for AuMC and MC\textsubscript{NaBH\textsubscript{4}}. Figure 7.14D shows a comparative absorption and emission spectra of reactants and products.
Bleach due to gold cluster absorption

Excited state of Dye

Energy transfer from gold to dye

~ 1.6 ps and 16 ps

-0.007

0.003

Stimulated emission of dye

Solvation

Substitution

Dye-ESA

Stimulated emission of dye

Bleach due to gold cluster absorption

Excited state of Dye

Energy transfer from gold to dye

~ 1.6 ps and 16 ps
Figure 7.14. A) Schematic of energy transfer pathway in AuMC. B) Transient Absorption of AuMC. C) Transient Absorption of MC_NaBH₄. D) Comparisson of UV-vis and emission spectra.

7.9. Conclusions

We successfully synthesized AuMPCs with fluorophores and characterized the coumarin passivated gold clusters with TEM, NMR, UV and electrochemistry measurements. We show that 7-mercapto-4-methylcoumarin is a reporter of thiol binding to the nanoparticle surface and that this process can be followed by common spectroscopic techniques. Fluorescence quantum yield increased dramatically when the dye is bound to gold clusters. The TPA cross-section of AuMC is eight times higher than MC_NaBH₄.

The reasons for the cooperative enhancements can be attributed to:

(i) rigidifying the chromophore or
(ii) increased energy transfer from gold to dye or both.
This system is an ideal example to illustrate the process of energy transfer. Two-photon measurements prove its further use as nanoparticle beacons and in the development of non-linear optical materials for biological imaging. AuMC can be used to develop optical and electrochemical sensor systems. All fluorescence upconversion and transient absorption measurements suggest that the energy transfer from the gold core to dye is responsible for the enhanced luminescence quantum yield and increased TPA cross-sections.
CHAPTER VIII

INVESTIGATING ELECTRON TRANSFER BETWEEN VIOLOGEN BASED THIOL AND GOLD CLUSTERS

8.1. Introduction

Supramolecular structures have attracted tremendous interest from a molecular machine perspective. Combining these with nanoparticles (NP) is an intriguing proposition due to the unique optical and electrochemical properties of nanoparticles, which are size dependent. Depending on the size and the nature of the surface of the nanoparticles they can be applied for constructing photovoltaic devices, nanoelectromechanical systems (NEMS) and targeted drug delivery for therapeutic uses\(^{439-443}\). Rigid molecular machine immobilization on resilient surfaces has revolutionized the way we apply mechano-stereochemical principles.

The building of these NP-based supramolecular architecture is an interesting and challenging field, as it challenges us to find suitable nanomaterials. Gold clusters (AuNP) are well known for their self-assembly properties and hence can be used as substrates for assembling mechanically interlocked catenanes, pseudorotaxanes or rotaxanes. Ambrogio\(^6\) et al. have recently stated that we can look at these assemblies based on a bow-and-arrow approach, the arrow being the axle/rotaxane and the nanoparticle as the bow which would complement each other. The surface of the AuNP, when altered with different ligands, causes dramatic changes to the electronic properties of the nanoparticles due to spectral overlap.
A rotaxane/pseudo rotaxane is a mechanically-interlocked molecular architecture consisting of a dumbbell shaped molecule referred to as a rod/axle, which is threaded through a macrocycle referred to as a wheel.

![Schematic of a rotaxane](image)

Figure 8.1. A) Schematic of a rotaxane B) redox-switching in a bistable neutral rotaxane

The two components are held together by intermolecular forces such as ion–dipole interactions, hydrogen bonding, π–π stacking, electrostatic interactions or coordination bonds. The position of the wheel along the axle can be switched in a controlled and reversible fashion, onset by external stimuli such as chemical (pH change, metal ion, etc.), electrochemical or light input.

Herein, we examine the interaction between glutathione protected AuNP with a new thiol-based methyl viologen (MV$^{2+}$-SH) axle molecule. We need to understand the mechanism of interaction between nanoparticles and the surface modifier. In this case the modifier is the axle (MV$^{2+}$-SH) with possible implication in building efficient sensors. The distance between donor - and acceptor is a vital cog in the development of sensors for metal ions. Coulston et al. have reported interactions of a 12-carbon modified paraquat and showed that there is a one electron transfer when it is loaded on gold nanoparticles.
**8.2. Characterization using UV-vis and steady state measurements**

