Gold And Silver Alloy Nanoparticles: Synthesis, Characterization And Atomic Structure

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GOLD AND SILVER ALLOY NANOPARTICLES:
SYNTHESIS, CHARACTERIZATION AND ATOMIC STRUCTURE

A Dissertation presented for the Doctor of Philosophy Degree
The University of Mississippi

Chanaka Kapila Kumara

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ABSTRACT

Nanotechnology is an emerging field of science with applications in digital electronics, medicine, catalysis and energy. Gold nanoparticles are nanomaterials that have less than 100 nm at least in one dimension. The composition and the structure/geometry of the nanoparticles determine the chemical properties and reactivity. Modern research focus on method development in atomically monodisperse nanoparticle synthesis. This dissertation describes the method development for synthesis and isolation of atomically precise gold and gold-silver alloy nanoparticles and their comprehensive characterization and atomic structure investigation.

Chapter one offers an introduction to the synthesis and isolation methods. These nanoparticles can be represented in the form of Auₙ(SR)ᵢ, where SR is the thiol ligand. The co-reduction method was used for alloy nanoparticle synthesis using a fixed total metal molar ratio. After considering various possible elements, silver were selected to study the formation of alloys to atomically precise gold nanoparticles.

Chapter two includes a discussion of the characterization methods used in the nanoparticles community including scanning transmission electron microscopy, X-ray techniques, UV-visible spectroscopy. Under X-ray techniques, powder X-ray diffraction, single crystal X-ray diffraction, small angle X-ray scattering and hard X-ray techniques were included. Mass spectrometry is commonly used for composition determination. Electron microscopy and small angle X-ray scattering experiments provide information on the size, shape and dispersity.
Alloying provides a way to tune the properties of materials which is very different from those of their monometallic counterpart. Chapter 3 describes such an effect on \( \text{Au}_{25}(\text{SR})_{18} \) gold nanomolecules by alloying with silver. The atomic arrangement of Au and Ag atoms in \( \text{Au}_{25-x}\text{Ag}_{x}(\text{SR})_{18} \) was determined by X-ray crystallography and it was found that Ag atoms were specifically localized in the 12 vertices of the icosahedral core.

Among ultra-small nanomolecules, \( \text{Au}_{38}(\text{SR})_{24} \) is one of highly attractive nanomolecules due to high stability, availability of single crystal X-ray structure, unique spectroscopic features and intrinsic chirality. Chapter 4 highlights alloying effect on \( \text{Au}_{38}(\text{SR})_{24} \) nanomolecules. Series of bimetallic \( \text{Au}_{38-x}\text{Ag}_{x}(\text{SR})_{24} \) nanomolecules were synthesized and its composition was determined to atomic precision. Incorporation of Ag atoms appears to smear out the distinct UV-visible features of \( \text{Au}_{38}(\text{SR})_{24} \). However, silver substitution decreased the stability of alloy nanoparticle when compared to the monometallic \( \text{Au}_{38}(\text{SR})_{24} \). The synthesized nanoalloy was subjected to a synchrotron based single crystal X-ray analysis. The substituted silver atom were concentrated in the core and preferentially occupy nine selected locations out of 38 possible sites. Furthermore, the independent compositional assignments were done using mass spectrometry.

\( \text{Au}_{144}(\text{SR})_{60} \) is one of the ultra-stable gold nanomolecule that occur within the size regime of molecules to plasmonic transition. Chapter 5 describes the modulation of chemical and physical property of \( \text{Au}_{144}(\text{SR})_{60} \) by silver doping. UV-visible spectroscopy shows the Ag incorporation affects the electronic structure of the nanomolecules. The maximum number of Ag
atoms substitute found to be 60.

Extensive efforts have been made to synthesize and isolate monodisperse atomically precise nanomolecules larger than \( \text{Au}_{144}(\text{SR})_{60} \). In general, size dispersity of the larger nanoparticle can be corroborated by TEM, SAXS and other commonly used nanoparticle analysis methods. However, electron microscopic analysis on ultra-small scale often alter the size, composition and atomic structure due to the beam induced aggregation and sintering.

Chapter 6 describes the first composition determination of super-stable plasmonic nanoparticles in the 2 nm (or 76.3 kDa mass region) and its alloying. This atomically monodisperse plasmonic molecule contains exactly 329 gold atoms and 84 ligands. Apart from the mass spectrometric composition, further characterization was conducted using scanning transmission electron microscopy equipped with high angle annular dark field imaging (HAADF-STEM), high energy X-ray based atomic pair distribution function (PDF) analysis and small angle X-ray scattering (SAXS).

Composition determination of large nanocrystals is challenging due to the lack of available techniques to quantify the number of atoms and literature protocols for high yields. Chapter 7 discusses the two largest nanocrystals produced at 2.4 and 2.9 nm with a composition of \( \text{Au}_{500\pm10}(\text{SR})_{120\pm3} \) and \( \text{Au}_{940\pm20}(\text{SR})_{160\pm4} \). Most importantly, we were able to successfully extend the mass spectrometric window up to 200 kDa for compositional determination and to study the molecular nature of nanocrystals. Furthermore the monodispersity of the nanocrystals was examined by SAXS and STEM. Possible atomic arrangements were investigated by HR-
PDF. Mass spectrometry was used to determine size and composition with additional supporting data from STEM, PDF and SAXS analysis. These atomically defined nanocrystals are very stable under ambient conditions and thermochemical treatment at 80 °C for over several days.

Chapter 8 highlight the contributions made from this dissertations toward the advancement of thiol protected nanoparticle research.
DEDICATION

This dissertation is dedicated to all of my family members, teachers and friends.

A special feeling of gratitude to my loving parents for their endless support and putting me through the best education possible. I dedicate this work to my beloved wife Buddika for her sacrifice and being with me through the joy and the pain and my loving son Vihange. Finally, I dedicate this work and give special thanks to my research advisor Dr. Amala Dass for his continue support and inspiration throughout the doctoral program. I will always appreciate all the people who help me in anyway.
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\( \text{Au}_{34.04}\text{Ag}_{3.96}(\text{SCH}_2\text{CH}_2\text{Ph})_{24} \) is the average structure obtained from many \( \text{Au}_{38-x}\text{Ag}_x(\text{SR})_{24} \) molecules within the single crystal. The Ag atoms were distributed in face shared bi-icosahedral vertex atoms, with significant preference for sites 4, 5, 6, 33, 34, 35 when compared with 20, 21 and 22...

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CHAPTER 1

INTRODUCTION TO GOLD AND ALLOY NANOPARTICLES
CHAPTER 1
INTRODUCTION TO GOLD AND ALLOY NANOPARTICLES

1.1 Gold and gold nanoparticles

Element gold (Au) is the 79th element that fall under the d block of the periodic table. The ground state electronic configuration of gold is \([\text{Xe}] \ 4f^{14} \ 5d^{10} \ 6s^1\). The metallic gold is chemically inert. It has six oxidation states ranging from -1 to +5 whereas +1 and +3 charge states occur more commonly. The inter-band electronic transition between 6s and 5d orbital of the gold atom leads to the distinct yellow color of metallic gold by absorbing the blue light of the visible spectrum.

The elemental gold was recognized as sign of wealth and power in early civilization. With the progression of technology similar to the iron and copper works, gold became a part of the human culture. The metallic gold is used as coins, jewelry and as a coating material not only due to its luster, natural beauty, malleability but also to its properties such as inertness, resistant to oxidation and corrosion. In the modern world, gold advanced in high technology applications and electronic devices due to its electrical and thermal conductivity.

Later, the elemental gold was introduced to the scientific community in its new form as nanoparticles. By definition, nanoparticles are particles that has dimension in the order of 100 nm or less at least in one dimension. Nanoscience deals with understanding and characterization of thses nanoscale materials. The use of gold nanoparticles dates back to the 4th century. Lycurgus Cup is a typical example which exhibits unique color upon lighting. Later in
1850, Michel Faraday synthesized a colorful tiny metallic particles that exhibit interesting optical properties. He recognized that these particles have the same order of magnitude of the wavelength of light. Unfortunately, there were limited techniques to evaluate the size of these particles except optical microscopy. Later, Gustav Mie theoretically explain this interesting phenomena in term of scattering and absorption of light by small particles.

The surface plasmon resonance (SPR) is the oscillation of the free electron in the nanoparticles by incident light. For an example gold nanoparticles absorbs light in blue-green region of the electromagnetic spectrum and reflects off the red color. Interaction of gold nanoparticles with incident light is influenced by size, shape and the native environment of the nanoparticles. In general, an increase in the particle size results in shifting of SPR to the longer wavelength. In case of gold nanoparticles, the SPR range from 500-550 nm. When particle the size was decreased to 2 nm or less, molecular like optical transition could be observed. Another important aspect is the SPR intensity decreased as the particles become smaller. Apart from the size and the shape, the refractive index of the environment also affect the SPR peak and the bandwidth.

Due to the recent advanced in the analytical instruments, these nanoscale materials became popular and opened a new area of research in science and technology so called “Nanoscience” and “Nanotechnology”. For example, gold nanoparticles are used in catalysis, energy harvesting, nano-devices, drug delivery and biomedical applications.

1.2 Molecules of Gold: Gold nanoclusters, nanomolecules and nanocrystals

Generally, there is a vast difference in size determination and characterization of gold nanoparticles. The powerful characterization tools in the study of ultra-small nanoparticles includes mass spectrometry and X-ray crystallography, while, larger (> 5 nm) gold nanoparticles
can be characterized by transmission electron microscopy (TEM), powder X-ray diffraction, small angle X-ray scattering (SAXS) and other techniques.

Figure 1.1. (Left) Mass spectrum of gold-clusters obtained by the laser-vaporization disc source. Spectra shows maximal signals around cluster sizes $n \sim 12, 35, 60$ and 90. (Right) Mass spectrum of the $\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{C}_6\text{H}_5)_{18}$.

There are several terminology used in these nanoscale gold materials. Nanomolecules, nanoclusters, nanoparticles and faradaurate are some of them. The term “clusters” is often used for the gas-phase materials that are protected by thiol ligands but not stable in the solid phase. Furthermore, these gas phase clusters does not have a fixed composition and cannot be isolated in molecular form in ambient environmental conditions. Figure 1.1 shows the mass spectrum of the gold clusters in contrast to the gold nanomolecules that have a fixed composition. The gold nanomolecules discussed here are protected by thiol ligands and have the generalized formula $\text{Au}_x(\text{SR})_y$ where $x$ and $y$ are number of gold atoms and thiol ligands. Interestingly, the synthesis protocol for these gold nanomolecules are robust and can be synthesis to atomic precision i.e.±0 atom variation. For an example, $\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{C}_6\text{H}_5)_{18}$ nanomolecule has 25 gold atoms and 18
thiolate ligands, altogether 369 atoms. The core size of this molecule is around 1.5 Å in diameter.

Figure 1.2. Gold nanomolecules, plasmonic Faradaurates, and nanoparticles. (a) The 1–100 nm size regime with nanomolecules (±0 Au atom variation) in the 1–2 nm region and nanoparticles in the 2–100 nm regime (where very good monodispersity has been achieved, but the size distribution is still ±1000s of atoms or a few nanometers). (b) Thiolated gold nanomolecules, such as Au$_{25}$, Au$_{38}$, Au$_{67}$, Au$_{144}$, and Au$_{329}$ with a precise number of metal atoms and organic ligands and Au$_{\sim 500\pm 10}(SR)\sim 120\pm 3$ and Au$_{\sim 940\pm 20}(SR)\sim 160\pm 4$. Only highly reproducible and phenylethane thiol-protected Au cores are shown. (c) Dashed line, between 329 and 500 atom sizes indicates the transition between the fixed composition containing nanomolecules region(±0 Au atom variation) versus polydisperse Au$_{\sim 500\pm 10}(SR)\sim 120\pm 3$ particles (±10 Au atom variation). The next largest size Faradaurate-940 has a variation of ±20 Au atom with a composition of Au$_{\sim 940\pm 20}(SR)\sim 160\pm 4$. Reprinted with permission from reference [2]. Copyright 2014 American
However, as the size of the particle is increased, the molecular nature cease and transition occur from fixed to polydisperse composition due to the existence of several possible conformations.\textsuperscript{15} The atomic arrangement of these larger nanoparticles may show amorphous or crystalline nature. These versatile gold-gold and gold-thiol arrangements include octahedral, decahedral and other stable geometric and therefore larger gold nanoparticles are refer to as gold nanocrystals.

Especially larger gold nanoparticles (> 2 nm) shows surface plasmon resonance. This SPR emerge in between 2-3 nm size range and is enhanced as the size increases. In 1956, Michael Faraday systematically studied gold nanoparticles that shows the SPR. Thus, the term faradaurate was given in honor of Michael Faraday who conducted the pioneering scientific studies on small sized gold particles.\textsuperscript{16}

Figure 1.2 shows the boundaries between the gold nanoparticle and nanomolecules which is between 2-3 nm. Figure 1.2b shows the expansion between 1-4 nm size regime where thiolate nanomolecules [atomically monodisperse molecules such as Au\textsubscript{25}(SR)\textsubscript{18}, Au\textsubscript{38}(SR)\textsubscript{24}, Au\textsubscript{144}(SR)\textsubscript{60}] and gold nanoparticles whose diameter is less than 4 nm. Monodisperse to polydisperse transition occurs at Au\textsubscript{329} and Au\textsubscript{500} (Figure 1.2c) where the size range is between 2 to 2.4 nm.

1.3 Synthesis of gold nanomolecules

There are two major types of gold nanoparticles namely colloidal gold nanoparticles and thiol protected gold nanoparticles. In general, colloidal gold nanoparticles have limited stability where aggregation and decomposition take place over time. Brust and Schriffin\textsuperscript{17} introduced a two phase method to synthesize the thiolate protected-gold nanoparticles which show extraordinary stability. Later, Whetten and coworkers introduced a method called
thermochemical etching that further narrows down the size dispersity of gold nanoparticles. This method produces atomically monodisperse Au-SR molecules, such as Au$_{25}$(SR)$_{18}$, Au$_{38}$(SR)$_{24}$, and Au$_{144}$(SR)$_{60}$. Interestingly, these nanoparticles are highly stable in air, at higher temperatures (~80 °C) and moisture. Also these can be dried, stored as a powder and re-dispersed in solvents. They are stable for several years while maintaining its chemical and physical properties.

The two phase Brust-Schiffrin method$^{17}$ use both aqueous and organic solvents and is widely employed to synthesize gold nanomolecules. Briefly, a mixture of gold salt (HAuCl$_4$.3H$_2$O) in distilled water and tetraoctylammonium bromide (TOABr) dissolved in toluene was stirred for 30 minutes until gold salt is transferred to the organic layer. To the organic layer, a calculated amount of thiol was added and stirred for 1 h. Then a reducing agent, sodium borohydride (NaBH$_4$) in ice-cold distilled water is added to the mixture to form nanomolecules. In the case of alloy synthesis, both gold and the other metal salts were added simultaneously in the initial synthesis under controlled total metal molar ratio. Figure 1.3 shows the steps involved in Au$_{144-x}$Ag$_x$(SR)$_{60}$ synthesis.
Figure 1.3. Steps involved in the synthesis of $\text{Au}_{144-x}\text{Ag}_x(\text{SR})_{60}$ alloy nanomolecules. **Step 1:** synthesis of crude nanoparticles mixture. **Step 2:** Thermochemical treatment. **Step 3:** Solvent fractionations

In general, mixtures of nanomolecules are produced in the initial crude product synthesis. Thus, the separation protocol is necessary to isolate nanomolecules species as shown in step two and three. Although, the formation of nanomolecules mixtures is difficult to avoid, the synthesis condition can be optimized to improve the yield of a certain nanomolecules size. Years of research have identified the following parameters to change the population of the desired nanomolecules.

(a) Choice of solvent system /one phase vs. two phase: One phase synthesis often yield small sized (<2 nm) nanomolecules whereas two phase method can be applicable for both small and larger sized nanoparticles synthesis.

(b) Gold to thiol ratio: Larger gold to thiol ratios like 1:10 are often used for the small
nanomolecules synthesis such as Au$_{25}$(SR)$_{18}$ and Au$_{38}$(SR)$_{24}$, whereas smaller ratio tend to yield larger plasmonic nanocrystals.

(c) Reducing agents: NaBH$_4$ is widely used for the gold nanomolecules synthesis. Apart from that slow reduction can be achieved by using NaBH$_3$CN. Citrate acid can be used as a reducing agent for naked and thiol protected nanoparticles synthesis. Also, the amount of reducing agent and rate of addition can affect the nanomolecules size distribution. The stirring rate and the reaction/reducing time also play a role when determining the nanomolecules of interest. For example Au$_{20}$(SR)$_{16}$ can be achieve by addition of excess NaBH$_4$ under slow stirring whereas Au$_{24}$(SR)$_{20}$ yield from rapid addition of a small amount of NaBH$_4$ under rapid stirring of the reaction mixture.$^{16}$ Also Au$_{25}$(SR)$_{18}$ can be selectively isolated at room temperature by controlling the reaction time of the one phase synthesis.$^{19}$

### 1.4 Isolation protocol for gold nanoparticles

The synthesis of the crude product may contain one major component or may result a mixture of nanoparticles depending on the experimental conditions. Further isolation or purification of the desired nanoparticle can be achieved by thermochemical treatment, solvent fractionation and size exclusion chromatography (SEC) separation. The final goal of these protocols is to isolate atomically precise single type nanomolecules out of polydispersed mixtures.

1.4.1 Thermochemical treatment

The thermochemical treatment can serve as both post-synthesis modification and isolation technique. The protocol involves thermal treatment of the nanoparticles mixtures in the presence of excess thiol. This technique was used to remove meta-stable nanomolecules. Step 2
in figure 1.3 shows the mass spectrometry of the nanomolecules before and after the thermochemical treatments. The first spectrum shows the nanomolecules species at ~7, ~12, ~26 and ~32 kDa which is the crude product mixture. Nanomolecules at ~12 and ~26 kDa seem to be meta-stable and decomposed upon thermochemical treatment at this point, leaving ~7 and ~32 kDa nanomolecules in the mixture.

Whetten and co-workers first introduced the thermochemical treatment or the etching step in 1999.\textsuperscript{20} This technique can be used to synthesize the nanomaterial via core size conversion. For example, Dass et al report the $\text{Au}_{130}(\text{SR})_{50}$ synthesis via core size conversion method. The nature of the ligand is also important in the etching process. For an example, the thermochemical treatment of $\text{Au}_{144}(\text{SCH}_2\text{CH}_2\text{Ph})_{60}$ with hexanethiol or dodecanethiol leads to the formation of $\text{Au}_{144}(\text{SC}_6\text{H}_{13})_{60}$ and $\text{Au}_{144}(\text{SC}_{12}\text{H}_{25})_{60}$ which is a simple ligand exchange. However, the same reaction with thiophenol and \textit{tert}-butyl benzene thiol yield $\text{Au}_{99}(\text{SPh})_{42}$ and $\text{Au}_{133}(\text{SPh-}t\text{Bu})_{52}$ respectively, which is core size conversion leading to completely different size and composition.\textsuperscript{21}

1.4.2 Solvent fractionation

Different nanomolecules have different chemical affinity towards solvents. $\text{Au}_{25}(\text{SR})_{18}$ is completely soluble in acetonitrile while $\text{Au}_{144}(\text{SR})_{60}$ is not. Thus, extraction with acetonitrile can separate pure $\text{Au}_{25}(\text{SR})_{18}$ from a mixture of $\text{Au}_{25}(\text{SR})_{18}$ and $\text{Au}_{144}(\text{SR})_{60}$. Furthermore, a mixture of solvents such as methanol/toluene or acetonitrile/toluene can be used in solvent fractionation to isolate the desired species. The number of ligands and the charge of the nanomolecule also plays a major role in determining the solubility. Step 3 in Figure 1.3 demonstrate the solvent fractionation based separation of $\text{Au}_{25}(\text{SR})_{18}$ and $\text{Au}_{144}(\text{SR})_{60}$.
1.4.3 Size Exclusion Chromatography (SEC)

The separation is based on the size of the nanomolecules. Bio Rad SX1 beads were used as the stationary phase and THF as the mobile phase. Several purification cycles can be performed as required. Mass spectrometry was used to monitor the separation process and purity of the SEC fractions.