In our case we have used a 4-carbon chain length distance from the MV$^{2+}$ core (synthesis details are in Chapter II). Further studies have to be carried out to determine the critical chain length required in order to prevent the electron transfer from Au core to surface ligand, thereby retain the positive core. For this study we used glutathione (SG)-protected AuNPs of two sizes, Au$_{25}$ and 4nm-Au (SG), which were altered via a ligand exchange reaction with the synthesized MV$^{2+}$-SH ligand. The ligand exchange reaction was carried out on a preformed gold – glutathione MPC to label it with the axle molecule (MV$^{2+}$ - SH). In order to characterize the sample we recorded the UV spectrum; after subtracting the Au absorption the number of axles/cluster was estimated, using the axle extinction coefficient. The axle molecule has absorption in the UV region, $\lambda_{\text{max}} = 270$ nm, Figure 8.3.
The fluorescence changes of these methyl viologen based molecules have a low quantum yield. The free axle has a charge transfer luminescence at 520 nm due to the interaction of the Br counter ion with the positively charged core of the axle and a feeble emission at 340 nm. When the axle is bound to a gold surface the luminescence at 340 nm is quenched and the charge transfer band completely disappeared or was quenched, Figure 8.4.
The next step was to assemble the crown ether/wheel with the axle molecule to complete the assembly of a pseudo-rotaxane or molecular machine. The UV-Vis spectra were recorded and this did not show any new band in the UV profile; which should have been present at a wavelength 450 nm under normal circumstances owing to the complexation/electrostatic interaction between the axle and the wheel, Figure 8.5. One explanation for its absence could be the very high extinction coefficients of AuMPCs. AuMPCs have an exponential decay from the UV-region into the near IR-region. So we tried using smaller AuMPCs such as Au$_{25}$ 1.1 nm in diameter instead of the 4 nm species. This resulted in similar inconclusive behavior with no new band in the absorption spectrum.

![Absorbance vs Wavelength graph]

Figure 8.5. Comparison of MV$^{2+}$-SH, axle and wheel modified AuMPC

This led us to believe that there is a high likelihood of electron transfer, which is neutralizing the positively charged bipyridinium core.
8.3. Electrochemical measurements

We looked toward electrochemistry to give us conclusive evidence. Voltammetric experiments were conducted using conventional 3-electrode systems and large sample volumes ~1mL. Figure 8.6A gives the square wave voltammogram results for the axle, MV$^{2+}$-SH. This showed typical molecule like charging with two reduction and oxidation peaks. The voltammograms for the axle modified 4nm-Au-GS MPC were also recorded, Figure 8.6B. From this we can see that the axle peaks could not be accounted for, further supporting the electron transfer theory. Also, the rest

![Voltammograms](image)

Figure 8.6. Square wave voltammograms of A) MV$^{2+}$-SH B) comparison between 4nm Au(SG)MV$^{2+}$-S, MV$^{2+}$-SH and Au modified with the wheel C) rest potential values
potential values moved in the positive direction, indicating that the Au is oxidized. The rest potential values for Au(SG) changed from 0.15 V to 0.175 V indicating oxidation of the Au cluster.

To confirm this hypothesis, an electrochemical titration was performed. As the synthesis of the ligand is cumbersome, we made use of a novel two electrode printed system made of silver nanoparticle ink to conduct a square wave voltammetric experiment. The electrodes and the electrochemical workstation were at the SMART sensor fabrication lab in Professor Masood Zandi Atashbar’s lab, our collaborator. The main advantage of this system is the use of small sample sizes of 10 µL for measurements. To see if the currently designed electrode worked credibly when compared to conventional electrochemical measurements [where we normally use a three-microelectrode system comprised of a working, a counter and a reference electrode in a closed vessel], we conducted an experiment with the printed two-electrode system on standard methyl viologen dihydrochloride (MV$^{2+}$) and MV$^{2+}$-SH. It should be noted that the sample volume is now reduced to 10 µL. These values were compared to reported values$^{449}$, Figure 8.7.

![Figure 8.7. SWV of 100mM MV$^{2+}$ and 100mM MV$^{2+}$-SH using 0.1M KCl](image)
The square wave voltammogram shows two peaks, at -0.64 V and -0.97 V, which are in agreement with published values measured in water\(^{450}\). In order to affirm the electron transfer an electrochemical titration was performed between AuNP [Au\(_{25}\) and 4nm-Au(SG)] against MV\(^{2+}\)-SH, while monitoring any changes due to size effect of the AuNP. For the 4nm-Au(SG) and Au\(_{25}\) we see the change in reduction potential toward the negative direction, displaying the probable electron transfer from the Au core to MV\(^{2+}\)-SH, Figure 8.8. If this electron tunneling from Au is prevented, the bipyridinium ring will remain a positively charged core, and enable subsequent assembly of the crown ether ring to complete the formation of the pseudo-rotaxane.

Figure 8.8. A) SWV of 1 mM MV\(^{2+}\)-SH vs 1.8e\(^{-5}\)M 4nm-Au(SG)  B) SWV of 1 mM MV\(^{2+}\)-SH vs 1.8e\(^{-5}\)M Au\(_{25}\) C) change in peak potential for Au\(_{25}\) and 4nm-Au(SG)
From the square wave voltammogram (SWV) we see that the 4nm-Au(SG) has less electron transfer effect when compared with Au$_{25}$ (Figure 8.8 A,B). This can be attributed to the percentage of SG being higher on the surface in the 1 nm cluster, Au$_{25}$. Hence there could be two sources from where an electron can shuttle, one being from the heteroatom N having a role to play in the electron transfer, apart from electron transfer taking place from the gold core to the MV$^{2+}$-SH. The change in peak potential toward a more negative value from -0.53V to -0.59V for 4nm-Au(SG) is a representative example (peak1), which is an indication of the electron transfer happening in the ground state (Figure 8.8 A,C). Similar changes are observed in the case of Au$_{25}$.

![Absorption spectrum](image)

Figure 8.9. Absorption spectrum of TOAB stabilized AuNP

Another set of experiments was carried out with hexanethiol AuMPC and TOABr passivated MPCs. Similar results were obtained with no evidence for the assembly of the pseudo-rotaxane, Figure 8.9. This rules out the possibility that the ligand glutathione had any part in the electron transfer.
transfer process. It indicates that the bipyridinium accepts electrons irrespective of the nature of the ligand.

8.4. Conclusions

Further experiments have to be performed to determine the critical carbon chain length necessary to prevent electron tunneling. Irrespective of the size of the AuMPC or nature of the passivating layer, electron transfer seems to be taking place. A novel printed electrode using silver nanoparticles can be used for studying complex systems in volumes as small as 5-10 μL, which would allow facile sample preparation and less time. These systems can lead to the development of handheld sensors and NEMS devices. Surface pre-organisation within robust self assembled monolayers can be exploited for selective electrochemical sensing of anions in both organic and aqueous media. Combining the processes of molecular-scale recognition and surface self-assembly offers a powerful route to the development of sensing systems and, more generally, new technological devices based on controlling and analyzing interactions at the nanometer scale.
CHAPTER IX

SUMMARY AND OUTLOOK

9.1. Summary

My research focused on the synthesis, characterization and the investigation of the optical and electrochemical properties of monolayer-protected quantum-sized gold clusters. Much attention was focused by us on the solution phase optical and electrochemical properties of these clusters. Highly monodisperse Au clusters with different types of ligands were successfully synthesized varying in size from 1 nm to 13 nm. Further, these preformed gold clusters were modified with fluorophores and pseudo rotaxanes for developing nonlinear optical materials for chemical sensing and biological imaging purposes. We have tried to focus on the interaction of the outer ligand shell, made of –S-Au-S-Au-S- bonds, with the core gold states. This alludes to changes in the optical footprint of these AuMPCs.