1.5 Salient features of gold nanomolecules

Nanomolecules (NMs) are molecular species that breach into the < 3 nm size. Typical examples include gold thiolate species which contains a specific number of Au atoms and thiolate ligands such as: Au$_{25}$(SR)$_{18}$, Au$_{38}$(SR)$_{24}$ and Au$_{144}$(SR)$_{60}$. Salient features of nanomolecules include: (a) fixed composition as determined by mass spectrometry, (b) size-dependent optical and electrochemical properties, (c) crystallographically characterized atomic structure, first examples include Au$_{102}$(SPh-COOH)$_{44}$ and Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$; (d) high stability under ambient environmental conditions and elevated temperatures; (e) stability in dry form and in solutions and can be dried and re-dispersed in various organic solvent and aqueous media; (f) the ability to add and tune functionality by ligand exchange, fluorescent or chiral ligand (g) and the ability to tune the properties of the metallic core by alloying with other metals such as Ag, Cu, Pd, Pt, while maintaining the fixed composition.

Mass spectrometry and X-ray crystallography have been two of the most important analytical tools to study nanomolecules. Commonly used nanoparticles characterization methods such as TEM, SAXS and powder diffraction provide limited details on these ultra-small molecules, but can be used as complementary and supporting evidence.


1.6 Atomic structure of gold nanomolecules

Investigation of nano-structures of thiolated gold and alloy nanoparticles at molecular level is important to determine their physical and chemical properties. This total structure determination will require diffraction quality single-crystal of the nanoparticles. Recently, significant progress has been made in the atomic structure determination of $\text{Au}_x(S\text{R})_y$ and $\text{Au}_x\text{M}_{x-y}(S\text{R})_z$ nanoparticles where “$x$” is the number of gold atoms, “$y$” is the number of incorporated heteroatoms, “$z$” is the number of ligands, and $\text{M}$ is the type of hetero metal atom. The first breakthrough in $\text{Au}_y(S\text{R})_y$ crystal structure was made by Kornberg and co-workers in 2007 with the structure of $\text{Au}_{102}(\text{pMBA})_{44}$ where pMBA is $p$-mercaptobenzoic acid. Later in 2008, Murray and Jin research groups independently determined the atomic structure of $\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}$. These advances in the structure elucidation provide an understanding of the three dimensional arrangement of gold atoms and ligand at the atomic level.
Figure 1.4. Crystal structure of $\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}^-$ nanomolecules (a) Total structure (b) $\text{Au}_{25}\text{S}_{12}$ framework (c) Shell structure of the $\text{Au}_{25}(\text{SR})_{18}$ nanomolecules; shell is formed by six $\text{Au}_2\text{(S)}_3$ staple motif (d) representation of core and one $\text{Au}_2\text{(S)}_3$ staple motif (e) $\text{Au}_{13}$ atom icosahedral core.

The crystal structure shows the central metal atom core surrounded by staple motif that consist of metal atoms and ligands. Staple motif can be explained in the form of $-\text{SR-}(\text{Au-SR})_n$
motif, where \( n = 1, 2, 3 \) etc. Two of the most common staple motifs are (a) monomeric staples in the form of \(-SR-Au-SR-\) units and (b) dimeric staple in the form of \(-SR-Au-SR-Au-SR-\) units. For example, \( Au_{25}(SCH_2CH_2Ph)_{18} \) has a 13-gold atom icosahedral core surrounded by six dimeric motifs. Figure 1.4 shows the structure of the \( Au_{25}(SCH_2CH_2Ph)_{18} \). Furthermore, the total structure of the \( Au_{25}(SCH_2CH_2Ph)_{18}, Au_{38}(SCH_2CH_2Ph)_{24} \) and its alloy nanomolecules is discussed in detail in chapter 3 and 4.

### 1.7 Alloying and alloy nanomolecules

Alloying provides another dimension in exploring the properties of materials which is very different from the properties of the monometallic component. Thus, combining the properties of individual metals into a single system as alloy nanoparticles will enhance the efficiency, multi-tasking ability and application. For example, Ag/Au alloy nanoparticles show enhanced catalytic active towards the oxidation of CO at low temperatures than that of monometallic Ag or Au nanoparticles.\(^\text{26}\) Further, SPR properties of Ag–Au alloy nanoparticles can be continuously modulated between 400 nm and 550 nm by changing the atomic composition.

Alloy nanoparticles can be prepared by several methods including co-reduction, digestive ripening, laser ablation, galvanic and anti galvanic replacement. However, recent challenges involve in size and composition control due to the complex synthesis routes for alloying than that of monometallic nanoparticles. This lead to the different mixing pattern such as core-shell, multi-shell and intermixing and based on chemical ordering and geometric structure. In core-shell configuration, one metal surrounds the other and in the intermixed alloying the metals are homogenously mixed at the atomic scale.
The atomic arrangement and mixing pattern of nano-alloy strongly depends on the one or more factors mentioned below and also depend on experimental conditions and synthesis protocol. The degree of alloying and atomic ordering in \( \text{A}_x\text{B}_y \) type bi-metallic nanoalloys depends on several factors, where A and B are hetero metal atoms. For example, if A−B bond is the strongest, this favors mixing or else segregation is favored. Surface energies of the elements determine the position of the atom in the nanoparticles and the element with lowest surface energy tends to segregate to the surface. Alloying also depends on the relative atomic size and relatively smaller atoms favor the more sterically confined central core than shell. In the case of ligand protected nanoparticles, whichever metal binds strongly to the ligand, tends to be on the surface. Apart from this factor, certain alloy size may stabilized by electronic shell structure.

In general, the composition determination of these alloy nanoparticles includes techniques such as EDX,\(^{27,28}\) XPS,\(^{28}\) EELS,\(^{28,29}\) AAS and ICP-MS analysis. These methods have limitations in that they can only determine the alloy composition as a percentage or a molar fractions with uncertainty. However mass spectrometry has the unique ability to determine exact atomic mass and recent reports account for synthesis and accurate alloy composition determination (±0 atoms) using mass spectrometry.\(^{5,30}\)

Among other nanoparticles, Ag nanoparticles have interesting optical properties, antibacterial and anti-fungal activity.\(^{31,32}\) It is also shown that small silver nanoparticles have enhanced antibacterial activity than larger silver nanoparticles.\(^{33}\) But silver nanoparticles are relatively unstable and easily decomposed in ambient conditions. Incorporation of silver into the ultra-stable gold nanoparticles will improve the stability of silver nanoparticles while enhancing the reactivity and electronic properties of gold nanoparticles.

Properties of silver can be encapsulated into the ultra-stable gold nanoparticles as
atomically precise nano-alloy molecules. Heteroatom incorporation will take place at specific energetically favorable sites within ultra–small nanomolecules. Further, ultra-small gold nanoparticles have size dependent optical properties and incorporation of single heteroatom is extremely sensitive to the electronic structure. Understanding of heteroatom incorporation to the ultra-small gold nanomolecules will have advantages in design of next generation molecular-level mixed-metal nanoparticles. Therefore, we used \( \text{Au}_{25}(\text{SR})_{18} \), \( \text{Au}_{38}(\text{SR})_{24} \) and \( \text{Au}_{144}(\text{SR})_{60} \) nanomolecules as template system to develop the protocol for silver atom incorporation. Then, the synthesized Au-Ag alloy nanomolecules were qualitatively and quantitatively studied to atomic precision. Structure characterizations of traditional large alloy system are limited by the diffraction quality single crystals. These ultra-small nanomolecules with atomic structure provide nice platform for determination of favorable locations for the silver atom substitution.

Table 1.1 properties of silver and gold that can be alloying\(^{34}\)

<table>
<thead>
<tr>
<th></th>
<th>Au</th>
<th>Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomic number</td>
<td>79</td>
<td>47</td>
</tr>
<tr>
<td>Electronic Configuration</td>
<td>[Xe] 4f(^{14}) 5d(^{10}) 6s(^{1})</td>
<td>[Kr] 4d(^{10}) 5s(^{1})</td>
</tr>
<tr>
<td>Atomic radius(Å)</td>
<td>1.440</td>
<td>1.445</td>
</tr>
<tr>
<td>Surface energy(meV Å(^{-2}))</td>
<td>96.8</td>
<td>78.0</td>
</tr>
<tr>
<td>Cohesive energy (eV)</td>
<td>-3.81</td>
<td>-2.95</td>
</tr>
<tr>
<td>Electro negativity</td>
<td>2.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Lattice constant(Å)</td>
<td>4.08</td>
<td>4.09</td>
</tr>
</tbody>
</table>

The table 1.1 summarizes the chemical and physical properties of elemental gold and silver. Au and Ag are coinage metals and can occur in nature as free metal and in complexes. In
solid phase, Au and Ag make a solid solution in all ratios. Also some of these alloys occur naturally. For an example, “electrum’ is a naturally occurring alloy of Au and Ag. Gold, silver and its alloy have cubic close packing system in the solid phase. Metal-metal bond length of Au-Au (2.88 Å) and Ag-Ag (2.90 Å) is very close and therefore Au-Ag systems show minimum strain-induced driving force toward segregation. However, low surface energy of Ag relative to the Au, makes Ag prefer surface enrichment. Au has a higher cohesive energy than Ag and therefore the bond strength of Au-Ag alloy system is in order of Au-Au> Au-Ag>Ag-Ag. However, these considerations are related to the bulk alloy. The chemical and physical properties may vary in the nano-scale alloy.

The alloy nanoparticles have a broad range of applications in opto-electronic, solar cells, fuel cells, catalytic and drug delivery, cancer cell detections and plasmonic applications. Therefore, it is important to obtain information relevant to the crystal structure of ultra-small and plasmonic nano-alloys for this well defined formula (atomic composition) to understand how the structure, optical and geometry vary with number of atoms or size. Followed by the $\text{Au}_{25-x}\text{Ag}_{x}$(SR)$_{18}$ alloy, recent reports on ultra-small alloy nanomolecules includes incorporation of $\text{Cu}_{35}$, $\text{Pd}_{36}$ and $\text{Pt}_{37}$ metals. But unfortunately the complete understanding of this system is hindered by lack of diffraction quality single crystals. Chapter 3, 4, 5, and 6 of the dissertation discuss the synthesis and structure characterization of $\text{Au}_{25-x}\text{Ag}_{x}$(SR)$_{18}$, $\text{Au}_{38-x}\text{Ag}_{x}$(SR)$_{24}$, $\text{Au}_{144-x}\text{Ag}_{x}$(SR)$_{60}$ and large plasmonic $\text{Au}_{329-x}\text{Ag}_{x}$(SR)$_{84}$, alloy nanomolecules respectively.
CHAPTER 2

CHARACTERIZATION METHODS OF GOLD NANOPARTICLES
CHAPTER 2
CHARACTERIZATION METHODS OF GOLD NANOPARTICLES

2.1 Mass Spectrometry (MS)

Molecular weight is a fundamental property to characterize the composition of the molecules. Mass spectrometry has been revolutionized in the fields of biology, chemistry, and physics. A mass spectrum provides a plot of ion abundance versus mass-to-charge (m/z) ratio. The mass of the molecular ion can be probed from the m/z values which correspond to the intact molecular ion or its fragments. MS is widely used in small molecule organic chemistry to determine the chemical composition, structural information, verifying the identity and purity of a molecule. MS characterizations were primarily applied to studies on small organic, inorganic and biological molecules.\textsuperscript{38} In 1996, Whettens’ pioneering work has made MS a reliable analytical tool for characterization of various nanoparticles. In recent works, applications of MS further extend towards the nanoparticles reaction mechanism, intermediates and charge state determination.\textsuperscript{39, 40}

Matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) and electrospray ionization mass spectrometry (ESI-MS) are the main MS tools utilized for the nanomaterials characterization to date. Prior to that, optical spectroscopy, electron microscopy and X-ray techniques were used to investigate the size, shape and physicochemical properties of the gold nanoparticles.

Whetten and co-workers initially used a laser desorption ionization mass spectrometry for
the characterizations using Nd:YAG laser (532 nm) and time of flight mass separator.\textsuperscript{38} However, the high energy laser irradiation cleaved the S-C and Au-S bond and also led to gold atom loss, resulting in the absences of intact molecular on peaks. This extensive fragmentation impedes the actual molecular formula determination resulting in a broad and complex mass spectrum.

\textit{2.1.1 MALDI-MS}

In 1987, Koichi Tanaka and coworker developed the MALDI-MS methodology that avoid inherit fragmentation by using a matrix in the laser desorption ionization (LDI). They were able to detect and analyze larger biomolecules (~34 kDa) using combined cobalt nanoparticles in glycerol as a matrix under nitrogen laser. In 2008, Dass and coworkers have extensively studied the different matrices including sinapinic acid (SA), 4′-hydroxyazobenzene-2-carboxylic acid (HABA), universal MALDI Matrix and \textit{trans}-2-[3-(4-\textit{tert}-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) for gold nanoparticles. Finally, they have successfully developed MALDI-MS methodology based on DCTB matrix, facilitating the complete molecular ion determination without fragmentation. Since then, most of the thiolated gold nanoparticles community used DCTB in their routine MS analysis. Figure 2.1 shows the MS of the \textit{Au}\textsubscript{25}(SCH\textsubscript{2}CH\textsubscript{2}C\textsubscript{6}H\textsubscript{5})\textsubscript{18} using several matrices. Most of matrices tested resulted in spectra dominated by fragment peaks except for DCTB that show intact molecular ion at 7394 Da. Efficient photon absorption, electron transfer to/from the analyte makes DCTB an effective matrix for the gold nanoparticles analysis. Here, we used toluene or THF as a solvent for both nanomolecule and DCTB matrix. It should be noted that matrix solution composition, pH, matrix crystal growth rate also effect the mass spectrometric response.
Figure 2.1. MALDI-TOF-MS spectra of (Applied Biosystems Voyager-DE Pro) Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$ in UMM, HABA, SA, and DCTB matrix showing the superiority of DCTB matrix in obtaining molecular ion over fragment ions. Reprinted with permission from reference [3]. Copyright 2008 American Chemical Society.

Figure 2.2 shows the potential of the DCTB matrix based MALDI-MS detection of the gold thiolated nanoparticles up to 200 kDa mass range. The nanomolecules were dissolved in toluene or THF and mixed with DCTB $^{41}$ (20 mM/ in THF ) and allow to co-crystallize on the MALDI plate. MALDI-MS was recorded using Bruker AutoFlex 1, data were acquired from 4-500 kDa mass range and analysis was performed using Bruker Flex analysis. 3.0.

Figure 2.2 inset shows the MS of nanoparticles up to 40 kDa which include Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$ [MW = 7394 Da], Au$_{38}$(SCH$_2$CH$_2$Ph)$_{24}$ [MW = 10778 Da], Au$_{130}$(SCH$_2$CH$_2$Ph)$_{50}$ [MW = 32466 Da] and Au$_{144}$(SCH$_2$CH$_2$Ph)$_{60}$ [MW = 36596 Da]. MALDI-MS of Au$_{25}$(SR)$_{18}$ and Au$_{38}$(SR)$_{24}$ show sharp peaks and enough resolution for MS identification.
As the size increase, MS analysis become challenging due to the resolution limit of the TOF tube. Thus, when compared to the smaller gold nanomolecules, larger Au_{130}(SR)_{50} and Au_{144}(SR)_{60} shows broad MALDI-MS signal. Both instrument resolution at high mass and laser induced fragmentation result in peak broadening. Thus, alternative soft ionization technique is required to overcome the laser induced fragmentation and detect the intact molecular ions for large gold nanoparticles analysis.

2.1.2 Electro spray ionization mass spectrometry

ESI technique is a much softer ionization technique and produced intact molecular ions without fragmentation in most cases. In contrast to the MALDI-MS, ESI required solution based sample introduction and spray based ionization techniques. Initially, Whetten and Tsukuda group successfully introduced ESI-MS technique to water soluble glutathione protected gold nanoparticles.\textsuperscript{42, 43} Many recent reports have used ESI-MS for the compositional assignment of
gold nanomolecules.

ESI involved three major steps in transferring the compound from solution to gas phase ions. (1) Formation of tiny spray of charge droplets; (2) desolvation; and (3) ion ejection from the charged droplets. A sample will be introduced to the MS through the tiny capillary which is maintained at a high voltage (e.g. 2.5 – 6.0 kV). As a result of the applied solution flow rate and voltage, tiny mist of highly charged droplets are generated.

The charged droplets will be generated at the tip of the capillary tube when suitable voltage reached and then directed to the atmospheric pressure spray chamber with the aid of the nitrogen gas. The elevated ESI source temperature and stream of nitrogen drying gas facilitate the charged droplets to reduce in size by solvent evaporation. This desolvation process lead to high surface charge density by decreasing the droplet size. Finally, as the solvent molecules evaporates, the charge density of the droplet reaches a critical point at which ions in the droplets will be ejected into the gaseous phase. Then, the emitted gas phase ions are extracted by skimmer cone and directed to the mass analyzer.

The advantage of the ESI process is the generation of multiply charged ions. It allows the analysis of large molecules and improves the sensitivity. However, multiply charge ions observed in the ESI does not reflect the actual charge state of the gold nanoparticles in the solution. It is slowly depend on the charge accumulation in the droplet and charge modification by the electrochemical process. Figure 2.3 show the comparison of the MALDI and ESI-mass spectra of Au$_{329}$(SR)$_{84}$. Figure 2.3 gray color spectrum is the MALDI-MS of Au$_{329}$(SR)$_{84}$ that shows one peak at $\sim$75 kDa region, corresponding to the 1+ molecular ion. It shows broad features due to the laser induced fragmentation occurring during the ionization process. The red color mass spectrum represent the ESI-MS of the Au$_{329}$(SR)$_{84}$ showing multiple charged 2-, 3-,
4- and 5- molecular ions. Blue color spectrum represent the deconvoluted 4- ions to the 1-
molecular ion which is sharp compared to that of MALDI-MS spectrum.

Figure 2.3. ESI-MS(red) and MALDI-MS(blue) spectra of Au$_{329}$(SR)$_{84}$ nanomolecules. A MALDI spectrum shows the one broad peak of 1+ charge state at 76.3 kDa. ESI-MS show the multiple charged 2-, 3-, 4- and 5- molecular ions. These multiply charge species can be deconvoluted to obtained mass of the molecular ion. Figure shows the deconvoluted 4- ions to the 1- molecular ion peak (blue). The 2-,3- and 5- molecular ion peak also can be deconvoluted to the 1- molecular ion peaks by multiplying the corresponding m/z value with the charge state.