In summary, ultrafast luminescence investigations on Au$_{25}$L$_{18}$ clusters with two ligands, glutathione (GS) and hexanethiol (C$_{6}$S), have revealed the nature of the higher excited states in these clusters. A low quantum-yield visible luminescence is observed in Au$_{25}$ clusters in addition to the NIR luminescence which is used as a probe to understand the excited state relaxation. The dynamics of the luminescence from Au$_{25}$ clusters have distinct wavelength-dependent growth and decay kinetics which is absent in larger gold nanoclusters and nanoparticles. The growth of luminescence is independent of the ligands and is ascribed to the luminescence from the Au$_{13}$ core states and the decay dynamics is mainly governed by its relaxation to S-Au-S-Au-S semi-ring states. Relaxation processes happen over several hundreds of femtoseconds and proceed
through a multitude of electronic energy levels. Investigations have shown that the higher excited-states are longer lived unlike what is predicted in a simple molecular excited state picture. This can be harnessed for electron transfer from hot states and can be utilized in solar energy harvesting and catalysis. Thus the electronic properties of Au\(_{25}\)L\(_{18}\) clusters suggest that they belong neither to the class of large nanoparticles nor do they bear any semblance to molecular behavior; they are best understood as a standalone “superatom complex” system. Comparative luminescence measurements on larger monolayer-protected gold clusters (2.2 nm Au(C\(_6\)S) and 2.2 nm Au(GS)) and gold nanoparticles (3 nm Au(C\(_6\)S) and 13 nm Au(citrate stabilized)) were also carried out. The excited state dynamics in Au\(_{25}\)L\(_{18}\) clusters is not a typical two-state relaxation from core to semi-ring states, but rather proceeds through a manifold of electronic states as the luminescence traces show wavelength-dependent growth and decay kinetics. Also, femtosecond time-resolved luminescence measurements of Au\(_{25}\)L\(_{18}\) have proved that the higher excited-states in monolayer-protected Au\(_{25}\) clusters decay with a finite lifetime (200 fs up to a few ps) that can be utilized for applications in solar-energy harvesting and catalysis, Figure 9.1
Temperature-dependent electronic absorption properties of the \( \text{Au}_{25}\text{L}_{18} \) and \( \text{Au}_{38}\text{L}_{24} \) clusters have been studied for the first time. So far, research has been carried out by the El-Sayed group at Georgia Tech on gold nanoparticles larger than 10 nm. Interesting absorption features such as shift in the absorption maxima to higher energies, sharper absorption peaks along with additional vibronic peaks, and increased oscillator strengths of the electronic transitions, are observed with decreasing temperatures. The results are explained on the basis of electron-phonon interactions involving core-gold electrons or excitons with semi-ring gold phonons. The temperature-dependent absorption maxima and oscillator strengths were fitted with models that describe the energy gaps in semiconductor quantum dots and quantum wells. Significantly larger phonon energies of \( \sim 400 \text{ cm}^{-1} \) were observed for the MPCs suggesting that they are arising out of the semi-ring gold. Also, increased exciton-phonon coupling strengths are observed for MPCs which
reduces the oscillator strength of electronic transitions at higher temperatures. The unique property of decreasing oscillator strength with increasing temperature is modeled on the Debye-Waller equation, which relates oscillator strength to the exciton-phonon interaction. We have demonstrated that this is observed only in quantum sized clusters and not in nanoparticles, Figure 9.2.
We also did extensive research on chromophore labeled Au clusters. We used pyrene and coumarin analogs as chromophores. Precisely formulated \( \text{Au}_{25}(\text{C}_6\text{S})_{17}\text{PyS} \) clusters have been synthesized with one pyrene species attached to each \( \text{Au}_{25} \) cluster and its electrochemical and optical properties have been investigated. It is unambiguously shown that \( \text{Au}_{25} \) clusters can work as electron donors with electrochemical and optical measurements. The interesting electron transfer ability of \( \text{Au}_{25} \) clusters can be utilized in making better sensors, catalysts and light harvesting systems. Significant fluorescence quenching is observed for pyrene attached to \( \text{Au}_{25} \) clusters, suggesting strong excited state interactions. Time-resolved fluorescence upconversion and transient absorption measurements are utilized to understand the excited state dynamics and possible interfacial electron and energy transfer pathways, Figure 9.3. Electrochemical investigations suggest the possibility of electron transfer from \( \text{Au}_{25} \) clusters to the attached pyrene. Fluorescence upconversion measurements have shown faster luminescence decay for the case of pyrene attached to \( \text{Au}_{25} \) clusters pointing toward ultrafast photoinduced electron/energy transfer pathways. Femtosecond transient absorption measurements have revealed the presence of the anion radical of pyrene in the excited state absorption, suggesting the directional electron transfer from \( \text{Au}_{25} \) clusters to the attached pyrene.
transfer from Au$_{25}$ clusters to pyrene. The rate of forward electron transfer from Au$_{25}$ cluster to pyrene is ultrafast (~580 fs) as observed with femtosecond fluorescence upconversion and transient absorption. This is the first report that shows the possibility of electron transfer from Au$_{25}$ to the fluorophore which attests that Au$_{25}$ is an electron donor.

![Diagram of synthesis pathway and electron transfer in Au$_{25}$(C$_6$S)(PyS)](image)

Figure 9.3. Schematic of synthesis pathway and electron transfer in Au$_{25}$(C$_6$S)(PyS)

We also used coumarin labels on preformed MPCs. They were synthesized by achieving organized binding of coumarin on gold clusters, and confirmed by electrochemistry and fluorescence. They are highly selective and sensitive turn-on fluorescent sensors for DCP. Enhancements in both one- and two-photon excited fluorescence is observed with DCP and this also suggests their application in biological imaging and photo ablation therapy. Two-photon fluorescence enhancement is greater than that of one-photon excitation, which is ascribed to the greater two-photon cross sections upon complexation and lower interference from DCP absorption. Mechanistically, the coumarin labeled clusters (AuNC) had greater association
constants with DCP as they complex with the imine nitrogen, thereby assisting in electron transfer, which gave rise to the observed luminescence, Figure 9.4. The free ligand is unstable when not bound to the gold core. The present architecture of AuNC can be further explored to obtain highly selective and sensitive sensors and develop optical limiting materials.