Mass spectral peaks can be interference by formation of adducts, cations, and anions. As an example, Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}^-$ shows a peak at 7394 Da corresponding to the singly charged negative ion, Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}^-$. But, the same nanomolecule in the positive mode yields a peak at 8326 Da, corresponding to the positive ion [Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$]·2(N(C$_8$H$_{17}$)$_4$) $^+$. Therefore it is important to obtain the mass spectrum in both positive and negative mode to account for possible counter-ions and adduct formation. Detail analysis of the interference from counter ions are discussed in chapter 6.
2.2 Optical spectroscopy

Spectroscopy is the measurement of the interaction of light with matter. UV-visible spectroscopy is used to study the optical properties of gold nanomaterial. When light is absorb by molecules, it is excite from ground state to the excited state. This absorption spectra can be analyzed qualitatively and quantitatively by measuring the incident and transmission radiation. Figure 2.4 shows the size dependent optical absorbance of the gold nanoparticles. Small gold nanoparticles show molecular like optical transition. However as the size (or as number of metal atom) increases, the step like features are diminished leading to monotones curve.

![Figure 2.4. UV-visible spectra of different size gold nanoparticles.](image)

The molecular like feature in small gold nanoparticles or nanomolecules is due to the presence of a large HOMO-LUMO gap. Figure 2.4 shows the optical absorbance spectrum of the $\text{Au}_{25}(\text{SR})_{18}$ (red). The peak at 750 nm is due to the HOMO-LUMO transition (sp-sp) and other peaks and shoulders correspond to the sp-sp and d-sp transition. However, as the size increase, this HOMO-LUMO gap and molecular nature cease to exist, resulting in a continuous electronic state. After a certain size (~2 nm), the surface plasmon resonance begin to appear due to the
sufficient number of free or weakly bound conduction electrons. Therefore, the optical spectra also offer indirect evidence for the size of the gold nanoparticles.

As the electromagnetic field oscillates, the weakly bound electron in the conduction band of the nanoparticles responds collectively. Then light will be absorbed when the incident light frequency matches with the free electron oscillation frequency. For nearly spherical gold nanoparticles, SPR peak is at 520 nm whereas silver exhibit at 400 nm. Alloying the gold nanoparticles with silver will facilitate the tuning of SPR band in between 400-500 nm. In 1999, El-Sayed and co-workers shows that the systematic shifts of SPR peak of ~20 nm gold nanoparticles with Ag alloying.\textsuperscript{11}

However, it also depends on the size and the shape of the nanoparticle. For example spherical nanoparticles have only one SPR band. Second low energy resonance band can be observed in nanorods due to the SPR oscillation in the longitudinal direction.\textsuperscript{44} Polycrystalline nature of the nanoparticles strongly affect for the SPR band. For example, more complex and non symmetric nanostructure results different mode of resonance frequencies resulting in broader plasmonic absorbance spectrum.\textsuperscript{45}

\subsection*{2.3 Electron microscopy}

Both optical and electron microscopes are used for visualizing structures that are too small to be seen with the naked eye. Electron microscopes use electrons beam instead of visible light for visualization. Electron microscopy has four types of lenses. Condenser lenses, objective lenses, intermediate lenses and projection lenses. Condenser lenses are used to make fine electron probe, control spot size and total beam current. Objective lenses mainly control the focus and determine the limit of resolution. Intermediate lens control the mode of operation and projection lenses control the magnification.
Typical STEM has scanning probe or raster that focus electron beam into a narrow spot. However, in an ideal microscopic system, the entire electron beam coming from the object plane should converge to the same point in the image plane, forming a clear image. Focusing of electron beams at different locations based on its radial distance from the lens center is called aberration. If there is no aberration, all parallel electron beam should focus at the same distance. However, it generates poor quality images at the atomic scale. Spherical aberration can be minimized by bending the lens into its best form or canceled by overcorrecting. These can be done by installing the aberration corrected lens system.

![Figure 2.5. Arrangement of the STEM raster and detectors system](image)

As shown in the figure 2.5 bright-field (BF) scanning transmission electron microscopy images can be acquired by positioning the electron detector on forward scattered beam. BF images are due to the coherently scattered electron. An annular dark field image formed at very high angle by incoherently scattered electrons (as opposed to Bragg scattered electrons). In contrast to the BF imaging, HAADF images are formed by energy lost in collisions with atoms.
It is sensitive to the atomic number and hence Z contrast can be achieved. Due to Z contrast in HAADF imaging, the low-Z atoms appear dark while the high-Z atom appear bright.

HR-STEM experiments were conducted in collaboration with the Oak Ridge National Laboratory. Imaging of the gold nanoparticles (smaller than 5 nm) is challenging due to electron beam induced aggregation and sintering. Structural deformation and inter-particle diffusion hinders the particle size and size dispersity analysis. STEM imaging parameters were optimized to minimize the electron dose and only first-pass images were used in the analysis. This is to minimize the electron beam induced effect. In higher resolution data acquisition mode, neighboring particles tends to coalescence. Low resolution images were acquired to study size dispersity and the aberration corrected high angel annular dark field (HAADF) image was recorded for the atomic structure analysis.

2.4 X-ray techniques

2.4.1. Powder X-ray Diffraction

Powder X-ray diffraction is a popular technique to use when high quality single crystals are not available. This is a non-destructive technique. It is widely used for identification of crystalline samples. XRD line width can be used to determine the size of the nanoparticles by using Debye-Scherrer formula. In general, as the size of the nanoparticles decrease, the line width of the diffraction peak broadened. Nanoparticles were dissolved in CH$_2$Cl$_2$ and placed on a quartz plate over several times to make a thick layer of nanoparticles. Then, the diffraction pattern was recorded using Bruker D8-focus diffractometer. The 2θ angles were varied from 2 to 150°, and continuous measurements were taken overnight to improve the signal to noise ratio.
2.4.2 Single crystal X-ray diffraction

Periodic nature of the molecules leads to the understanding of the atomic arrangement along with physical and electronic properties through the Bragg diffraction. Mass spectrometry allows formulating the nanomolecules composition while atomic arrangement still remains unsolved. The total structure of the nanoparticles can be determined using single crystal X-ray analysis. Growing high quality single crystals of thiolate-protected nanoparticles is challenging. However, several breakthroughs have been accomplished such as \( \text{Au}_{102}(\text{SPhCOOH})_{44}, \text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}, \text{Au}_{38}(\text{SCH}_2\text{CH}_2\text{Ph})_{24}, \) and \( \text{Ag}_{44}(\text{SPh})_{30}^{4-}. \)

Single crystal X-ray structure determinations line up with crystallization set up, crystal screening, data acquisition and finally structure refinement.

1. Crystallization set up: Several crystallization techniques are available. However, solvent diffusion method works best for the gold nanoparticles. First, nanoparticles are dissolved in solvent such as toluene. Then, a non-solvent was vapor diffused to the nanoparticles mixture over time in a closed system. Depend on the conditions, crystals may grow or precipitate out. If precipitated, depending on the nature of the precipitation, other crystallization conditions and solvents can be attempted. Growing diffraction quality single crystals is not only a science but also an art.

2. Crystal screening and data acquisition: A good crystal should be large enough, pure in composition, and should be of high quality (without imperfections, multi-crystals or twinning). Diffraction quality crystals should produce regular pattern of reflections.

3. Data refinement and fitting: Diffraction patterns obtained from different orientations of the crystal is converted into a three dimensional electron density model using method call Fourier synthesis. Finally, data obtained with chemical information will be refined with a predicted
model to obtain the crystal structure.

Crystals were selected under ambient conditions and attached to the tip of a MiTeGen MicroMount. The crystal was mounted in a stream of cold nitrogen at 100 K and centered in the X-ray beam manually with the aid of a video camera. The crystal evaluation and data collection were performed on a Bruker SMART APEXII diffractometer with Mo Kα (λ = 0.71073 Å) radiation at diffractometer to crystal distance of 4.96 cm. The initial cell constants were obtained from three series of ω scans at different starting angles. Exposure time was varied depending on the quality of the crystals. The reflections were indexed by an automated indexing routine built in the APEXII program. The data were collected by using the full sphere data collection routine to survey the reciprocal space to the extent of a full sphere to a resolution of at least 0.80 Å. The structure was solved and refined with the Bruker SHELXTL and Olex2 software package.

2.4.3 High energy X-ray based pair distribution function (PDF) analysis

It is often extremely difficult to obtain diffraction quality crystals for atomic structure investigation using single crystal XRD. For example, structural information on poorly crystallized or amorphous material is inaccessible by conventional diffraction techniques due to lack of long range atomic ordering or poor quality diffraction pattern. In this case, high energy X-ray and pair distribution function analysis can be used to determine the material properties such as particle shape, size, diameter and atomic order. Both Bragg and diffused scattering are taken into account in this technique.
Figure 2.6. Representative atomic ordering (left) and corresponding PDF pattern (right). PDF resolves the distance of each atom pair. The area under the peaks related to the number of neighboring atoms.

Typically PDF is obtained from the synchrotron powder X-ray diffraction data via a Fourier transform of the normalized scattering intensity \( S(Q) \). PDF describes the probability of finding any two atoms at a given distance \( r \) and produce a histogram of all the atom-atom distances within the molecule.

\[
G(r)=4\pi \int_0^r \rho(r) r dr
\]

Eq. 2.1

In the above equation, \( \rho \) (\( r \)), \( \rho \) and \( r \) represents the local, average atomic number densities, and the radial distance respectively. Figure 2.6 shows the short range ordered atoms and its PDF curve. PDF peaks are typically broad due to the thermal motion of the atoms. Peak width and area reveals information on local distortions and number of nearest neighboring atoms. The PDF techniques has an advantage of describing the short and medium-range local structure information over the XAFS technique which is capable of determining only short range inter-
atomic distances like first and second nearest neighbor. Also PDF have advantage in determining the structure of liquid, glass and amorphous materials that does not have long range atomic ordering.

Nanoparticles were loaded into kapton (or polyimide with ~1 mm diameter) tube for PDF analysis. The total scattering data suitable for PDF analysis were collected at 11-ID-B beamline in the Advanced Photon Source at Argonne National Laboratory using high energy synchrotron X-rays radiation ($\lambda = 0.2128 \text{ Å, } E \sim 58 \text{ keV}$). A large area detector (PerkinElmer amorphous silicon) was used to detect the total scattering data to high values of momentum transfer ($Q$). The two-dimensional total scattering images were reduced to one-dimensional diffraction data as a function of $Q$ within fit2d. The data were corrected for background and Compton scattering using pdfgetX2 program. Fourier transform of the scattering data gave the PDFs, $G(r)$. The calculated atomic PDF and PDF fitting was performed using PDFgui.

### 2.4.4 Small angle X-ray scattering

Several regions of the electromagnetic radiation, including X-rays or UV-radiation, can be used to obtain information on materials. Small angle X-ray scattering (SAXS) delivers information on shape, size, and size dispersity of the nanomaterials. SAXS consist of irradiating a sample with an X-rays and measuring the resulting intensity as a function of angle between the incoming beam and scattered beam. Then, the resulting scattering pattern was analyzed based on the differences in electron density. This scattering signal is generated by the difference in the average electron density of the incident sample. SAXS is extensively used on proteins to study the size, particle shape, intra-particle distance, degree of folding, denaturation and disorder of the structure. Further, low resolution particle shape can be reconstructed using ab initio method.
SAXS profile facilitates the direct identification of the spherical particle that gives the wave-like scattering pattern with maxima and minima. Anisometric particles yield slow decay monotones scattering profile. However, the scattering curves sometime failed to distinguish between spherical and hollow structures and are less detailed. Distribution function, p(r), inverse Fourier transformation of the scattering profile, provide more detail information regarding the size, shape and structural information. Globular particles (red curve-right) display bell-shaped p(r) functions with a maximum at about $D_{\text{max}}/2$. In contrast, hollow globular particle (blue) shows the skewed distributions but the maximum shifted towards distances larger than $D_{\text{max}}/2$. Rod shaped particles have skewed distributions with a maximum at small distances corresponding to the radius of the cross-section (green). Disk-like particles (yellow) results in arc shaped distribution function with shifted distances to less than $D_{\text{max}}/2$. 

Figure 2.7. Scattering profile and distribution function of different geometrical structures.\textsuperscript{50}
Guinier plot ($\ln(I(q))$ versus $q^2$) provide qualitative information on nanoparticle aggregation or concentration-dependent scattering. It analyzes the scattering data at very small scattering angles. Guinier plot should be a linear function for the homogenous sample. Figure 2.8 show the representative Guinier plot for three different samples. Monodisperse particle shows linear Guinier (Figure 2.8a). Exponential Guinier curve imply polydisperse nature of the samples (figure 2.8b). Guinier curve with scattering linear plot imply the small amount of particle aggregation.

The SAXS measurements were performed at the 12-ID-B beamline at Argonne National Laboratory using 12 keV X-ray energy. The SAXS data were collected with a Pilatus 2 M area detector. The beam size with 0.05×0.2 mm$^2$ and 1 s exposure times were used for the measurement. The data were fully corrected, reduced to intensity versus scattering vector (q) profiles, and background subtracted. The three-dimensional molecular envelope were derived from the SAXS data using an ab initio program DAMMIN. Pair distance distribution function, $p(r)$, derived from SAXS data using the program GNOM.
CHAPTER 3

BIMETALLIC ALLOY MOLECULES: INSIGHT INTO ATOMIC STRUCTURE OF MIXED METAL $\text{Au}_{25-x}\text{Ag}_x(\text{SR})_{18}$

Part of the text and figures in this chapter are extracted from the following publication:

CHAPTER 3

BIMETALLIC ALLOY MOLECULES: INSIGHT INTO ATOMIC STRUCTURE OF MIXED METAL Au$_{25-x}$Ag$_x$(SR)$_{18}$

3.1 Abstract

Alloying modualte the properties of the monometallic gold molecules. Crystallization of these alloy nanomolecules into ordered array of single crystals facilitate the total structures determination including the special arrangement of the each atom in thee dimension space. Silver alloy of Au$_{25}$(SR)$_{18}$ were able to synthesize and charaterized using optical spectroscopy and mass spectrometry. The atomic arrangement of Au and Ag atoms in Au$_{25-x}$Ag$_x$(SR)$_{18}$ was determined by X-ray crystallography. Ag atoms were selectively incorporated into the 12 vertices of the icosahedral core. The central atom and the metal atoms in the six staple units [−SR–Au–SR–Au–SR−] were exclusively gold, with 100% Au occupancy. The composition of the crystals determined by X-ray crystallography was Au$_{18.3}$Ag$_{6.7}$(SCH$_2$CH$_2$Ph)$_{18}$. This composition is in reasonable agreement with the composition Au$_{18.8}$Ag$_{6.2}$(SCH$_2$CH$_2$Ph)$_{18}$ measured by electrospray mass spectrometry. The structure can be described in terms of shells as Au$_1$@Au$_{5.3}$Ag$_{6.7}@6×$[−SR–Au–SR–Au–SR−].

Author Contributions

Chanaka Kumara synthesized Au$_{25-x}$Ag$_x$(SCH$_2$CH$_2$Ph)$_{18}$, and set up crystallization and initial crystal screening and analysis.
3.2 Introduction

Among the various ultra-small molecules, \( \text{Au}_{25}(\text{SR})_{18} \) is one of the most commonly studied due to its interesting optical\(^{52} \) and electrochemical\(^{53} \) properties and high stability. Crystal structures of the \( \text{Au}_{25}(\text{SR})_{18} \) molecule were independently reported by both Murray and Jin groups in 2008.\(^{14,54} \) In the \( \text{Au}_{25}(\text{SR})_{18} \) family of molecules, several alloys have been synthesized by different research groups including \( \text{Au}_{25-x}\text{Ag}_x(\text{SR})_{18}, \) \(^{4} \) \( \text{Au}_{24}\text{Pd}(\text{SR})_{18}, \) \(^{55} \) \( \text{Au}_{25-x}\text{Cu}_x(\text{SR})_{18}, \) \(^{56} \) and \( \text{Au}_{24}\text{Pt}(\text{SR})_{18}. \)\(^{37} \) These reports discussed the effect of doping with the metal of choice and extent of alloying. The central atom of the 13-atom icosahedral core can be replaced by Pd in thiols protected ultrasmall gold nanoparticles. Among these reports, Ag atom doping exhibits maximum heteroatom incorporation, which is up to 12 silver atoms. Similar atomic radii, lattice parameters and electronic properties between Au and Ag facilitate this interesting maximum atom incorporation.

The fundamental question arising from these alloy nanomolecules is the position of incorporated silver atoms in the actual structure. There are two ways of answering this question:

1. **Computational /theoretical studies.** For example, Walter et al.\(^{57} \) and Guidez et al.\(^{58} \) showed that the surface of the icosahedral core is energetically preferred for silver. 2. **Experimental determination using single crystal X-ray analysis.** While these predictions based on theoretical/computational calculations exist, there is a lack of experimental evidence for the location of the doped atoms. Here single crystal X-ray analyses were used to determine the location of silver atom substitution.

In 2010, Negishi\(^{4} \) reported the synthesis of \( \text{Au}_{25-n}\text{Ag}_n(\text{SR})_{18}, \) where \( n \) is the number of silver atoms, determining the number of silver atom incorporation qualitatively and
quantitatively using mass spectrometry as shown in the figure 3.1. They have produced series of \( \text{Au}_{25-n}\text{Ag}_n\text{(SR)}_{18} \) molecule by changing the incoming Au:Ag molar ratio. Further, they observed the continuous modulation of electronic structure upon silver incorporation. Inspired by Negishi’s report, \( \text{Au}_{25-x}\text{Ag}_x\text{(SR)}_{18} \) nanomolecules were synthesized to investigate the behavior of silver atom substitution in term of atomic structure.

Figure 3.1 Negative-ion MALDI mass spectra of 1–6. The inset shows the expanded spectrum of the main peaks of 2. The red peaks indicate the calculated spectra for \( \text{Au}_{25}\text{(SC}_{12}\text{H}_{25})_{18} \) and \( \text{Au}_{24}\text{Ag}(\text{SC}_{12}\text{H}_{25})_{18} \). Optical absorption spectra of the toluene solutions of \( \text{Au}_{25}\text{(SC}_{12}\text{H}_{25})_{18} \) and 1–6. Reproduced from Ref. [4] with permission from The Royal Society of Chemistry.

The locations of silver atoms in the \( \text{Au}_{25-x}\text{Ag}_x\text{(SCH}_{2}\text{CH}_{2}\text{Ph})_{18}^- \) structure were able to determine experimentally, based on X-ray crystallography. The \( \text{Au}_{25-x}\text{Ag}_x\text{(SCH}_{2}\text{CH}_{2}\text{Ph})_{18}^- \) molecules were synthesized using a modified procedure reported by Negishi.\(^4\) Briefly, the synthesis involves two steps. The first step is the synthesis of crude product that contains polydisperse Au–Ag clusters. The second step is solvent fractionation to isolate \( \text{Au}_{25-x}\text{Ag}_x\text{(SCH}_{2}\text{CH}_{2}\text{Ph})_{18}^- \) molecules. There are three different distinct locations where the metal atom
can be doped into the Au$_{25}$(SR)$_{18}$ structure, (1) the single central atom of the icosahedral core, (2) the 12 atoms in the vertices of the icosahedral core, and (3) the 12 Au atoms forming the six dimeric [-SR-Au-SR-Au-SR-] units.