![Diagram showing fluorescence emission spectra of DCP and AuNC](image)

Figure 9.4. Coumarin labeled Au clusters for two - photon sensing of DCP

We tried alternative methods to get organized assembly of fluorophores on AuMPCs. We successfully synthesized AuMPCs directly with fluorophores, by the Brust-Schiffrin Synthesis, with some residual TOABr. We have shown that 7-mercaptop-4-methylcoumarin is a reporter of thiol binding to the nanoparticle surface and that this process can be followed by common spectroscopic techniques, Figure 9.5. This system is an ideal example for the process of energy transfer. All the fluorescence upconversion and transient absorption measurements suggest that
the energy transfer from the gold to dye is the reason behind enhanced luminescence quantum yield and increased TPA cross-sections. Two-photon measurements illustrate its further use as nanoparticle beacons and in the development of non-linear optical material for biological imaging. This also shows that AuMPCs can be made to glow if we achieve the desired core size.

Figure 9.5. Schematic of possible pathways for making AuMC glow

We also tried to assemble molecular machines/rotaxanes on our preformed Au clusters. Further experiments have to be performed to determine the critical carbon chain length necessary to prevent electron tunneling. Irrespective of the size of the AuMPC, or nature of the passivating layer, electron transfer seems to be taking place, hampering the threading of the electron-rich crown ether wheel component, Figure 9.6. A novel printed electrode using silver nanoparticles was used for studying the electron transfer in small sample volumes, as small as 5-10 µL, which circumvented cumbersome sample preparation and more time in carrying out measurements which entails regular voltammetric experiments. These systems can lead to the development of handheld sensors for metal ions and NEMS devices. Combining the processes of molecular-scale
recognition and surface self-assembly offers a powerful route to the development of sensing systems and, more generally, new technological devices based on controlling and analyzing interactions at the nanometer scale.

Figure 9.6. Electron transfer from gold to axle molecule

The realm of monolayer-protected metal nanoclusters, in this case gold MPC, lies between bulk metal and discrete atoms, making it an interesting intermediate, bridging physics, chemistry and material science. Despite extensive work over several decades by different research groups, (the Murray group, the Whetten group and more recently the Jin group), there still remains unanswered questions pertaining to:

- Size controlled synthesis of Auₙ(L)ₘ clusters
- Single crystal structure determination of Auₙ(L)ₘ
- Origin of photoluminescence
- Critical number of electrons needed to observe a surface 183lasmon band
- Origin of magnetism
- Catalytic properties of $\text{Au}_n(L)_m$
- The origin of stability of $\text{Au}_n(L)_m$
- The role of the nature of the passivating layer, the mixed ligand Au outer shell in determining the optical properties
- Non-linear optical properties

In light of the above mentioned properties of gold clusters the following projects can be pursued.

9.2. Project 1: Development of sensors for pesticides

**Goal:** To develop multimodal electrochemical-fluorescent sensor systems to detect NA mimics using small gold clusters in order to make ultra-thin film detectors.

**Proposed Study:** We proposed to develop highly sensitive detectors for nerve agents based on gold nanoclusters (AuNC). These AuNC could be used as both optical and electrochemical sensors. Nerve agents are very toxic; therefore we will employ diethyl chlorophosphate (DCP) and dimethylmethylphosphonate (DMMP) as models for this purpose. Also we will test these systems with the organothiolate pesticides like parathion, fenthion, malathion, and ethion; they have similar activity. These sensors are designed to undergo change in absorption/emission and in electrochemical signal in the presence of the target analyte.

**Current Results:** We have synthesized and characterized two systems **System I** ($\text{AuMC; Au - gold core; MC - coumarin ligand}$) and **System II** ($\left[\text{Au}_{25}(\text{PPh}_3)_{10}(\text{FcS})_3\text{Cl}_2\right]^{2+}$; **Au- gold core**;
FcS - ferrocene ligand on the surface). The results of System I and II are presented in Figure 9.7 and 9.8, respectively.