3.3 Method

3.3.1: Synthesis of Au$_{25-x}$Ag$_x$(SCH$_2$CH$_2$Ph)$_{18}^-$

The Au$_{25-x}$Ag$_x$(SCH$_2$CH$_2$Ph)$_{18}^-$ were synthesized via the previously reported procedure by Negishi$^4$ in two steps: Aqueous solution (30mL) containing HAuCl$_4$ and AgNO$_3$ (total metal concentration was set to 30mM) was mixed with a toluene solution (30 mL) of tetraoctylammonium bromide, TOABr (1.1 mmol). The initial mole ratios of Au:Ag precursors were varied up to 1:0.66. After stirring for 30 min, the turbid organic phase was separated and phenylethane thiol (10 mmol) was added and further stirred for 30 min at room temperature. This solution was cooled in ice bath for 30 minutes. An aqueous solution of NaBH$_4$ (20 mmol, 20 mL) cooled to 0 °C, was rapidly added to the reaction mixture under vigorous stirring. After 3 hours, the organic layer was separated and evaporated to dryness. The product was washed with methanol to remove excess thiol, NaBH$_4$, TOABr and other by-products. The residual mixture was extracted with CH$_3$CN.

The product mixture was dissolved in toluene and then solvent fractionation was performed using CH$_3$CN to isolate Au$_{25-x}$Ag$_x$(SCH$_2$CH$_2$Ph)$_{18}^-$. Several crystallization setups were conducted using toluene or CH$_2$Cl$_2$ as a solvent and EtOH or MeOH as a non-solvent. The crystallization setups with higher Au:Ag ratios either decomposed or precipitated without forming crystals. However, crystals were found in dried form in the synthesis batch of Au:Ag 1:0.25 incoming molar ratio. This batch of crystals was used for X-ray crystallography.
3.3.2: Mass spectrometry

ESI mass spectra were obtained from Waters SYNAPT mass spectrometer in 50/50 toluene/CH$_3$CN solvent mixture or THF. ESI-MS calibration was performed with 50:50 isopropanol:water solution of NaI. Calibration check was performed with Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$ and Au$_{144}$(SCH$_2$CH$_2$Ph)$_{60}$.

3.3.3 Single crystal X-ray analysis

A black crystal with approximate dimensions $0.08 \times 0.07 \times 0.02$ mm$^3$ was selected under oil at ambient conditions and attached to the tip of a MiTeGen MicroMount©. The crystal was mounted in a stream of cold nitrogen at 100(1) K and centered in the X-ray beam by using a video camera. The crystal evaluation and data collection were performed on a Bruker Quazar SMART APEXII diffractometer with Mo K$_\alpha$ ($\lambda = 0.71073$ Å) radiation and the diffractometer to crystal distance of 4.96 cm. The initial cell constants were obtained from three series of $\omega$ scans at different starting angles. Each series consisted of 12 frames collected at intervals of 0.5° in a 6° range about $\omega$ with the exposure time of 20 seconds per frame. The reflections were successfully indexed by an automated indexing routine built in the APEXII program suite. The final cell constants were calculated from a set of 9092 strong reflections from the actual data collection. The data were collected by using the full sphere data collection routine to survey the reciprocal space to the extent of a full sphere to a resolution of 0.80 Å. A total of 58305 data were harvested by collecting 4 sets of frames with 0.5° scans in $\omega$ and $\varphi$ with exposure times of 40 second per frame.
3.4 Selective silver atom incorporation to $\text{Au}_{25}(\text{SR})_{18}$ structure

Figure 3.2 (left) represent the optical image of the needle like $\text{Au}_{25-x}\text{Ag}_x(\text{SCH}_2\text{CH}_2\text{Ph})_{18}$ crystals and its unit cell arrangement as repeating pattern that crystallizes in a triclinic $P\overline{1}$ space group. The structure was refined to a resolution of 0.8 Å and to an $R_1$ value of 4.1%.

Figure: 3.2 (Right) $\text{Au}_{25-x}\text{Ag}_x(\text{SCH}_2\text{CH}_2\text{Ph})_{18}$ crystals as can be seen in the optical microscope (Left) Arrangement of the $\text{Au}_{25-x}\text{Ag}_x(\text{SCH}_2\text{CH}_2\text{Ph})_{18}$ molecules within a crystals as triclinic unit cells. Cell parameter, $a = 16.191$ Å, $b = 17.244$ Å, $c = 18.613$ Å, $\alpha = 105.871^\circ$, $\beta = 106.252^\circ$, $\gamma = 90.74^\circ$, $V = 4776$ Å$^3$, $Z = 1$
Figure 3.3  X-ray crystal structure of Au$_{25-x}$Ag$_x$(SCH$_2$CH$_2$Ph)$_{18}$ anion. (a) Total structure of Au$_{25-x}$Ag$_x$(SCH$_2$CH$_2$Ph)$_{18}$.(b) Au$_{25}$S$_{18}$ framework showing the six [-SR-Au-SR-Au-SR-] dimeric units, where the dimeric units exclusively contain Au atoms, with 100% Au occupancy. (c) The 13-atom icosahedral alloy core geometry, showing a central Au atom (yellow) with 100% Au occupancy surrounded by 12 atoms that show partial occupancy of Au or Ag atoms, as shown in the inset table. Table 3.1 shows the degree of compositional disorder in the alloy core. The inset table and (c) shows that the 12 atoms at the icosahedral vertices are partially occupied by Ag or Au. The X-ray crystallographic structure is an average structure. Thus the X-ray composition,
Au_{18.3}Ag_{6.7}(SCH_2CH_2Ph)_{18}^{-} is the average value obtained from diffraction of many Au_{25-x}Ag_{x}(SR)_{18} molecules in the crystal. The composition obtained independently by ESI-MS is Au_{18.8}Ag_{6.2}(SCH_2CH_2Ph)_{18}, in reasonable agreement with X-ray crystallographic composition. This can be written as Au_{1}@Au_{5.3}Ag_{6.7}@6\times[-SR-Au-SR-Au-SR-]

Figure 3.3 shows the X-ray crystallographic structure of Au_{25-x}Ag_{x}(SCH_2CH_2Ph)_{18}. Specifically, the crystals considered in this study have the composition, Au_{18.3}Ag_{6.7}(SCH_2CH_2Ph)_{18}. The central atom was shown to be exclusively Au (occupancy factor =1.00), as predicted. The twelve atoms in the vertices of the icosahedral Au_{12-x}Ag_{x} core show dual occupancy, containing either Au atoms or Ag atoms, as shown in Figure 3.3c. The overall composition of the bimetallic core from the X-ray crystallography is Au_{1}@Au_{5.3}Ag_{6.7}. The composition was independently verified using mass spectrometry. The compositional disorder of Au and Ag atoms yield fractional occupancies. For example, the site 2 is occupied by either atom Ag2 or Au2 with the Ag2 atom being present 59% of the time. This is denoted as Ag2/Au2 – 59% / 41%. Figure 3.3 inset table shows the following percentages of Ag and Au atoms:, Ag3/Au3 – 45%/ 55%, Ag4/Au4–61%/ 39%, Ag5/Au5–58% / 42%, Ag6/Au6– 53% / 47%, and Ag10/Au10– 56% / 44%. Therefore, the mixed-metal core composition can be formulated as Au_{5.3}Ag_{6.7}. The metal atoms in the dimeric staple are exclusively gold, with 100% Au occupancy.

The dopant positions of silver atom in the 13-atom icosahedral core of Au_{25} have been computationally predicted before but have not been determined experimentally until this. Partially occupied counterions and solvent molecules were observed. There is clear evidence for the presence of tetraoctylammonium counterion, but it was not modeled in this work. It is very likely that the counterions and solvent molecules are disordered over several positions. This may
be due to solvent loss or disordered solvent channel that increases the mosaicity of the crystals, hindering the identification of the exact location of the counterions.

When compared with the monometallic Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}^-$ crystal structures, major structural features (Metal$_{13}$ icosahedral core protected by six [-SR-Au-SR-Au-SR-Au-] units) were preserved upon silver doping. The silver atoms are simply substituted among the 12 vertices of the icosahedron core. In general, bond elongations were observed from the central Au atom to icosahedral surface atoms as the Ag content increased.

![Figure 3.4](image.png)

Figure 3.4 Electro-spray ionization mass spectrum of the Au$_{25-x}$Ag$_x$(SCH$_2$CH$_2$Ph)$_{18}^-$ crystals.

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Table 3.2 Average composition determined by mass spectrometry data.

<table>
<thead>
<tr>
<th>nanomolecules</th>
<th>% Ag as total</th>
<th>contribution of silver from each species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au$_{21}$Ag$<em>4$(SR)$</em>{18}$</td>
<td>3.3</td>
<td>0.13</td>
</tr>
<tr>
<td>Au$_{20}$Ag$<em>5$(SR)$</em>{18}$</td>
<td>19.4</td>
<td>0.97</td>
</tr>
<tr>
<td>Au$_{19}$Ag$<em>6$(SR)$</em>{18}$</td>
<td>42.3</td>
<td>2.54</td>
</tr>
<tr>
<td>Au$_{18}$Ag$<em>7$(SR)$</em>{18}$</td>
<td>28.8</td>
<td>2.02</td>
</tr>
<tr>
<td>Au$_{17}$Ag$<em>8$(SR)$</em>{18}$</td>
<td>6.1</td>
<td>0.49</td>
</tr>
<tr>
<td>total number of Ag from ESI-MS</td>
<td>6.15 ≈ 6.2</td>
<td></td>
</tr>
</tbody>
</table>
Crystals of the Au_{18.8}Ag_{6.2}(SCH_2CH_2Ph)_{18} nanomolecules were analyzed using ESI-MS. This will facilitate the determination of the composition that is complementary to but independent of X-ray crystallographic composition. Figure 3.4 shows the ESI mass spectrum, containing peaks corresponding to Au_{17}Ag_8(SR)_{18}, Au_{18}Ag_7(SR)_{18}, Au_{19}Ag_6(SR)_{18}, Au_{20}Ag_5(SR)_{18}, and Au_{21}Ag_4(SR)_{18} nanomolecules, present in 0.061, 0.288, 0.423, 0.194, and 0.033 fractions, respectively. Then, the percentages of each of these species were calculated from the peak height corresponding to each species in the ESI-MS spectrum, as shown in Table 3.2. Finally, using these percentages, the average composition was calculated and found to be Au_{18.8}Ag_{6.2}(SR)_{18}. This can be written as Au_1@Au_{5.8}Ag_{6.2}@ 6×[-SR-Au-SR-Au-SR-]. X-ray crystallography also yields the average structure and average composition which is independently derived as 6.7 silver atoms, with the composition Au_{18.3}Ag_{6.7}(SCH_2CH_2Ph)_{18}. Therefore, mass spectrometry and X-ray crystallography show 6.2 and 6.7 Ag atoms respectively. This is a reasonable agreement, and the differences may be due to the fact that the ESI peak heights are correlated to ionization efficiencies and may slightly differ from what is present in solution.

### 3.5 Conclusion

The crystal structure of Au_{25-x}Ag_x(SCH_2CH_2Ph)_{18} is presented, which is the first X-ray crystallography report of a Au_{25}(SR)_{18} alloy family. We show that the central atom is exclusively Au with 100% occupancy, while the 12-atom icosahedral vertex atoms are partially occupied by Ag or Au atoms. The six staples are exclusively occupied by Au, with the [-SR-Au-SR-Au-SR-] structure. Ag incorporation shows site specific distribution in the Au_{25}(SR)_{18} structure leading to Au-(Au/Ag)-Au mixed-metal atom configuration, more specifically the Au_1@Au_{5.3}Ag_{6.7}@ 6×[-

**Crystal Data** for C\textsubscript{144}H\textsubscript{162}Ag\textsubscript{6.6587}Au\textsubscript{18.3413}S\textsubscript{18} \((M = 6800.70)\): triclinic, space group P-1, \(a = 16.191(7) \text{ Å}\), \(b = 17.244(7) \text{ Å}\), \(c = 18.613(7) \text{ Å}\), \(\alpha = 105.871(12)^{\circ}\), \(\beta = 106.252(13)^{\circ}\), \(\gamma = 90.74(2)^{\circ}\), \(V = 4776(3) \text{ Å}^3\), \(Z = 1\), \(T = 100.01 \text{ K}\), \(\mu(\text{Mo } K\alpha) = 14.917 \text{ mm}^{-1}\), \(D_{\text{calc}} = 2.364 \text{ g/mm}^3\), 58305 reflections measured \((2.38 \leq 2\Theta \leq 56.718)\), 23796 unique \((R_{\text{int}} = 0.0426)\) which were used in all calculations. The final \(R_1\) was 0.0411 \((I > 2\sigma(I))\) and \(wR_2\) was 0.1065.
CHAPTER FOUR

SYNTHESIS AND ATOMIC STRUCTURE OF ALLOY MOLECULES WITH 38 METAL ATOMS

Part of the text and figures in this chapter are extracted from the following publication


CHAPTER FOUR

SYNTHESIS AND ATOMIC STRUCTURE OF ALLOY MOLECULES WITH 38 METAL ATOMS

4.1 Abstract

Au\textsubscript{38-x}Ag\textsubscript{x}(SCH\textsubscript{2}CH\textsubscript{2}Ph)\textsubscript{24} alloy nanomolecules were synthesized, purified and characterized by MALDI-TOF mass spectrometry to atomic precision. Ag atoms incorporation results in smearing out of distinct features of the Au\textsubscript{38}(SR)\textsubscript{18} optical spectrum. Further, silver preference sites of Au\textsubscript{38-n}Ag\textsubscript{n}(SCH\textsubscript{2}CH\textsubscript{2}Ph)\textsubscript{24} alloy were determined by X-ray crystallography. The primary structure of monometallic Au\textsubscript{38}(SR)\textsubscript{24} consists of two central Au atoms, twenty one Au atoms forming a bi-icosahedral core protected by six dimeric and three monomeric staple units. In Au\textsubscript{38-x}Ag\textsubscript{x}(SR)\textsubscript{24} structure where x range from 1 to 5, the silver atoms are selectively incorporated into the Au\textsubscript{21} bi-icosahedral core. Within the core, silver atoms preferentially occupy nine selected locations; (a) the two vertex edges – three atoms on each edge and six atoms total, and (b) the middle face shared three-atom ring, adding to a total of nine locations. X-ray crystallography yielded a composition of Au\textsubscript{34.04}Ag\textsubscript{3.96}(SCH\textsubscript{2}CH\textsubscript{2}Ph)\textsubscript{24}. The crystal structure of the alloy can be described in terms of shells as Au\textsubscript{2}@Au\textsubscript{17.04}Ag\textsubscript{3.96}@6×[-SR-Au-SR-Au-SR]3×[-SR-Au-SR-].

Author Contributions

Chanaka Kumara synthesized Au\textsubscript{38-x}Ag\textsubscript{x}(SCH\textsubscript{2}CH\textsubscript{2}Ph)\textsubscript{18}, and set up crystallization, initial crystal screening and analysis. Kevin Gagnon performed final single crystal X-ray measurement and analysis at Advanced Light Source at Lawrence Berkeley National Laboratory.
4.2 Introduction

Among the metal nanoparticles, ultra small gold nanoparticles (< 2 nm) have been extensively studied due to their stability, resistance to oxidation and interesting optical properties.\textsuperscript{38,60,61} Metal nanoparticles of molecular definition, referred to as nanomolecules, such as Au\textsubscript{25}(SR)\textsubscript{18}, Au\textsubscript{38}(SR)\textsubscript{24}, Au\textsubscript{102}(SR)\textsubscript{44} and Au\textsubscript{144}(SR)\textsubscript{60} have already been reported.\textsuperscript{7,62,63,64,65,66} In terms of stability, thermochemical treatments show that Au\textsubscript{38}(SR)\textsubscript{24} and Au\textsubscript{144}(SR)\textsubscript{60} possess extraordinary stability.\textsuperscript{7,65,67,68}

Alloy nanoparticles have opto-electronic properties which differ from their monometallic nanoparticles,\textsuperscript{34} further extending its applications in biosensing and catalysis. Ag atoms have been incorporated into Au\textsubscript{144}(SR)\textsubscript{60} and Au\textsubscript{25}(SR)\textsubscript{18} nanomolecules resulting in Au\textsubscript{144-x}Ag\textsubscript{x}(SR)\textsubscript{60} and Au\textsubscript{25-x}Ag\textsubscript{x}(SR)\textsubscript{18} alloy nanomolecules.\textsuperscript{69} Negishi et al \textsuperscript{4} showed continual modulation of electronic structure by controlling the number of silver atoms incorporated into Au\textsubscript{25}(SR)\textsubscript{18}. For the same Au\textsubscript{25}(SR)\textsubscript{18} nanomolecule, doping with Pd, replaces the central Au atom, in the Au\textsubscript{13} core to form Pd\textsubscript{1}Au\textsubscript{24}(SR)\textsubscript{18}, which is found to be independent of the incoming Au:Pd ratio.\textsuperscript{70}

Initially identified\textsuperscript{20} as the 8 kDa core size, later Tsukuda and co-workers\textsuperscript{71} determined its composition as Au\textsubscript{38}(SR)\textsubscript{24} to atomic precision using ESI-MS. The synthesis almost always seems to result in a mixture of Au\textsubscript{38} and Au\textsubscript{40} species. However, size exclusion chromatography was successfully employed to separate both Au\textsubscript{38}(SR)\textsubscript{24} and Au\textsubscript{40}(SR)\textsubscript{24} from other nanomolecules.\textsuperscript{72}

Recently, crystal structure of Au\textsubscript{38}(SR)\textsubscript{24} was reported as triclinic space group, \textit{P}\textit{\textsubscript{\textup{i}}} and unit cell consists a pair of enantiomers.\textsuperscript{73} Au\textsubscript{38} consist of six long staples (-SR-Au-SR-Au-SR-) and three short staples (-SR-Au-SR-) altogether 15 gold atoms, which reflect the Au\textsubscript{23}/Au\textsubscript{15} core-shell structure. Au\textsubscript{38} is extensively studied due to the interesting optical, electrochemical and
luminescence properties coupled with its high stability. Burgi and co-workers used chiral HPLC to separate the two enantiomers of Au$_{38}$(SR)$_{24}$ and its Pd alloy Pd$_2$Au$_{36}$(SR)$_{24}$ as its chirality was predicted before.

Although several other experimental and theoretical works on alloys of nanomolecules exist, what is generally lacking for gold based thiolated alloy molecules are the experimental data that shows the exact locations of the doped Ag, Cu and Pd atoms. Understanding the heteroatom doping locations are very important in the active catalytic sites determination of the alloy nanoparticles. Apart from hetero atom incorporation, recent progress has been made to understand the ligand exchange protocol via crystallization the partially ligand exchanged products of Au$_{102}$(SR)$_{44}$ and Au$_{25}$(SR)$_{18}$ to investigate the active ligand exchange sites.

This chapter presents the synthesis and isolation of Au$_{38-x}$Ag$_x$(SR)$_{24}$ alloy nanomolecules protected by phenylethane thiol. Composition, including the number of silver atoms incorporated was studied by MALDI mass spectrometry. The change in optical properties with silver incorporation was examined by UV-visible spectroscopy. In general, the features of the optical spectra were diminished with silver incorporation. Alloy stability in relation to the degree of silver atom incorporation is discussed.

Finally, X-ray crystal structure of the Au$_{38-x}$Ag$_x$(SR)$_{24}$ molecules were determined. Ag atoms are selectively incorporated into nine specific location of the face shared bi-icosahedrons core. Independent compositional assignments made using mass spectrometry data confirm the X-ray results. Finally, decrease in stability of Au$_{38-x}$Ag$_x$(SR)$_{24}$, when the number of Ag atoms exceed nine, were discussed from a structural basis.
4.3 Method

4.3.1 Synthesis

Synthesis of the Au_{38-x}Ag_{x}(SR)_{24} involves three steps. The first step is the synthesis of crude product that contains polydisperse Au-Ag clusters using the method reported by Negishi et al.\textsuperscript{4} The second step is the thermo-chemical treatment of the crude product with excess thiol resulting in mixtures with Au_{38-x}Ag_{x}(SR)_{24} alloy nanomolecules. Finally, pure Au_{38-x}Ag_{x}(SR)_{24} were isolated by solvent fractionation.

Step 1. Aqueous solution (30 mL) containing HAuCl\textsubscript{4} and AgNO\textsubscript{3} (total metal concentration was set to 15 mM) was mixed with toluene solution (30 mL) of tetraoctylammonium bromide, TOABr (0.55 mmol). The initial mole ratios of Au:Ag precursors were set to 1:0, 1:0.05, 1:0.10, 1:0.15, 1:0.20, 1:0.30, 1:0.35 and 1:0.40 in different synthesis batches. After stirring for 30 min, the turbid organic phase was separated and phenylethane thiol (5 mmol) was added. The stirring was continued for 30 min at room temperature. The reaction mixture was then cooled to 0 °C in ice bath for 30 min. An aqueous solution of NaBH\textsubscript{4} (10 mmol, 20 mL) cooled to 0 °C, was rapidly added to the reaction mixture under vigorous stirring and reaction carried out at 0 °C. After 3 hours, the organic layer was separated from aqueous layer and evaporated to dryness. The product was washed with methanol to remove excess thiols and other byproducts. The residual mixture was extracted with toluene. Smaller clusters less than ~10 kDa [(Au/Ag)\textsubscript{n}(SR)\textsubscript{m} particles] were removed by solvent fractionation and remaining product ( nanoparticle size range from 10-40 kDa) was subjected to second step.

Step 2: Nanoparticles separated from step 1 (~20 mg of 10-40 kDa) was dissolved in 0.50 mL of toluene and etched with excess phenylethane thiol (0.50 mL) at 80°C under stirring. Aliquots from the reaction were monitored using MALDI-MS at different time intervals. When pure Au_{38-}
nAgₙ(SR)₂₄ nanoalloy was observed in MALDI (12-24 h), the reaction was stopped and washed with methanol. Then product was subjected to solvent fractionation to isolate pure Au₃₈₋ₓAgₓ(SR)₂₄ nanoalloy by removing other species such as Au₁₄₄₋ₓAgₓ(SR)₆₀.

*Step 3:* Then, the product was subjected to following solvent fractionation steps to isolate pure Au₃₈₋ₓAgₓ(SR)₂₄ nanomolecules. First step is to remove clusters larger than 10 kDa from the product. Product obtained from thermo-chemical treatment was dissolved in toluene (0.20 ml) and 0.50 mL of acetone was added slowly. Then, acetonitrile was added very slowly (in 0.25 mL increments), allowing precipitates to form, which was monitored with MALDI-MS. When Au₃₈₋ₓAgₓ(SR)₂₄ nanomolecules start to precipitate, soluble part was separated and solvent evaporated. In the second step, clusters less than 10 kDa were removed from the mixture. The soluble part was re-dissolved in acetone (0.50 ml) and acetonitrile was added very slowly (as a fraction of 0.05 to 0.10 mL) while monitoring with MALDI-MS. When most of the Au₃₈₋ₓAgₓ(SR)₂₄ nanomolecules were seen in the insoluble portion, process was stop. This insoluble portion contain pure Au₃₈₋ₓAgₓ(SR)₂₄ nanomolecules. Almost all the (Au/Ag)ₙ(SR)ₙ that are less than 10 kDa was removed before theromchemical treatment. This facilitate the isolation of Au₃₈₋ₓAgₓ(SR)₂₄ nanomolecules using solvent fractionation with minimal interference of Au₂₅₋ₓAgₙ(SR)₁₈.

### 4.3.2 Characterization

UV-visible spectra were measured in toluene solutions using Varian UV-vis-NIR spectrophotometer in the 300–1200 nm range. MALDI mass spectra were acquired with a Bruker Daltonics Autoflex mass spectrometer using DCTB matrix at optimal laser fluence. Spectral analyses were done using Bruker Daltonics flexAnalysis version 3.0.
4.3.3 Crystallizations

Vapor diffusion methods were employed for the crystallization of \( \text{Au}_{38-x}\text{Ag}_x\text{(SCH}_2\text{CH}_2\text{Ph)}_{24} \). Crystallization setup was made using the samples that synthesized using various Au:Ag incoming molar ratio. Briefly, \( \text{Au}_{38-x}\text{Ag}_x\text{(SCH}_2\text{CH}_2\text{Ph)}_{24} \) nanoparticles (0.50 mg) were dissolved in toluene (0.20 mL) and kept in small glass vial (5 mL). Then, the small vial was kept inside a larger (20 mL) glass vial with ethanol (10 mL) and closed with a cap. After several days, needle like crystals were observed, which is the Au:Ag incoming ratio of 1:0 and 1:0.1 with the \( \text{Au}_{35}\text{Ag}_3\text{(SR)}_{24} \) composition.

4.3.4 Single Crystal X-ray analysis and structure refinement

A black crystal with approximate dimensions 0.02 x 0.01 x 0.005 mm\(^3\) was isolated and mounted on the tip of a 10 µm MiTeGen Micromount. The crystal was mounted in a 100(2) K nitrogen cold stream, provided by an Oxford Cryosystems 700+ series, on a D8 goniostat equipped with a PHOTON 100 CMOS detector operating in shutterless mode at Beamline 11.3.1 at the Advanced Light Source (Lawrence Berkeley National Laboratory) using synchrotron radiation (\( \lambda =0.7749 \) Å) from a channel cut silicon (111) monochromator. The data frames were collected using the program APEX2 and processed using the program SAINT v8.34a. The data were corrected for absorption, volume, and beam corrections based on the multi-scan technique as implemented in SADABS 2014/4. Data were collected using a combination of \( \phi \) and \( \omega \) scans at exposure times of 1 second/degree for \( 2\theta = 0 \) degrees, and 5 seconds/degree for \( 2\theta = -45 \).

Significant efforts were made to improve the data quality by collecting data at higher angle, growing crystals of different batches, and by screening many crystals. Several data collections and structure solution on many crystals have attempted. All non-disordered metal and sulfur atoms were refined with anisotropic displacement coefficients. The disordered atoms and
all the carbon atoms were refined isotropically.

Structure refinement shows the formula to be $\text{Au}_{34.04}\text{Ag}_{3.96}\text{S}_{24}\text{C}_{192}\text{H}_{216}$. The Ag atoms are specifically incorporated into nine locations of the face shared bi-icosahedron core. The nine dual occupancy sites are 4, 5, 6, 20, 21, 22, 33, 34, and 35. The S18 and S21 atoms show positional disorder over two positions with major component contribution of 53.8(8)% each. The thiol group at S2 site is disordered over two positions; however, only the average position could be stably modeled. Therefore, this is the only position which is contained in the refinement. The result is reasonably large electron density in the vicinity. The structure was deposited under the Cambridge crystallography data centre database under the CCDC number 1038715.

4.4 Mass spectrometry of $\text{Au}_{38-x}\text{Ag}_x(\text{SCH}_2\text{CH}_2\text{Ph})_{24}$ alloy

MALDI-TOF mass spectrometry was used for characterization as the molecular ions were of sufficient resolution to distinguish the Ag alloys from the parent $\text{Au}_{38}(\text{SR})_{24}$ nanomolecules. The bottom black spectrum in Figure 4.1 shows the MALDI spectrum of monometallic $\text{Au}_{38}(\text{SR})_{24}$. Introducing silver precursor in the starting material lead to the formation of atomically precise $\text{Au}_{38-x}\text{Ag}_x(\text{SR})_{24}$ alloy nanomolecules. Mass difference between the peaks corresponds to the mass difference between gold and silver atom, which reflects the replacement of gold with silver atoms in the formation of $\text{Au}_{38-x}\text{Ag}_x(\text{SR})_{24}$. Silver having apparently equal atomic volume compared to gold is a good candidate for incorporation into $\text{Au}_{38}(\text{SR})_{24}$. As the incoming silver mole ratio was increased in initial synthesis, the number of silver atoms incorporated also increases as expected. But, it plateaus at 1:0.20, Au:Ag mole ratio.
Figure 4.1 (Left) MALDI-TOF mass spectra (positive mode) of $\text{Au}_{38-x}\text{Ag}_x\text{(SR)}_{24}$ nanomolecules for Au:Ag precursor ratios of 1:0 (black), 1:0.05 (red), 1:0.10 (blue), 1:0.15 (olive) and 1:0.20 (orange), 1:0.30 (purple), 1:0.35 (dark yellow) and 1:0.40 (brown) in the starting material. The mass difference between the peaks in nanoalloys corresponds to the Au (196.97 Da) and Ag (107.87 Da) mass difference, $\Delta m=89$ Da. The number of Au and Ag atoms of most dominant peak is denoted above each distribution of peaks. (Right) Full MALDI-TOF mass spectra (positive mode, 4-40 kDa) of isolated $\text{Au}_{38-x}\text{Ag}_x\text{(SR)}_{24}$ nanomolecules. Reproduced from [6] with permission from The Royal Society of Chemistry.

For example, the average and maximum number of silver atoms in 1:0.20 and 1:0.30 mole ratios remain almost constant at ~6 and 10 atoms. Experiments performed at incoming mole ratio of 1:0.35 and 1:0.40 produce a minor fraction of $\text{Au}_{38-x}\text{Ag}_x\text{(SR)}_{24}$ that could be observed at early reaction times, as shown in the figure 4.1 (top two spectra), but could not be
able to isolated and purified. The average and maximum number of silver atoms incorporation in Au:Ag, 1:0.40 molar ratios is found to be ~9 and 12 atoms. In the experiments performed at higher molar ratios > 1: 0.40, Au$_{38-x}$Ag$_x$(SR)$_{24}$ could not be detected. Although atomically precise alloy nanomolecules are achieved in direct synthesis, it is challenging to synthesize alloys with a definitive number of doped foreign X atoms (especially for X=Ag) due to the, similar lattice parameters, atomic radii and electronic properties which facilitate the replacement for Au atom. Thus, although total metal atom count is fixed to 38, x varied with initial Au:Ag incoming molar ratio.

4.5 Tuning the optical properties of the Au$_{38-x}$Ag$_x$(SR)$_{24}$ nanomolecules

![Absorbance graph](image)

Figure 4.2 UV-visible spectra of Au$_{38-x}$Ag$_x$(SR)$_{24}$ nanomolecules in toluene with Au:Ag precursor ratios of 1:0 (black), 1:0.05 (red), 1:0.10 (blue), 1:0.15 (olive) and 1:0.20 (orange), 1:0.30 (purple) in the starting material Reproduced from [6] with permission from The Royal Society of Chemistry.
Figure 4.2 clearly shows that optical properties of Au$_{38}$(SR)$_{24}$ are sensitive to the number of silver atom incorporation. Similar results were observed in case of Au$_{25}$(SR)$_{18}$ and Au$_{144}$(SR)$_{60}$	extsuperscript{4,69}. Au$_{38}$(SR)$_{24}$ exhibits 1.33 eV, HOMO-LUMO energy gap as observed from UV-visible and electrochemical measurements. Au$_{38}$(SR)$_{24}$ nanomolecules have distinct bands at 1050, 750, 630, 478 nm (major) and shoulders at 517 and 400 nm. Silver incorporation into Au$_{38}$(SR)$_{24}$ leads to blue-shift in UV-visible spectrum. The band at 1050 nm present in monometallic Au$_{38}$ diminishes with Ag incorporation in the case of 1:0.05 and gradually disappears with increasing number of Ag atoms. The bands at 1050 and 750 nm may solely be due to pure Au$_{38}$ fraction. Thus incorporation of silver may not facilitate the low energy transition in Au$_{23-x}$Ag$_x$ core. Incorporation of six silver atoms resulted in broadening and shifting of 630 nm peak to 610 nm. However, diminishing of Au$_{38}$ features upon silver incorporation is in contrast to the observation in Au$_{144-x}$Ag$_x$(SR)$_{60}$ nanomolecules,	extsuperscript{69} where the plasmon-like features emerge upon Ag incorporation.

Murray et al examined the effect of substituent in the ligand (SPhX where X is different substituent groups) on the optical properties of the Au$_{25}$(SPhX)$_{24}$ and found it to be negligible and consistent with inductive effect of the ligand.	extsuperscript{84} Thus, electronic properties mainly originate from the core. Therefore smearing of the optical properties upon silver incorporation suggests that the Au atoms are replaced in the Au$_{23}$ core.

4.6 Site specific silver atoms incorporation

Based on the Au$_{38}$ crystal structure, there are several possible sites for silver atom incorporation: (1) silver atoms can be incorporated into staple motifs exclusively (2) silver atoms could be incorporated into both staple motifs and the core (3) silver atoms selectively incorporated into the core alone. Option 1 accounts for the maximum incorporation of 12 silver
atoms into long staple units, but cannot explained the diminishing optical properties of \( \text{Au}_{38}(\text{SR})_{24} \) nanomolecules. In option 3, silver atoms may be distributed in two face fused \( \text{Au}_{13} \) cores. Several crystallization setup were made to investigate the atomic structure of the \( \text{Au}_{38-x}\text{Ag}_x(\text{SR})_{24} \) alloy. Figure 4.3 shows the needle like crystals obtained from the alloy. Figure further represents the simples repeating unit in the alloy crystals. It crystallizes in triclinic \( P\overline{1} \). As expected, the unit cell dimensions and volume were nearly identical to the monometallic \( \text{Au}_{38}(\text{SR})_{24} \).\(^{73} \)

Figure 4.3 (Left) \( \text{Au}_{38-x}\text{Ag}_x(\text{SCH}_2\text{CH}_2\text{Ph})_{24} \) crystals as can be seen in the optical microscope (Right) Arrangement of alloy molecules within a crystals as triclinic unit cells. Cell parameter, 
\[ a = 18.8585(7) \, \text{Å}, \quad b = 22.2711(8) \, \text{Å}, \quad c = 29.2473(12) \, \text{Å}, \quad \alpha = 96.472(2)^\circ, \quad \beta = 106.952(3)^\circ, \quad \gamma = 111.124(3)^\circ, \quad V = 10629.0(7) \, \text{Å}^3, \quad Z = 2. \]

Figure 4.4 shows the atomic structure of a \( \text{Au}_{38-x}\text{Ag}_x(\text{SCH}_2\text{CH}_2\text{Ph})_{24} \) molecule. X-ray crystallogegetic refinements yield a composition of \( \text{Au}_{34.04}\text{Ag}_{3.96}(\text{SCH}_2\text{CH}_2\text{Ph})_{24} \) with \( R_1 \) value of 4.92%. The compositional disorder in nine metal atom sites yield fractional occupancies. There are four distinct locations where the metal atom possibly can be doped: (1) two central atoms of the face shared bi-icosahedral core, (2) the 21 atoms in the vertices of the bi-icosahedral core, (3) the 12 Au atoms of the long staple: six dimeric \([-\text{SR}\text{-Au}\text{-SR}\text{-Au}\text{-SR}]\) units and (4) the three Au atoms of the short staple: monomeric \([-\text{SR}\text{-Au}\text{-SR}]\) units.
Figure 4.4 X-ray crystal structure of Au$\text{38-x}$Ag$_x$(SCH$_2$CH$_2$Ph)$_{24}$. The X-ray crystallography based composition is Au$_{34.04}$Ag$_{3.96}$(SCH$_2$CH$_2$Ph)$_{24}$. This can be written as Au$_2$@Au$_{17.04}$Ag$_{3.96}$@ 6[-SR-Au-SR-Au-SR-Au-SR-] @ 3[-SR-Au-SR-] (a) The 23-atom face shared bi-icosahedra Au$_{38-x}$Ag$_x$(SCH$_2$CH$_2$Ph)$_{24}$ alloy core geometry, showing Au atom (light brown) with 100% Au occupancy and partial occupied Au or Ag atoms (gray) (b) Side view of the alloy core. (c) Au$_{23}$S$_{24}$ framework showing the six [-SR-Au-SR-Au-SR-] dimeric units and three [-SR-Au-SR-] monomeric units, where the staples exclusively contain Au atoms. (d) and (e) Front and side view of the 23-atom face shared bi-icosahedra monometallic Au$_{38}$(SCH$_2$CH$_2$Ph)$_{24}$ core geometry.[17] Pink: Au in the staple, light brown: Au in the bi-icosahedrons core, gray: Ag or Au in the core, yellow: Sulfur. The inset table and (a) shows that the 6 atoms at the edges of the icosahedra vertices and middle 3 atoms ring are partially occupied by Ag or Au. Therefore, Ag
atoms selectively occupy the nine specific locations of the face shared bi-icosahedrons core. \( \text{Au}_{34.04}\text{Ag}_{3.96}(\text{SCH}_2\text{CH}_2\text{Ph})_{24} \) is the average structure obtained from many \( \text{Au}_{38-x}\text{Ag}_x(\text{SR})_{24} \) molecules within the single crystal. The Ag atoms were distributed in face shared bi-icosahedral vertex atoms, with significant preference for sites 4, 5, 6, 33, 34, 35 when compared with 20, 21 and 22.

The two central atoms in the bi-icosahedral core were shown to be exclusively Au, as shown in Figure 4.4. Within the \( \text{Au}_{21} \) core, the silver atoms preferentially occupy nine selected locations; (a) the two vertex edges–three atoms each and six atoms total and (b) the middle face-shared three-atom ring, adding to a total of nine locations. In other words, among the 21 atoms in the vertices of the face shared bi-icosahedral \( \text{Au}_{23-x}\text{Ag}_x \) core, 9 selected sites show dual occupancy, as shown by gray colored atoms in figures 4.4a, b and c. The nine dual occupancy sites are 4, 5, 6, 20, 21, 22, 33, 34, and 35. Dual occupancy means that the same site is occupied by either Au or Ag in different molecules of the crystal. For example, site 4 was occupied by Ag or Au, with Ag atom being present 45.8% of the time. This is denoted as \( \text{Ag}_4/\text{Au}_4–45.8\%/54.2\% \), as percentages in Figure 4.4 inset table. The actual ratio of Ag/Au atoms with the corresponding ESDs are: \( \text{Ag}_4/\text{Au}_4–0.458(10)/0.542(10), \text{Ag}_5/\text{Au}_5–0.498(10)/ 0.502(10), \) \( \text{Ag}_6/\text{Au}_6–0.517(10) /0.483(10), \) \( \text{Ag}_20/\text{Au}_20– 0.277(10) / 0.723(10), \) \( \text{Ag}_21/\text{Au}_21 – 0.385(10)/0.615(10), \) \( \text{Ag}_22/\text{Au}_22 –0.352(10)/ 0.648(10), \) \( \text{Ag}_33/\text{Au}_33–0.506(10)/0.494(10), \) \( \text{Ag}_34/\text{Au}_34–0.452(10) / 0.548(10), \) and \( \text{Ag}_35/\text{Au}_35–0.515(10)/0.485(10). \) However, when the diffraction data for the entire crystal is acquired, the reflections are for the average structure, which contains contributions from both Au and Ag atoms for the same site. From above analysis, the average bimetallic alloy core composition can be formulated as \( \text{Au}_{19.04}\text{Ag}_{3.96} \). The metal atoms in both the monomeric and dimeric units are also exclusively Au.
In the 38-atom structure, the Ag atoms occupy the vertices of the icosahedral core instead of the center or the protecting units, as was the case in \( \text{Au}_{25-x}\text{Ag}_x(\text{SCH}_2\text{CH}_2\text{Ph})_{18} \). \(^5\) \( \text{Au}_{38-x}\text{Ag}_x(\text{SR})_{24} \) synthesis shows that the average Ag incorporation reaches a plateau near six atoms in both 1:0.20 and 1:0.30 Au:Ag incoming molar ratio and reached the maximum of 9 silver atoms. This is in good agreement with the crystallography data, which exhibit the possible 9 locations for silver atom incorporation. When the Au:Ag ratio in the synthesis was increased beyond 1:0.30, more Ag atoms were incorporated, but the samples decomposed on thermochemical treatment. The structural information presented here suggests that the stability is structure related as shown in the figure 4.6 and related text. The geometry of the nanomolecules (icosahedron vs. face shared bi-icosahedrons) may also directly affect the Ag dopant location of the nanomolecules.