Figure 9.7. System I (AuMC) A) UV-vis spectra of free ligand (MC) and product (AuMC) B) fluorescence spectra C) fluorescence change on adding nerve gas analogs D) TEM image

Figure 9.7 A, B, D, indicate the formation and characterization of System I; demonstrating the difference between the free and bound ligand. In Figure 9.7A and B we see a red shift in peak maximum from 320 to 380 nm in the UV-Vis and 380 nm to 470 nm in the fluorescence spectra for the product. From Figure 9.7C we can see that it shows preferential quenching in terms of fluorescence with DCP (analyte), thus it could be used as a detector for DCP in nanomolar concentrations.
Figure 9.8. System II ([Au$_{25}$(PPh$_3$)$_{10}$ (FcS)$_5$ Cl$_2$]$^{2+}$) A) UV-vis spectra of free ligand(FcSH) and product ([Au$_{25}$(PPh$_3$)$_{10}$ (FcS)$_5$ Cl$_2$]$^{2+}$) B) TEM Image C) & F) Fluorescence change on adding nerve gas analogs to System II and FcSH respectively D) Square wave voltammogram of System II and FcSH E) change in oxidation potential on adding nerve gas analogs
Figures 9.8A, B, and D, indicate the formation and characterization of System II, demonstrating the difference between the free ligand and the bound ligand. The large band gap of 1.8 eV in Figure 9.8D indicates the core size to be 1 nm which complements the TEM measurement. Also the shift in oxidation potential from 0.2 eV to 0.8 eV determines that the FcSH ligand is bound to the gold core. Figure 9.8E summarizes the shift in oxidation potential of System II on adding various analytes; it shows maximum change with respect to fenthion. Figure 9.8C shows preferential fluorescence quenching with fenthion (analyte) of System II, which coincides with the electrochemical results in Figure 9.8E. Figure 9.8F shows the fluorescence activity of the free ligand (FcSH). It can be clearly understood that there is no change in the free ligand towards the analytes.

**Significance**

These systems will overcome complex separation processes such as sample pretreatment, expensive equipments, time, and using toxic organic solvent, which all of the current methods involve.

- These systems absorb light at higher wavelengths i.e. in the near IR/ visible region we can use low energy radiations instead of harmful high energy radiation (UV light).
- These systems are good two-photon absorbers; hence can be used as bioimaging agents as well (Figure 9.7D).
- We will finally develop a handheld detector by constructing a solid electrode which could be one possible extension of the project.
- Electro-analytical sensors are alternative and viable methods because of their compact nature, easy handling in field trials, low cost and low power requirements. They can also produce selective responses (Figure 9.8E) within a very short time with respect to those methods described in the background section.
**Further analysis:** To fully understand the mechanism which promotes the detection of specific analytes such as DCP and fenthion, Transient absorption measurements will have to be done. Transient-absorption spectroscopy is an extension of absorption spectroscopy. Also, to determine the nature of quenching of fluorescence; be it static or dynamic Stern-Volmer plots will have to be drawn and the quenching constants have to be determined. Binding constants will have to be calculated in order to project the strength of the bond that is formed between the AuNC substrate and the analyte. Currently, we have a 1.3 nm AuMC cluster (System I) and a 1 nm ([Au$_{25}$($	ext{PPh}_3$)$_{10}$(FcS)$_5$Cl$_2$]$^{2+}$) system (System II). We will have to synthesize other sized AuNC with the MC and FcSH ligands and assess their sensing capabilities by comparing them with System I and II, to determine the role of core size and ligand structure. Also from a structural point of view it has to be determined if the AuFcS system is a true quantum sized gold cluster or a simple organometallic complex, since at this point in time the fluorescence upconversion results indicate that the cluster/crystallite has no Au core (Figure 9.9). The comparison between the two Au$_{25}$ molecules will have to be pursued as System II does not have the semi-ring structure.

![Figure 9.9. Fluorescence upconversion of [Au$_{25}$($	ext{PPh}_3$)$_{10}$(FcS)$_5$Cl$_2$]$^{2+}$](image-url)
9.3. Project 2: Drugs for targeted cancer therapy and imaging

Goal: To develop a new system with the dual function of labeling and destroying of cancer cells using quantum sized [diameter < 10 nm] gold clusters, AuNC, less than 2 nm diameter, modified with a dye and antibody. This will thereby result in increasing the payload per kilogram, increasing specificity and minimizing invasive surgery.

Proposed study: To develop versatile dual functionality endowed AuNC with dyes and drugs for targeted drug delivery and labeling. Cytotoxicity studies will be performed to determine drug efficacy, as a collaborative project [Mr. Amr Izzat Mohammed Mahmoud, fellow group member]. A two-photon study has to be done to assess their imaging capabilities.