There is concerted disorder in the structure resulting in significant isotropic displacement parameters in the phenylethandithiol groups. Significant efforts were made to improve the resolution and data quality. Data collection on various crystals have attempted and the best data set resulted in an \( R_1 \) value of 4.92, with a resolution of 0.94 Å. Diffraction data beyond this limit was observed; however, the inherent disorder in the structure results in weak and diffuse data with poor statistics in this range. Thus, this structure solution and resolution was used for further analysis. A total of 38 metal atom sites, 24 sulfur atoms with 24 complete \(-\text{CH}_2\text{-CH}_2\text{-Ph}\) carbon frameworks of the ligands were determined successfully here via structure solution and least-square refinements. There is a positional disorder in the alloy structure. The thiol groups attached to the S18 and S21 atoms show positional disorder over two positions with a major component contribution of 53.8(8)% for each.
Figure 4.5 MALDI–MS spectra of Au$_{38-x}$Ag$_x$(SR)$_{24}$ used for crystallization. The spectrum shows that the sample are actually a mixture of Au$_{37}$Ag$_1$(SR)$_{24}$, Au$_{36}$Ag$_2$(SR)$_{24}$, Au$_{35}$Ag$_3$(SR)$_{24}$, Au$_{34}$Ag$_4$(SR)$_{24}$, and Au$_{33}$Ag$_5$(SR)$_{24}$. The average composition was calculated from the peak height of each species and found to be Au$_{35.0}$Ag$_{3.0}$(SCH$_2$CH$_2$Ph)$_{24}$. The inset shows the expansion of the sample in 4-40 kDa mass range. Table show the composition determination by mass spectrometry.

Table 4.1 Composition of Au$_{38-x}$Ag$_x$(SR)$_{24}$ Determination by Mass Spectrometry

<table>
<thead>
<tr>
<th>Nanomolecules</th>
<th>Ag percent of total</th>
<th>Contribution of silver from each species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au$_{37}$Ag$<em>1$(SR)$</em>{18}$</td>
<td>7.7</td>
<td>0.08</td>
</tr>
<tr>
<td>Au$_{36}$Ag$<em>2$(SR)$</em>{18}$</td>
<td>25.8</td>
<td>0.52</td>
</tr>
<tr>
<td>Au$_{35}$Ag$<em>3$(SR)$</em>{18}$</td>
<td>36.0</td>
<td>1.08</td>
</tr>
<tr>
<td>Au$_{34}$Ag$<em>4$(SR)$</em>{18}$</td>
<td>23.4</td>
<td>0.94</td>
</tr>
<tr>
<td>Au$_{33}$Ag$<em>5$(SR)$</em>{18}$</td>
<td>7.0</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Total number of Ag determined by mass spectrometry $2.97=\sim3.0$

MALDI-MS is widely used to determine the composition of gold nanomolecules,$^3$ analyze a mixture of nanoparticles$^8$ and for monitoring of ligand exchange reactions.$^{86,87}$ Capabilities of MALDI extend to the qualitative and quantitative investigation of ultra-small
alloy composition.\textsuperscript{4, 6} Figure 4.5 shows the MALDI mass spectrum of the initial sample used for crystallization. The percentages of each of these species were calculated from the peak heights corresponding to each species in the mass spectrum as shown in the Table 1.1.

For each $\text{Au}_{38-x}\text{Ag}_x(S\text{CH}_2\text{CH}_2\text{Ph})_{24}$ species in the crystal, the composition is one of these species: $\text{Au}_{37}\text{Ag}_1(S\text{R})_{24}$, $\text{Au}_{36}\text{Ag}_2(S\text{R})_{24}$, $\text{Au}_{35}\text{Ag}_3(S\text{R})_{24}$, $\text{Au}_{34}\text{Ag}_4(S\text{R})_{24}$, and $\text{Au}_{33}\text{Ag}_5(S\text{R})_{24}$ with relative abundance of 7.7%, 25.8%, 36.0%, 23.4% and 7.0% respectively, as shown in the MALDI-MS spectrum. Then, the relative silver contributions from each species to the total were calculated. The peaks corresponding to $\text{Au}_{37}\text{Ag}_1(S\text{R})_{24}$, $\text{Au}_{36}\text{Ag}_2(S\text{R})_{24}$, $\text{Au}_{35}\text{Ag}_3(S\text{R})_{24}$, $\text{Au}_{34}\text{Ag}_4(S\text{R})_{24}$, and $\text{Au}_{33}\text{Ag}_5(S\text{R})_{24}$ nanomolecules contain 0.08, 0.52, 1.08, 0.94 and 0.35 fractions of silver, respectively.\textsuperscript{88} Finally, the average composition was calculated to be $\text{Au}_{35.0}\text{Ag}_{3.0}(S\text{R})_{24}$. The purpose of mass spectrometry result is to independently verify the X-ray crystallographic composition. The differences may be due to the fact that MALDI-MS peak heights are correlated with ionization efficiencies of each species, and because the later measurements were performed on bulk samples, while crystallography was performed on one crystal. Preferential aggregation of certain $\text{Au}_{38-x}\text{Ag}_x(S\text{R})_{24}$ upon crystallization is also possible. On the other hand, according to the MALDI-MS data, Ag atoms variation in the sample is $3\pm2$ atoms. Thus, the composition and site occupancy may be specific to the particular single crystal used for diffraction measurement, although the deviation from a representative bulk sample should be minimal.
Figure 4.6 (Left) Anatomy of the Au$_{19.04}$Ag$_{3.96}$ alloy core (Selected color code alone with the dotted line represents the four different ring systems present in the structure. The central atoms in the bi-icosahedrons core are shown in red. Green and blue color atoms indicate the ring system 2,2’ and 3,3’ respectively. The fractionally occupied Au and Ag sites are indicated by gray color in ring 1, 4 and 1’. Two pseudo icosahedra ring hold together by atomic layer 4: base or connecting point of two icosahedra and atomic layer 2, 2’: base for the monomeric staples that hold the two icosahedra. (Right) Anatomy of Au$_{19.04}$Ag$_{3.96}$ alloy core shown in the top view (a, b, c). In b) a breakdown of the structure is shown by omitting some of the vertex atoms, so that the silver doping position can be observed clearly. The atomic layer denoted as 4 can be treated as backbone or base that hold two pseudo icosahedra ring together. d, e and f are side view of the silver preference sites.

Au$_{38-x}$Ag$_x$(SR)$_{24}$ (where x=1,2,3,4,5) nanomolecules shows extraordinary stability for prolong thermochemical treatment (2-3 days) at 80°C. Although further Ag incorporation is still possible, nanomolecules become unstable when X exceed more than nine and decompose upon
thermochemical treatment. Figure 4.6 represent the anatomy of the $\text{Au}_{23-x}\text{Ag}_x$ alloy core with different atomic ring systems and their interaction to the monomeric and dimeric staple unites. Atoms in the layer 1, 1’, 3 and 3’ act as base for the dimeric staple units while layer 2 and 2’ for that of monomeric staple units. According to the alloy crystal structure, monomeric staple units not directly associated with the silver atoms where as dimeric staple does. It is well known that stability of the thiolated gold nanoparticles is due to the protecting monolayer of the thiol group and strong Au-S bonding. The $\text{Au}_{23}$ core or two face shared bi-icosahedral structure are held together by (a) three gold atoms in the layer 4 (b) three monomeric staple motif connected to the atomic layer 2 and 2’. Ag incorporation leads to the weak bonding when compared to the Au-Au interaction. Absence of Ag occupancy at atomic layer 2 and 2’ imply that the stability of the $\text{Au}_{38-x}\text{Ag}_x(\text{SR})_{24}$ alloy more affected by factor (b). However, six dimeric staple units are associated with mixed Au/Ag sites (either Au or Ag in the layer 1 and 1’) and pure Au sites (in the layer 3 and 3’) at each end.

Significant Au/Ag-Au bond length deviation could not observed compared to the monometallic $\text{Au}_{38}(\text{SR})_{24}$ crystal structure. This may be due to the partial occupancy of Ag (maximum upto ~52%) and similar atomic radii (Au: 1.44 Å and Ag: 1.45 Å). However, within the crystal structure, Au/Ag-S bond length (bond length =2.411±0.008 Å) seem to be longer than the Au-S bond (bond length=2.388±0.018 Å). Note that the only the metal atoms in layers 1,1’, 3 and 3’ were considered for above calculation due to the atoms in the layer 2 does not contributed to the dimeric staple and atoms in the layer 4 does not involve in the Au/Ag-S bonding. Corresponding Au-S bond length for the monometallic nanomolecules is slightly shorter (2.369±0.019 Å) than bimetallic $\text{Au}_{38-x}\text{Ag}_x(\text{SR})_{24}$ nanomolecules.
4.7 Conclusion

Atomically precise Au_{38-x}Ag_x(SCH_2CH_2Ph)_24 alloy nanomolecules were synthesized and formula determined by MALDI-TOF mass spectrometry. Some optical features were diminished when alloying with silver. The crystal structure of the Au_{38-x}Ag_x(SR)_24 were analysis to investigate the silver atom incorporation sites. Samples were crystallized in triclinic P\textsuperscript{T} space group and structure refinement yield R\textsubscript{1} value of 4.92%. Silver atoms are found to be preferentially occupying nine selected locations. This structural study will be pave the way to fundamental understanding of the intrinsic chirality, racemization, catalytic active sites and stereo-selective catalysis of thiolated 38 metal atom alloy by knowing the active silver atom incorporation sites.

Crystal Data for C\textsubscript{192}H\textsubscript{216}Ag\textsubscript{3.96}Au\textsubscript{34.04}S\textsubscript{24} (M =10424.98 g/mol): triclinic, space group P-1 (no. 2), a = 18.8585(7) Å, b = 22.2711(8) Å, c = 29.2473(12) Å, α = 96.472(2)°, β = 106.952(3)°, γ = 111.124(3)°, V = 10629.0(7) Å\textsuperscript{3}, Z = 2, synchrotron (λ = 0.7749) T = 100(2) K, μ(synchrotron) = 29.751 mm\textsuperscript{-1}, Dealc = 3.257 g/cm\textsuperscript{3}, 58308 reflections measured (4.238° ≤ 2Θ ≤ 42.97°), 18653 unique (R\textsubscript{int} = 0.0741, R\textsubscript{sigma} = 0.1065) which were used in all calculations. The final R\textsubscript{1} was 0.0492 (I > 2σ(I)) and wR\textsubscript{2} was 0.1125. CCDC number is 1038715.
CHAPTER 5
SILVER ALLOY OF Au$_{144}$(SR)$_{60}$ WITH ENHANCED OPTICAL PROPERTIES

Part of the text and figures in this chapter are extracted from the following publication:

CHAPTER 5
SILVER ALLOY OF Au$_{144}$(SR)$_{60}$ WITH ENHANCED OPTICAL PROPERTIES

5.1 Abstract

Recent progress in high-resolution ESI–MS experiments enable the composition assignment of larger nanomolecules at high mass range of 30-36 kDa such as Au$_{130}$(SR)$_{50}$, Au$_{137}$(SR)$_{54}$ and Au$_{144}$(SR)$_{60}$. However, Au$_{144}$(SR)$_{60}$ is extensively studied due to its stability, interesting optical and electrochemical properties which lying between molecule to bulk transition. Au$_{144-x}$Ag$_x$(SR)$_{60}$ alloy nanomolecules were synthesized and characterized by ESI mass spectrometry to atomic precision. The number of Ag atoms could be varied by changing the incoming total metal ratio. Optical spectrum shows that the electronic structure of the nanomolecules can be modulate by silver atoms incorporation. The optical spectra of the Au$_{144-x}$Ag$_x$(SR)$_{60}$ nanoalloy with high silver contents shows distinct features at around 310, 430 and 560 nm. Syntheses with higher Au:Ag ratios like 1:1 or beyond not feasible for stable Au$_{144-x}$Ag$_x$(SR)$_{60}$ formation and decomposed upon thermochemical treatment. Maximum silver atom incorporation platues near 60 atoms. Based on the proposed 3-shell structure of Au$_{144}$(SR)$_{60}$, the Ag atoms may selectively incorporated into the symmetry equivalent 60-atom shell—having Au$_{12}$, Au$_{42}$, Ag$_{60}$ concentric shells with 30×(–SR–Au–SR–) protecting units.
5.2 Introduction

Ultra-small gold nanoparticles (<2 nm) are molecular in nature. They have unique molecular weights, physical and chemical properties.\textsuperscript{38} Typical examples of nanomolecules with specific number of gold atoms and organic thiol ligand include molecules such as \( \text{Au}_{25}(\text{SR})_{18} \), \( \text{Au}_{38}(\text{SR})_{24} \), \( \text{Au}_{102}(\text{SR})_{44} \) and \( \text{Au}_{144}(\text{SR})_{60} \).\textsuperscript{14, 24, 71} Metallic gold nanomolecules are attractive due to their chemical stability and the ability to characterize precisely using commercially available mass spectrometers and a variety of spectroscopic instruments.\textsuperscript{40, 91} \( \text{Au}_{25}(\text{SR})_{18} \) and \( \text{Au}_{144}(\text{SR})_{60} \) have been studied extensively due to their exceptional stability and optical properties.\textsuperscript{7, 92}

While monometallic nanomolecules are extremely stable, and are easily processed and characterized, alloys have unique advantages such as enhanced optical properties and catalytic activity.\textsuperscript{34} In terms of Au-metal containing alloy nanoparticles, Murray et al. synthesized a 3–5 nm monolayer protected metal alloy by doping Au with Ag, Cu and Pt.\textsuperscript{93} El-Sayed et al. showed the formation of 20 nm gold–silver alloy nanoparticles with different compositions by co-reduction of Au and Ag precursors in varying proportions with sodium citrate.\textsuperscript{11} Wilcoxon studied the optical properties of Au–Ag alloy nanoparticles and its composition dependence and size.\textsuperscript{94} These reports, mostly in the 3–20 nm size regime show that the Au–Ag nanomaterials are indeed alloys and that the position of the SPR band depends on the ratio of Au and Ag starting precursors.\textsuperscript{11, 94, 95} The size of these alloy are not atomically monodisperse and based on mean size determine by techniques such as \(^1\text{H} \) NMR line broadening, electron microscopy, SAXS techniques. Further, composition determinations are not atomically precise and mainly focus on techniques like EDS, XPS that may leaves ±1000 atoms uncertainly depend on size. The properties depend on composition and structure of the nanoparticles. Therefore, it is important to develop method for atomically monodisperse nanoparticles to directly address the structure and
property relationship.

Although Au$_{144}$(SR)$_{60}$ has been abundantly synthesized and extensively studies, the total-structure determination remained inconclusive due to the lack of diffraction quality single crystal. However, in 2009 Hannu and co-worker proposed multi-shell structure for Au$_{144}$(SR)$_{60}$ using Density-functional theory computations.$^7$ The proposed structure consists of three inner cores with 12, 42 and 60 atoms and outer shell with 30 monomeric staple units. This layer-by-layer arrangement of the Au$_{144}$(SR)$_{60}$ shown in the figure 5.1.

Figure 5.1 (A)–(C) show the concentric 12-atom (hollow), 42-atom and 60-atom Au shells of the 114-atom gold core, respectively. (D) Shows the S-Au-S arrangement of the 30×-RS-Au-SR-units covering the surface (blue) of the 114-atom gold core and (E) shows all the atoms. Color code in (D), (E): S: yellow, Au(I): orange, C: gray, H: white. Reprinted with permission from [7]. Copyright 2009 American Chemical Society

This chapter discusses the alloying of one of a large and highly stable 144 metal atom nanomolecules. Au$_{144-x}$Ag$_x$(SR)$_{60}$ alloy nanomolecules were synthesized and characterize by ESI mass spectrometry to the atomic precision. Silver substitution into the monometallic Au$_{144}$(SR)$_{60}$
does not depend linearly with the Ag ratio of the starting material. UV-visible spectroscopy shows the Ag incorporation affects the electronic structure of the nanomolecules. Ag incorporation into the Au$_{144}$ nanomolecules have discussed based on a proposed atomic structure.$^7$

5.3 Method

5.3.1 Synthesis

Synthesis of the Au$_{144-x}$Ag$_x$(SR)$_{60}$ involves three steps. The first step is the synthesis of crude product that contains polydisperse Au/Ag nanoparticles. The second step is the thermochemical treatment of the crude product with excess thiol to form Au$_{144-x}$Ag$_x$(SR)$_{60}$ alloy nanomolecules. Last step is the isolation of Au$_{144-x}$Ag$_x$(SR)$_{60}$ using solvent fractionation.

Step 1: Aqueous solution (30 mL) containing HAuCl$_4$ and AgNO$_3$ (total metal concentration was set to 30 mM) was mixed with toluene solution (30 mL) of tetraoctylammonium bromide, TOABr (1.1 mmol). The initial mole ratios of Au:Ag precursors were set to 1:0, 1:0.25, 1:0.50, 1:0.66 and 1:0.75. After stirring for 30 minutes, the turbid organic phase was separated and phenylethane thiol (10 mmol) was added and further stirred for 30 minutes at room temperature. This solution was cooled in ice bath for 30 minutes. Then, an aqueous solution of NaBH$_4$ (20 mmol, 20 mL) cooled to 0 $^\circ$C, was rapidly added to the reaction mixture under vigorous stirring. After 3 hours, the organic layer was separated from aqueous layer and evaporated to dryness. The product was washed with methanol to remove excess thiol, NaBH$_4$, TOABr and other by-products. The residual mixture was extracted with toluene. Smaller nanoparticles in the 7–10 kDa mass range were removed by CH$_3$CN extraction.

Step 2: A 20 mg of CH$_3$CN insoluble portion was dissolved in 0.50 mL of toluene and etched with excess phenylethane thiol (0.5 mL) at 80 $^\circ$C under stirring, while monitoring the MALDI-
MS. When pure $\text{Au}_{144-x}\text{Ag}_x(\text{SR})_{60}$ nanoalloy was formed (~3 hours), the reaction was stopped and washed with methanol and extracted with toluene.

Step 3: The toluene soluble fraction of the product was subjected to solvent fractionation using toluene/methanol mixture to isolate pure $\text{Au}_{144-x}\text{Ag}_x(\text{SR})_{60}$ alloy in pure form.

5.3.2 Characterizations

UV-visible spectra were obtained in dichloromethane solutions using Shimadzu UV-1601 spectrophotometer/UV probe 2.0 software in the 300–1200 nm range.

MALDI mass spectra were acquired with a Bruker Daltonics Autoflex mass spectrometer using DCTB matrix at optimal laser fluence. Positive and negative mode gave identical results. Spectral analyses were done using Bruker Daltonics flexAnalysis version 3.0. ESI mass spectra were obtained from a Waters Synapt mass spectrometer in 50:50, toluene:CH$_3$CN solution in negative mode. ESI calibration was performed with 50:50 isopropanol:water solution of NaI. Calibration check was performed with $\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}$ and $\text{Au}_{144}(\text{SCH}_2\text{CH}_2\text{Ph})_{60}$.

5.4 Results and discussion

5.4.1 Qualitative and quantitative determination of silver atom incorporation

Electrospray ionization mass spectra of the isolated nanomolecules show that the composition of the nanoalloy follows generic formula of $\text{Au}_{144-x}\text{Ag}_x(\text{SR})_{60}$ and the Au and Ag content varies with the incoming molar ratio of HAuCl$_4$ and AgNO$_3$. Figure 5.2 shows the mass region focused on the 3- ions of the nanomolecules that were predominant in the ESI experiments. The Bottom black curve shows the monometallic $\text{Au}_{144}(\text{SR})_{60}$ that has extraordinary stability among various monometallic nanomolecules.
Figure 5.2 (Left) Electrospray ionization (ESI) mass spectrometry (3- ions) of Au$_{144-x}$Ag$_x$(SR)$_{60}$ nanomolecules for Au:Ag precursor ratios of 1:0 (black), 1:0.25 (red), 1:0.50 (blue), 1:0.66 (olive) and 1:0.75 (orange) in the starting material. The mass difference between the peaks in nanoalloys corresponds to the Au (196.97 Da) and Ag (107.87 Da) mass difference, $\Delta m = 89$ Da. Average number of Au and Ag atoms is denoted above each distribution of peaks. (Right) MALDI-TOF mass spectra (positive mode) of Au$_{144-x}$Ag$_x$(SR)$_{60}$ nanomolecules for Au:Ag precursor ratios of 1:0 (black), 1:0.25 (red), 1:0.50 (blue), 1:0.66 (olive) and 1:0.75 (orange) in the starting material. The 3- ESI mass spectra was deconvoluted and plotted as 1- peaks in grey. The MALDI peaks are significantly broader and slightly lower in mass than ESI peaks due to fragmentation from the loss of ligand.

Incorporating silver precursor, AgNO$_3$ in the synthesis led to the formation of Au$_x$Ag$_y$(SR)$_{60}$ nanoalloys while the core size was maintained at 144 total atoms, i.e., $x+y =144$. The distribution of peaks is due to the different number of Au and Ag atoms as denoted by the 89
Da mass difference between the peaks ($\text{Au} = 196.97 \text{ Da}, \text{Ag} = 107.87 \text{ Da}, \Delta m = 89.1 \text{ Da}$). The fraction of Ag atoms in the nanoalloy product does not reflect the mole fraction of the precursor starting materials, HAuCl$_4$ and AgNO$_3$ as discussed later. The silver fraction in the nanoalloy seems to plateau at 37% for the 1:0.75 Au:Ag incoming mole ratio. Experiments at higher silver ratios (e.g., 1:1, Au:Ag) do not lead to stable Au$_{144-x}$Ag$_x$(SR)$_{60}$ alloy formation. Synthesis from 1:0.25, Au:Ag mole ratio also form Au$_{145-x}$Ag$_x$(SR)$_{59}$ alloys species as a minor fraction. The distribution of silver substituted Au$_{144-x}$Ag$_x$(SR)$_{60}$ alloy species reminiscent the binomial distribution found in ligand exchange experiments reported earlier. 

MALDI-TOF mass spectrometry easily ionizes a variety of analyte especially at high laser fluence. This makes MALDI data more suitable for mixtures and hence facilitates attaining a more accurate representation of the nanomaterial composition. Ionization in electrospray can be selective depending on the spray conditions. Figure 5.2 (right) shows the MALDI-MS data of the Au$_{144-x}$Ag$_x$(SR)$_{60}$ nanomolecules and confirms their purity and monodispersity. The 3- ESI peaks were deconvoluted to 1- ions (grey peaks) and plotted along with MALDI 1+ ions as shown in Figure 5.2(Right). The MALDI peaks are significantly broader and slightly lower in mass due to fragmentation from the loss of ligands by the breakage of S–C bonds.

5.4.2 Tuning the electronic structure of the nanoalloys

Optical properties of nanoalloys including Au/Ag have been intensely studied especially the effect of the proportion of Ag metal on the surface plasmon resonance (SPR) peak, where the core sizes of nanoalloys are in the 5–50 nm diameter range. Nanoparticles that are less than 2 nm in diameter show distinct features instead of SPR peaks, due to quantum size effects. These features are size dependent and are less distinct with larger sizes such as Au$_{144}$ before the emergence of an SPR peak for nanoclusters around 5 nm in diameter. While the optical spectrum
of monometallic Au$_{144}$(SR)$_{60}$ nanomolecules is relatively monotonous, Ag incorporation affects the electronic structure and leads to a 425 nm peak reminiscent of the silver SPR band and broad shoulders at 560 and 310 nm (figure 5.3). Au$_{144-x}$Ag$_x$(SR)$_{60}$ alloy nanomolecules can be synthesized with various protecting thiol groups such as phenylethane thiol, dodecane thiol and hexane thiol, though the pK$_a$ of these thiols should be very similar. Figure 5.2 (right) demonstrates the reproducibility of the Au$_{144-x}$Ag$_x$(SR)$_{60}$ nanoalloy formation during the synthesis.

Figure 5.3: (Right) UV-visible spectra of Au$_{144-x}$Ag$_x$(SR)$_{60}$ nanomolecules in CH$_2$Cl$_2$ for Au:Ag precursor ratios of 1:0 (black), 1:0.25 (red), 1:0.50 (blue), 1:0.66 (olive) and 1:0.75 (orange) in the starting material. (Left) UV-visible spectra of Au$_{92}$Ag$_{52}$(SR)$_{60}$ nanomolecules for Au:Ag precursor ratios of 1:0.66 with phenylethanethiol (dotted), hexanethiol (dashed), and dodecanethiol (solid).

The structure arrangement of atoms in Au$_{144}$(SR)$_{60}$ most likely is similar to the experimental XRD crystal structure of Pd$_{145}$(CO)$_x$(PEt$_3$)$_{30}$.$^7$,$^{99}$,$^{100}$ The proposed Au$_{144}$(SR)$_{60}$ structure shows a good fit with the experimental X-ray scattering data. The relaxed structure
shows three concentric shells of 12, 42 and 60 atoms protected by $30 \times (\text{--SR--Au--SR--})$ staple groups. As the silver mole ratio is increased in the starting material, the number of Ag atoms in the $\text{Au}_{144-x}\text{Ag}_x(\text{SR})_{60}$ alloy increases as expected. However, the Ag atoms seem to plateau around 1:0.66, Au:Ag incoming mole ratio. The average and maximum number of Ag atoms in both the 1:0.66 and 1:0.75 ratios are 52 and 60 respectively. Based on the crystal structure prediction, there are two possible options for Ag atom incorporation (1) The Ag atoms are present both in the staple units as $\text{--SR--Ag--SR--}$ and partially occupy at the equivalent 60 atom shell (2) The staple units have Au atoms, but Ag atoms are only exclusively incorporated into the equivalent 60 atom shell. Case 1 cannot account for the plateau of 60 Ag atoms, while case 2 does. Synthesis using a high Ag precursor ratio such as 1:1, Au:Ag mole ratio leads to unstable clusters in the 144 atom region.

Motivated by $\text{Au}_{144-x}\text{Ag}_x(\text{SR})_{60}$ alloy report, Häkkinen and coworkers studies these system with their proposed $\text{Au}_{144}(\text{SR})_{60}$ model. They implement DFT calculations to examine the structures of several $\text{Au}_{144-x}\text{Ag}_x(\text{SR})_{60}$ nanomolecules where $x = 0, 30, 54,$ and 60. Figure 5.4 structure 1 shows the optimized structure of monometallic $\text{Au}_{144}(\text{SR})_{60}$. Systems 2-5 represent the optimized alloy nanomolecules where silver distribute over different locations. For examples, system 2 represent a $\text{Ag}_{54}\text{Au}_{60}(\text{RSAuSR})_{30}$, where the silver atoms localized into inner 54-atom metal core and structure 3 for $\text{Au}_{54}\text{Ag}_{60}(\text{RSAuSR})_{30}$, where the 60 Ag atoms are randomly distributed over 114 core. System 4 denote $\text{Au}_{54}\text{Ag}_{60}(\text{RSAuSR})_{30}$, where the 60 Ag atoms concentrated to 60 atom shell and structure 5 for $\text{Au}_{114}(\text{RSAgSR})_{30}$, where 30 silver atoms are in the staple motif. The calculated formation energies ($E_{\text{form}}$) for the different systems show in the graph 5.4(left) and result shows the minimal $E_{\text{form}}$ for the structure 4. According to the theory, energetically optimal structure will have the lowest (positive) formation energy. DFT
calculations imply that the nanomolecules with Ag substituted to inner 54-atom icosahedrons and monomeric staples are energetically less stable than monometallic Au$_{144}$(SR)$_{60}$. Lowest $E_{\text{form}}$ value for system 4 imply that the alloying prefer segregation or separate shells inside the core rather than random distributions. Experimental investigation of atomic structure of Au$_{144-x}$Ag$_x$(SR)$_{60}$ has been hindered by lack of diffraction quality single crystals.

Figure 5.4: (left) Optimized structures of the considered clusters 1–5 of composition Au$_{144-x}$Ag$_x$(SH)$_{60}$. brown, gold; shiny gray, silver; yellow, sulfur. The hydrogen atoms are not shown for clarity. (Right) Formation energy per metal atom, $E_{\text{form}}$, of clusters 1–5. The smallest value indicates optimal formation of cluster 4. Reprinted with permission from [23]. Copyright 2011 American Chemical Society.
5.5 Conclusion

Series of $\text{Au}_{144-x}\text{Ag}_x(\text{SR})_{60}$ alloy nanomolecules were synthesized and characterized by optical spectroscopy and ESI mass spectrometry. Substituted silver atoms were determined qualitatively and quantitatively using the mass difference between gold and silver. In the $\text{Au}_{144-x}\text{Ag}_x(\text{SR})_{60}$ alloy systems, the maximum number of Ag atoms substitution found to be $x = 60$. Syntheses with higher ratios (Au:Ag, 1:1 and beyond) did not produce stable $\text{Au}_{144-x}\text{Ag}_x(\text{SR})_{60}$. Optical features enhanced upon silver substitution and exhibit a “plasmonic-like” peak around 430 nm and two shoulders around 310 and 560 nm. Computational modeling of these ultra-small alloy systems provides insights into the stability and energetically favorable position for atom incorporation. $\text{Au}_{144-x}\text{Ag}_x(\text{SR})_{60}$ alloys has greater potential for interesting catalytic applications compared to analogous monometallic nanomolecules.
CHAPTER 6

ATOMICALLY MONODISPERSE PLASMONIC Au$_{329}$(SR)$_{84}$ NANOMOLECULES AND ITS ALLOY

Part of the text and figures in this chapter are extracted from the following publication:


CHAPTER 6
ATOMICALLY MONODISPERSE PLASMONIC \text{Au}_{329}(SR)_{84} \text{ NANOMOLECULES AND ITS ALLOY}

6.1 Abstract

Chemical composition of the plasmonic gold nanomolecules at 76.3 kDa were determined to the atomic precision. Electrospray ionization (ESI) Q-TOF mass spectrometry of the 76.3 kDa nanoparticles using three different thiols yield the 329:84 gold to thiol compositional assignment. Positive and negative mode ESI-MS spectra show identical peaks denoting that they are no counter ions, further reinforcing the accuracy of the assignment. Cs$^+$ ions were intentionally added to show that the \text{Au}_{329}(SR)_{84} is the base molecular ion, with several Cs$^+$ adducts. A comprehensive investigation including analysis of the compound with three ligands, in positive and negative mode and Cs$^+$ adduction leads to a conclusive composition of \text{Au}_{329}(SR)_{84}. This formula determination will facilitate the fundamental understanding of emergence of surface plasmon resonance in \text{Au}_{329}(SR)_{84} with 245 free electrons.

Then comprehensive characterization of the \text{Au}_{329}(SR)_{84} were present. The size homogeneity was examined by mass spectrometry, electron microscopy and X-ray scattering experiments. \text{Au}_{260} truncated octahedral atomic arrangement were proposed for the core, based on experiments and modeling of a total scattering based atomic pair distribution function analysis. HAADF-STEM images show fcc-like 2.0±0.1 nm diameter nanomolecules.
Finally, synthesis and characterization of largest atomically precise $\text{Au}_{329-x}\text{Ag}_x(\text{SCH}_2\text{CH}_2\text{Ph})_{84}$ alloy nanomolecules are discussed. Alloy composition was determined using high resolution ESI-MS. In contrast to larger (>10 nm) Au/Ag nanoparticles, no major SPR peak shifts observed upon silver incorporation. The intensity of SPR peak also varies in an intriguing manner, where a dampening is observed with medium silver incorporation, and a significant sharpening is observed upon higher Ag content. The content outlines (a) an unprecedented advance in nanoparticle mass spectrometry of high mass at atomic precision and (b) the unexpected optical behavior of Au/Ag alloys in the region where nascent SPR emerges.

**Author Contributions**

Chanaka Kumara synthesized $\text{Au}_{329}(\text{SR})_{84}$ and characterized by mass spectrometry. Both Dr. Amala Dass and Chanaka analyzed the PDF data. STEM image were acquired and analyzed by Dr. David Cullen at Oak Ridge National Laboratory. SAXS data were acquired and analyzed by Dr. Xiaobing Zuo at Argonne National Laboratory.
6.2 Introduction

Gold nanomolecules are ultra-small gold nanoparticles (< 3 nm) with a precise number of metal atoms and organic thiolate groups. They display interesting optical, magnetic, electrochemical properties with applications in catalysis, sensing and biomedical devices. Typical examples include \( \text{Au}_{25}(\text{SR})_{18} \), \( \text{Au}_{36}(\text{SR})_{24} \), \( \text{Ag}_{44}(\text{SR})_{30} \), \( \text{Au}_{67}(\text{SR})_{35} \), and \( \text{Au}_{144}(\text{SR})_{60} \). In 2011, Dass et al reported a larger nanomolecule with 76.3 kDa mass with ~320 gold atoms that is the smallest Au nanoparticles to support the plasmon resonance. One of the main objective of this work is to determine the elemental composition of the 76.3 kDa nanomolecule, that is to establish a molecular formula in the form of \( \text{Au}_x(\text{SR})_y \).

Mass spectrometry (MS) is the most common method to study the composition of thiolated gold nanomolecules. This is due in part to (a) the ability to analyze mixtures (b) the ability to use commercially available mass spectrometers and (c) the simple and robust nature of the sample preparation and analysis. Whetten’s seminal report in 1996 on LDI-MS of Au nanoparticles, partly inspired by MS of fullerenes provided conclusive information on the mass of the Au core, that is \( \text{Au}_x \) (where \( x \) is number of gold atoms). But the organic ligands underwent extensive fragmentation hindering full compositional assignment in the form of \( \text{Au}_x(\text{SR})_y \), where \( y \) is number of ligand attached. The Murray group in 2007 reported two major advances in nanoparticles MS, that transformed the field by facilitating the formation and detection of fully intact molecular nanoparticle ions. The first advance deals with electrospraying molecular ions in an organic solvent, with and without the presence of counter ions such as \( \text{Cs}^+ \). The second deals with the generation of intact ions in MALDI-TOF using aprotic DCTB matrix. Together these two methods with minimal modification, account for the
majority of today’s nanoparticle literature worldwide, for intact nanoparticle identification. MS methods successfully applied, including MALDI, ESI and FAB ionization, to study the synthesis,\textsuperscript{19, 108, 109} ligand exchange,\textsuperscript{74, 110, 111} dithiol crosslinking,\textsuperscript{86} fragmentation pattern,\textsuperscript{22} ion-mobility MS\textsuperscript{112, 113} and identity of several gold,\textsuperscript{16, 65, 85, 89, 114, 115} silver\textsuperscript{31, 116} and Au-metal alloy\textsuperscript{4, 6, 36, 70, 117} nanomolecules.

As the size of the nanomolecule increases, the ionization and detection of intact molecular ions become increasingly difficult. The large size, in addition to issues of adduct formation due to counter ions makes the assignment of larger (>200 atom, >40 kDa) nanomolecules non-trivial. Therefore, recent work\textsuperscript{16} in 2011 simply identified the Faradaurates, which is a plasmonic nanomolecule, as having a mass of 76.3 kDa. In early 2012, Jin’s group independently reported\textsuperscript{118} on a nanoparticle of nearly identical mass, ~76 kDa. Based on ESI experiments performed in the presence of Cesium acetate salt, they assigned its composition as Au\textsubscript{333}(SCH\textsubscript{2}CH\textsubscript{2}Ph)\textsubscript{79}.

The first part of the chapter discussed the composition assignment of the 76.3 kDa nanomolecules using ESI-Q-TOF MS. Synthesizing the nanomolecules with three different thiols, the Au\textsubscript{329}(SR)\textsubscript{84} composition were obtained using mass difference method. After establish the composition, further experiment were progresses for comprehensive characterization using synchrotron X-ray total scattering based atomic PDF method, small angle X-ray scattering and aberration-corrected HAADF-STEM images to extract size, shapes, size dispersity and atomic structure information. Comparing the experimental PDF data with simulations show that the structural arrangement is fcc-like, with either a truncated octahedral (TO) or cuboctahedral (CO) core.
The later part of the chapter describe the silver atom substitution to the \( \text{Au}_{329}(\text{SCH}_2\text{CH}_2\text{Ph})_{84} \). Series of silver incorporated \( \text{Au}_{329-x}\text{Ag}_x(\text{SCH}_2\text{CH}_2\text{Ph})_{84} \) alloy nanomolecules/ nanoparticles were synthesized by varying the incoming Au:Ag molar ratio in the initial synthesis. Non-linear dependence of SPR peak intensity with increasing Ag content were observed in contrast to the larger Au-Ag alloy nanoparticles. This is the first instance in which ESI-MS mass spectrometry was employed to determine composition of bimetallic plasmonic nanoparticles to the atomic level.

Scheme 6.1 Compositional determination and mass spectrometric methods used in the study.

6.3 Methods

6.3.1 Synthesis

Synthesis of the \( \text{Au}_{329}(\text{SR})_{84} \) and \( \text{Au}_{329-x}\text{Ag}_x(\text{SR})_{84} \) involves three steps. The first step is the synthesis of crude product that contains polydisperse nanoparticles according to a modified version of the recent report.\(^{16}\) The second step is the thermochemical treatment of the crude
product with excess thiol to remove meta-stable clusters. The final step is the solvent fractionation to isolate mono and bimetallic nanomolecules.