In this case, pyrene-N-hydroxysuccinimide or fluorescein-N-hydroxysuccinimide, can be used as fluorophores which provides optical communication between the system and the environment. Importantly, the rate of electron/energy transfer is much faster than the luminescence [fluorescence emission]. Figure 9.10A is the schematic structure of the AuNC explaining the system; firstly the AuNC is synthesized using Glutathione (SG) a low molecular weight poly peptide. SG was chosen as it is overexpressed in cancerous cells. This will enhance cellular uptake. Then this will be coupled with the antibody receptor which could be a surface receptor or internal receptor depending on the type of cancer.
Figure 9.10B explains the photochemistry phenomenon. When light is absorbed by the AuNC, the vacancy created by the excitation of electron in the fluorophore is filled by transfer of electron from the gold core to the ground state of the dye. Then the excited state electron undergoes relaxation to the triplet state which matches the ground state of molecular oxygen. At this stage there is a quick energy transfer to the oxygen, which in turn generates the free radicals which act on the tumor leading to apoptosis. When there is no oxygen in the vicinity, the electron moves back to the ground state emitting radiation. This can be monitored via a fluorimeter and will be used for labeling purposes. Also, the two-photon absorbing properties will have to be measured to assess their near IR fluorescing capabilities.

**Current Results:** We have synthesized and characterized three systems **System I** (AuMC; MC - coumarin ligand), **System III** (Au_{25}GS) and **System IV** (Au_{314}GS); GS – glutathione ligand on the surface, and further modified with DOX = doxorubicin hydrochloride, a common anticancer (leukemia)drug and PyNHS( a chromophore). The results of System I, III and IV
so far are presented in Figure 9.11, 9.12 and 9.13. Cytotoxic studies were performed on various cell lines related to breast cancer. Curcumin was used as the standard for comparison. Figure 9.11 shows the comparison between the standard and AuMC. It can be understood that even at 1 mg concentrations, System I shows remarkable anti cancer activity. Besides, the molecule is selective toward cell lines SK-BR-3 and MDA-MB-231, within a 48 hr. incubation period. This is promising and further investigations are under way.

![AuMC vs Curcurmin](A) (B) schematic of AuMC

Figure 9.11. (A) Comparison of cytotoxicity of Curcurmin vs. AuMC (cell culture experiments performed by Mr. Amr Mohammed) (B) schematic of AuMC

From Figure 9.12, it is observed that AuNC behaves differently when the surface is modified with dye PyNHS (pyrene) The UV- vis peak is blue shifted when the chromophore is bound to the gold cluster via amide bonds, indicating electron transfer from the gold core to the dye. The lifetimes have two components, and this proves that there is a chemical bond formed. System IV was tested for cytotoxic activity versus curcumin and DOX and AuNC coupled with DOX. From the results, in Figure 9.13, we see that Au-GS by itself has higher cellular uptake in the BT-474 cell line. These results are promising in that, it give us the impetus to design the molecule with increased DOX loading. Secondly we have to decrease the diameter of AuNC to 1 nm from 2 nm
(which was used in this study) and see if cell uptake is enhanced. This will promote increased anti-cancer activity.
Figure 9.12. UV/vis spectra of (A) 1.1 nm AuNC. (B) 2 nm AuNC. (C) Lifetime profiles of the coupled products (D) Fluorescence upconversion results (E) Emission Spectrum. \( \text{Au}_{25}\text{GS}_{18}\text{PyNHS}_8 \) and \( \text{Au}_{314}\text{GS}_{101}\text{PyNHS}_{25} \) are the molecular formula of the dye modified AuNC; the suffix 8 and 25 indicate the no. of dye per AuNC; the suffix 18 and 101 are the no. of GS and 25 and 314 refer to the no. of Au atoms on the core.

### Comparison between Au(GS), Au\(_{314}\)(GS)-(DOX) and DOX vs Curcurmin

Figure 9.13. Comparison of cytotoxicity of Curcurmin vs. DOX, GS, Au\(_{314}\)-GS and Au\(_{314}\)GS-DOX (cell culture experiments performed by Mr. Amr Mohammed)
Figure 9.14. Schematic of the synthetic reaction (Chapter II)

Figure 9.15. Transient absorption results of PyNHS modified Au$_{25}$GS and Au$_{314}$GS and PyNHS
From the transient absorption results we see that there is a likelihood of electron transfer taking place from gold to dye resulting in the formation of pyrene anion, reminiscent of the molecule we investigated in Chapter V. A further transient absorption result of GS-PyNHS has to be carried out to fully understand the mechanism of electron transfer.

**Significance**

- A system with dual function greatly decreases metal poisoning due to drug administration. The uses of AuNC conjugated with specific antibodies helps enhance tumor specificity and provide targeted apoptosis.

- Light is delivered only to the specific targeted tissues that a physician wishes to treat. In the absence of light, there is no activation of the photo-sensitizer and no cell killing.

- PDT can be much cheaper than the alternative radiotherapy or surgical operation and after care.

- We can ultimately envision the utilization of nanoparticles as nanovehicles to deliver therapeutics to sites of disease notoriously difficult to treat, such as, the brain. Minimum accumulation of the drug can be achieved thereby reducing the chances of the cells developing resistivity to the drug.

- Since this project deals with the development of sub nanometer sized spherical systems this will overcome the size and shape barrier and facilitate easy uptake. This also increases the surface area tremendously (i.e. the surface-to-volume ratio). This will escalate the payload per kilogram, thereby delaying the onset of drug resistance. It also will diminish side effects
drastically as the amount of foreign substance in the body will be reduced and therefore rejection is nullified.

- Devise effective treatments for all stages of skin cancer.

- AuMC so far is selective toward cell lines SK-BR-3 and MDA-MB-231, within a 48 hr. incubation period.

**Further Analysis:** Initial tests have been conducted for System I, III and System IV modified with DOX. These do look promising. The diameter of the current nanoparticle studied is 2 nm. Similar studies have to be done with Au_{25}GS, which is 1 nm in diameter. It will be interesting to compare them and compare the kinetics and see if there is a size effect. Besides, since the two photon measurements of these samples are promising we will need to do an investigation involving *in vitro* and *in vivo* cell imaging. Cytotoxic studies have to be done with normal cells and also the DOX-modified systems have to be studied on blood cancer cells in addition to breast cancer cells.
REFERENCES


204


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APPENDIX A

TEM-micrographs of different core size AuMPCs

\[ \text{Au}_{25}(\text{SC}_6)_{18} \]

\[ \text{Au}_{38}(\text{SC}_6)_{24} \]

\[ \text{Au}_{144}(\text{SC}_6)_{60} \]

\[ \text{Au}_{309}(\text{SC}_6)_{92} \]
AuC₆S(PyS)  \[\text{[Au}_{25}\text{(PPh}_3\text{)}_{10}\text{(FeS)}_5\text{Cl}_2]^2+\]

\[\text{Au}_{2406}\text{(SC}_6\text{)}_{326}\]  Au-Citrate
Au(C₆S₅) and Au NC [Coumarin labeled]

Au₂₅GS₁₈

1.1 ± 0.2 nm

Au₂₅GS₈PyNHS₁₈

1.2 ± 0.2 nm

Core Size (nm) 2.4 ± 0.4 nm
Au-Cysteine clusters

UV-vis spectral characterization of Au Cysteine clusters

![Graph showing UV-vis spectral characterization of Au-Cysteine clusters]
$^{1}$HNMR spectra

**Au$_{25}$C$_{6}$$_{18}$P YSH$_{3}$ in CD$_{2}$Cl$_{2}$ after iodine treatment**

- 9 Aryl H from PYSH
- CH$_{3}$ from C6 Thiol

**Au$_{25}$G S$_{18}$PyNHS$_{18}$ in D$_{2}$O**

- 10 H from GSH
- 9 Aryl H from PyNHS
$^1$H-NMR of MC$_2$CH-MC
$^{1}$H-NMR of AuMC
$^1$H-NMR of MC
### APPENDIX B

Representative example of a two-photon calculation table

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APPENDIX C

X-ray crystal structure of MC_CH2_MC
4-Methyl-7-[(4-methyl-2-oxo-chromen-7-yl)sulfanyl-methyl-sulfanyl\ chromen-2-one
APPENDIX D

List of publications


Contributed Work

vi. Fasil Abebe, Carla Sue Eribal, **Mary Sajini Devadas**, Ekkehard Sinn, “ Novel Turn-Off Nerve gas sensors based on Coumarin Schiff-base derivatives” *Sensors and Actuators B*, Accepted

vii. Binu Narakathu, **Mary Sajini Devadas**, Isurika Fernando, Guda Ramakrishna, Gellert Mezei, Ekkehard Sinn, Masood Zandi Athashbar, “Square Wave Voltammetry on a Novel Screen Printed flexible Sensor of a modified bipyridinium-thiol rod” manuscript under preparation.

viii. Lars Kohler, **Mary Sajini Devadas**, Guda Ramakrishna, Elke Schoffers, “Photophysical properties of Novel Phenanthroline Derivatives” *Journal of PCCP*, manuscript under preparation.