Step 1: An aqueous solution (30 mL) containing HAuCl$_4$ (0.9 mmol/ 0.3566 g) was mixed with toluene solution (30 mL) of tetraoctylammonium bromide, TOABr (1.1 mmol/ 0.6050 g). For Au$_{329-x}$Ag$_x$(SR)$_{84}$ alloy synthesis both HAuCl$_4$ and AgNO$_3$ (total metal concentration was set to 15 mM) was mixed in the initial synthesis. (The initial molar ratios of Au:Ag precursors set to 1:0, 1:0.01, 1:0.05, 1:0.10, 1:0.20, 1:0.33, 1:0.50, 1:0.66 and 1:1). After stirring for 30 minutes, the organic phase was separated and phenylethane thiol (0.90 mmol) was added and further stirred for 30 minutes at room temperature (gold to thiol molar ratio was set to 1:1). Note that the 1:1 Au to thiol ratio is important in the formation of the 76.3 kDa species. The 1:1 ratio is different than the 1:3 Au to thiol ratio commonly used to prepare smaller <200 atom nanomolecules. This solution was cooled in an ice bath for 30 minutes. An aqueous solution of NaBH$_4$ (20 mmol, 20 mL) cooled to 0°C, was rapidly added to the reaction mixture under vigorous stirring. After 3 hours, the organic layer was separated and evaporated to dryness. The product was washed with methanol to remove other byproducts. The residual mixture was extracted with toluene. Step 2: Then, ~100 mg of crude products was dissolved in 1 mL of toluene and subjected to thermochemical treatment with excess phenylethanethiol (1 mL) at 80°C under stirring, while monitoring with MALDI MS. After the meta-stable clusters disappeared (~3h to 8 days), the thermochemical treatment was discontinued and the product mixture was washed with methanol several times. Step 3: Finally, the product was dissolved in toluene and subjected to solvent fractionation with toluene /MeOH mixture to isolate the pure 76.3 kDa gold or alloy nanomolecules.
Figure 6.1 (Left) MALDI-MS spectra of thermochemical treatment of crude product as a function of time. (Right) MALDI-MS spectra showing the solvent fractionation of thermochemically treated product to isolate $\text{Au}_{329}(\text{SR})_{84}$ nanomolecules.

6.3.2 Mass spectrometry

MALDI mass spectra were acquired with a Bruker Daltonics Autoflex 1 mass spectrometer using DCTB matrix$^{107}$ at optimal laser fluence. At least 500 individual spectra were collected and averaged. MALDI-MS spectral analyses were done using Bruker Daltonics flexAnalysis version 3.0. ESI mass spectra were obtained from a Waters Synapt HDMS in 50:50 toluene:CH$_3$CN or 50:50 THF:CH$_3$CN solvent mixture. ESI-MS spectra were collected over a 5 min time period and averaged to acquired data with best possible signal to noise ratio.
Calibration check was performed with \( \text{Au}_{25} (\text{SCH}_2\text{CH}_2\text{Ph})_{18} \) and \( \text{Au}_{144} (\text{SCH}_2\text{CH}_2\text{Ph})_{60} \).

6.3.3 Optical spectroscopy

UV-visible spectra were recorded in toluene solutions using Shimadzu UV-1601 spectrophotometer/UV probe 2.0 software in the 300–1100 nm range.

6.3.4 Scanning transmission electron microscopy analysis

STEM samples were prepared by drop casting a toluene solution of nanoparticles suspension onto lacey carbon films supported on 3 mm Cu grids. HAADF-STEM images were acquired in a JEOL JEM-2200FS with a CEOS aberration corrector on the probe-forming system. Images were analyzed using ImageJ software (version 1.46r).

6.3.5 Synchrotron base high energy X-ray and pair distributions functional analysis

The total scattering data were collected using synchrotron X-ray at beam line 11-ID-B at the Advanced Photon Source at Argonne National Laboratory. Samples loaded in polyimide tubing of ~1 mm diameter and large area detector (Perkin-Elmer a-Si) was combined with high energy X-rays (\( \lambda = 0.21218 \, \text{Å}, \, E \sim 58\text{keV} \)) to collect data to high values of momentum transfer (Q).

6.3.6 X-ray scattering analysis

The solution X-ray scattering measurements were performed at the 12-ID-B beam line of APS using 12 keV X-ray energy. The SAXS camera employed with Pilatus 2M detector and energy cut-off was set as 10 keV to eliminate possible fluorescence background. The beam size with 0.05 x 0.2 mm and exposure times of 1s used for the measurement. The data were fully corrected and background subtracted and finally reduced to intensity vs scattering vector (q) profiles.
6.3.7 Powder XRD analysis

Nanomolecules dissolved in CH$_2$Cl$_2$ and placed on a quartz plate over several time in order to make a thick layer of nanoparticles. Powder XRD patterns were recorded on Bruker D8-Focus XRD instrument. Angle varied from 2 to 150 degree and continues measurements were taken over night.

6.4 Determination of the chemical composition

Figure 6.2 (Left) ESI-MS (red) and MALDI-MS (blue) spectra of 76.3 kDa nanomolecules synthesized using HSCH$_2$CH$_2$C$_6$H$_5$ thiol. The lines at the top indicate the theoretical values for the 1, 2, 3, 4 and 5 charge states of Au$_{329}$(SCH$_2$CH$_2$Ph)$_{84}$. (Right) Deconvolution: deconvoluted 4- ions to the 1- molecular ion peak (blue). The 2-, 3- and 5- molecular ion peaks also can be deconvoluted to the 1- molecular ion peaks by multiplying the corresponding m/z value with the charge state.

Figure 6.2 shows the MALDI-TOF mass spectrum of the 76.3 kDa nanomolecule. There is a broad peak centered at ~76.3 kDa corresponding to 1+ molecular ion. The features are broad due to the inherent resolution limitations of the linear TOF tube in the MALDI instrument. ESI
Q-TOF MS of the samples showed multiply charged peaks at 38, 25, 19 and 15 kDa/z peaks corresponding to 2-, 3-, 4- and 5- ions respectively. The deconvolution of the 2-, 3-, 4- and 5- peaks all point consistently to the 76.3 kDa molecular ions. (Figure 6.2 left)

**6.4.1 Composition determination by varying the mass of the ligand**

An independent method to determine the chemical composition involves the mass spectrometric measurement of the same nanomolecule with different stabilizing organic thiols. It is now generally recognized that for many stable nanomolecules, the same fixed composition Auₙ(SR)ₙ is obtained even when different thiol groups are used in the synthesis. For example, the Au₂₅(SR)₁₈, Au₃₈(SR)₂₄, Au₆₇(SR)₃₅ and Au₁₄₄(SR)₆₀ are obtained when the R group is varied to either –SCH₂CH₂Ph, -SC₆H₁₃ or –SC₁₂H₂₅. Therefore, if the same nanomolecule Auₙ(SR)ₙ is obtained using two different thiols, one can determine the number of ligands, y. From y, number of Au atoms, x can be back calculated, leading to the full elemental composition, Auₙ(SR)ₙ.

The nanomolecule was prepared using one of the three thiols each, namely –SCH₂CH₂Ph (137 Da), -SC₆H₁₃ (117 Da) or –SC₁₂H₂₅ (201 Da). Figure 6.3 (left) shows the MALDI-MS of these nanomolecules. All three MALDI spectra show one peak in the ~75 kDa region, corresponding to the 1+ molecular ion, indicating the purity of the sample. MALDI spectra show broad features when compared to the ESI mass spectrometry. This is due to the laser induced fragmentation occurring during the ionization process. This involves possible Au-S or S-C bond breaking within the Au₃₂₉(SR)₈₄ nanomolecules. The Au₃₂₉(SC₁₂H₂₅)₈₄ spectra on top actually shows a lower mass peak than other ligands, even though this is supposed to be heavier in mass.
This is due to the significant chemical differences between the three ligands. More specifically, the intermolecular interactions are dramatically enhanced in the long chain -SC₁₂H₂₅ ligand. This is distinctly manifest during the synthesis and processing of the SC₁₂H₂₅ protected title compound. The dodecanethiol ligand is waxy and very difficult to purify and process. The intermolecular interactions and the waxy nature of the ligand alone with the laser intensity significantly affects the matrix assisted laser desorption ionization process. This is the reason for the unusually broad feature for the MALDI spectra of the Au₃₂₉(SC₁₂H₂₅)₈₄ nanomolecules. Therefore, ESI-MS were recorded to determine the mass of the intact molecular ions without fragmenting.

Figure 6.3 (right) also shows the expansion of the 3- ions for ESI-MS of the Au₃₂₉(SR₂₅)₈₄ compound with the three thiols. From the mass difference of the three nanomolecules, and the mass difference of the ligand, the number of ligands, y, can be calculated. The number of ligands was calculated to be ‘84’ using the three independent mass differences a, b, and c, as shown in Figure 6.3. From the value of y=84, the number of Au atoms, x, was found to be 329. There are many literature precedents for using the difference in mass of ligands to accurately determine the compositional assignment. Many of these reports are independently verified later by complementary techniques such as thermogravimetric analysis and X-ray crystallography. Considering the signal of all three nanomolecules, the 3- ions were chosen for the compositional assignment.
Figure 6.3 Ligand count determination by synthesis of 76.3 kDa species using various ligands. (Left) MALDI-MS of the 76.3 kDa nanomolecule synthesized using dodecanethiol (olive), phenylethanethiol (blue) and hexanethiol (red); (Right) ESI-MS Q-TOF of 3- ions of the 76.3 kDa nanomolecules using three ligands. Assuming that the number of metal atoms is constant, for the 76.3 kDa nanomolecule for these three ligands (as in the case of Au$_{25}$(SR)$_{18}$, Au$_{38}$(SR)$_{24}$ and Au$_{144}$(SR)$_{60}$), the number of ligands is calculated by the mass difference of the intact ESI ions. The ligand count was found to be 84 in all cases, which then leads to the Au atom count of 329 atoms. Peaks marked by asterisk indicate remaining impurities not removed during purification. Dotted lines indicate the theoretical mass of the 3- ions of corresponding Au$_{329}$(SR)$_{84}$ nanomolecules. The mass error of the 3- ions of the nanoparticles synthesized using hexanethiol, phenylethanethiol and dodecanethiol was found to be 341, 118 and 96 ppm respectively.

Significant efforts were made to assign the ESI-MS peaks that are at lower mass than the
principal peaks. The minor peak to the right of the $\text{Au}_{329}(\text{SC}_2\text{H}_4\text{C}_6\text{H}_5)_{84}$ compound is closely related to the $\text{Au}_{329}(\text{SC}_2\text{H}_4\text{C}_6\text{H}_5)_{85}$ assignment and peak to the left is closely related with the assignment of $\text{Au}_{328}(\text{SC}_2\text{H}_4\text{C}_6\text{H}_5)_{84}$. Other lower mass peaks are less pronounced and were not observed repeatedly in the samples that were synthesized using three different thiols and in both positive and negative mode in MS analysis. Due to the low signal intensity and the lack of reproducible observations, it was not possible to assign these minor species. For smaller nanoparticles like $\text{Au}_{25}(\text{SR})_{18}$ and $\text{Au}_{38}(\text{SR})_{24}$ there exists only one main species. However, for larger nanoparticles, such as $\text{Au}_{144}$, there are reports on many closely related species such as $\text{Au}_{144}(\text{SR})_{59}$, $\text{Au}_{144}(\text{SR})_{60}$, $\text{Au}_{146}$ (SR)_{59} etc. However $\text{Au}_{144}(\text{SR})_{60}$ is the dominant species, and the others are present in very small amounts (<10%). Negishi showed that the existence of several possible configurations with the thiol protected $\text{Au}_{130}(\text{SR})_{50}$ and $\text{Au}_{187}(\text{SR})_{68}$ nanomolecules base on mass spectrometric analysis.\textsuperscript{89} Based on these previous observation on other sizes, it is possible to have many closely related species at 76.3 kDa range. Fragmentation or dissociative species were not observed in the control ESI-MS spectra of $\text{Au}_{144}(\text{SC}_2\text{H}_4\text{C}_6\text{H}_5)_{60}$ under same experimental conditions.

6.4.2 Interference from adducts, cations and anions

Easily misinterpreted mass spectral adduct peaks frequently arise due to the sum of the molecular ion of interest and some counter ion. For example, $\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}^-$ shows a peak at 7394 Da in the negative mode corresponding to the singly charged negative ion, $\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}^-$. However, the same sample in the positive mode yields a peak at 8326 Da, corresponding to $[\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}^-\cdot2(\text{N}(\text{C}_8\text{H}_{17}))_4]^+$. This is because the molecular ion with two tetraoctylammonium cations results in an adduct, whose overall charge is +1. Therefore, it is
critical to obtain the mass spectrum in both positive and negative mode to check for counter ions and adduct formation. Figure 6.4(Left) shows the $\text{Au}_{329}(\text{SCH}_2\text{CH}_2\text{C}_6\text{H}_5)_{84}$ nanomolecule in both positive and negative mode. Both positive and negative mode shows peaks at the same mass of 19.079 kDa in the 4- region. This suggest that the entire mass in the 19.079 kDa peak corresponds to Au atoms and organic ligands, and the absence of counter ions or adducts like $\text{N}^+(\text{C}_8\text{H}_{17})_4$, $\text{Cs}^+$ or $\text{Na}^+$. Despite the adduct formation in $\text{Au}_{25}(\text{SR})_{18}$, larger nanoparticles such as $\text{Au}_{67}$ and $\text{Au}_{144}$ show peaks in the same mass in both positive and negative modes. The observation of ions at identical mass in both positive and negative mode confirms that the entire mass of 19.079 kDa peak is due to only Au atoms and SR organic ligands. Hence, the $\text{Au}_{329}(\text{SR})_{84}$ assignment is not affected by counter ions such as $\text{N}^+(\text{C}_8\text{H}_{17})_4$, $\text{Cs}^+$ or $\text{Na}^+$. Figure 6.4 show the positive and negative mode ESI spectra of three different synthetic batches (CK2-118, CK2-108, CK2-120), that is a total of six representative spectra. These six spectra, all agree with the $\text{Au}_{329}(\text{SR})_{84}$ assignment and point towards the reproducibility of the data.

Figure 6.4 (Right) shows the deconvoluted spectra of 2-/+ 3-/+ 4-/+ and 5-/+ ions to the 1-/+ molecular ion. The good match among these peaks in agreement with the $\text{Au}_{329}(\text{SR})_{84}$ assignment adds confidence to the composition. The variation in the signal to noise ratio is due to the signal intensity variation at various mass ranges of the multiply charged peaks. In particular, the lower signal/noise for 2+/- and 5+/- peaks is due to the low signal for these peaks in the original spectrum.

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Figure 6.4 (Left) Positive (red) and Negative (blue) mode ESI Q-TOF mass spectra of Au$_{329}$(SCH$_2$CH$_2$C$_6$H$_5$)$_{84}$ nanomolecules of three different synthetic batches (CK2-118, CK2-108 and CK2-120). (B) Expansion of the 4- ESI peak region of Au$_{329}$(SCH$_2$CH$_2$C$_6$H$_5$)$_{84}$. The spectra shows that all the samples in both positive and negative mode show the same peak, denoting the absence of adducts (counter ions, Na$^+$ or Cs$^+$ ions). (Right) Deconvolution of 2-, 3-, 4-, 5-, 2+, 3+, 4+ and 5+ ions to the corresponding 1-/+ molecular ions, adding confidence to the Au$_{329}$(SCH$_2$CH$_2$C$_6$H$_5$)$_{84}$ assignment. (the number in the parenthesis represent the m/z values of the particular multiply charge species and it’s deconvoluted mass value). Dotted line shows the theoretical value (76329 Da) of the Au$_{329}$(SCH$_2$CH$_2$C$_6$H$_5$)$_{84}$ nanomolecules.

Cesium acetate is commonly added to the nanoparticle mass spectrometric analyses to facilitate the formation of ions by Cs$^+$ adduction. This was first reported by the Murray group, where Li$^+$, Na$^+$, Rb$^+$ and Cs$^+$ adducts of Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$ were observed in ESI-MS$^{120}$ In general, an envelope of nanoparticles corresponding to Au$_{25}$(SR)$_{18}$$\cdot$nCs$^+$, where n = 0, 1, 2, 3, can be observed. Subsequently, Cs$^+$ adducts of other nanoparticles that were not easily ionized in
their native state were reported.\textsuperscript{92, 121,118} The Cs\textsuperscript{+} adduction improves the signal by enhancing ionization and works well for previously known or well studied species.\textsuperscript{92, 121} Nevertheless, when applying this to a new nanomolecule with previously unknown composition can be complicated due to the difficulty in identifying the molecular ion without any cesium.

Figure 6.5 \(\text{Au}_{329}(\text{SCH}_2\text{CH}_2\text{C}_6\text{H}_5)_{84}\) with negative mode (NV, red), positive mode (PV, blue), in the presence of cesium acetate (PV, olive) and further addition of cesium acetate (PV, purple). The positive and negative spectra shows the 4\textsuperscript{+} and 4\textsuperscript{-} molecular ions respectively, while the addition of cesium acetate leads additional peaks due to the formation of Cs\textsuperscript{+} adducts, such as \(\text{Au}_{329}(\text{SCH}_2\text{CH}_2\text{C}_6\text{H}_5)_{84}\text{Cs}^+\), \(\text{Au}_{329}(\text{SCH}_2\text{CH}_2\text{C}_6\text{H}_5)_{84}\text{Cs}^+\text{Cs}^+\), \(\text{Au}_{329}(\text{SCH}_2\text{CH}_2\text{C}_6\text{H}_5)_{84}\text{Cs}^+\text{Cs}^+\text{Cs}^+\). Further addition of Cs\textsuperscript{+} acetate leads to adducts with 4, 5, 6 and 7 Cs\textsuperscript{+} ions.

However, to compare with other literature work, cesium was added intentionally to show that cesium adduction can be observed. Envelope of peaks were observed corresponding to \(\text{Au}_{329}(\text{SR})_{84}\text{nCs}^+\), where n = 0, 1, 2 and 3 as can be seen in the figure 6.5. The addition of more
cesium completely shifts the envelope to even higher mass. Both mass difference method and Cs$^+$ ions addition experiment shows the formula to be 329:84. Another report on a similar sized plasmonic nanoparticle has assigned this nanoparticle to Au$_{333}$(SCH$_2$CH$_2$Ph)$_{79}$·nCs$^+$, using cesium acetate to promote ionization.\textsuperscript{118} This result in an envelope of peaks as expected with many cesium adducts. If the number of cesium ions is not accurately assigned, then the ions with Cs$^+$ adduct can lead to erroneous assignment. It remains to be seen if the Au$_{333}$(SCH$_2$CH$_2$Ph)$_{79}$ assignment could be reproduced without Cs$^+$ ions and obtain the same composition. This will help in determining if there are two closely related species, namely, Au$_{329}$(SR)$_{84}$ and Au$_{333}$(SR)$_{79}$, or if the Au$_{333}$(SR)$_{79}$ composition needs to be corrected to properly account for the number of Cs$^+$ ions.

\textbf{6.5 Optical properties}

The UV-visible spectra of Au$_{329}$(SR)$_{84}$ nanomolecules synthesized using three different thiols (R groups) shows plasmonic band at around 495 nm. For a neutral charge state, the number of free electrons was calculated to be 245 (329-84). There must be threshold number of free electrons before the surface plasmon resonance (SPR) can be supported due to the collective oscillation of conduction electrons. This threshold value is apparently reached or exceeded at 245 free electron counts, and maybe crucial in determining if a certain size would support SPR. The optical spectrum of smaller nanomolecules, Au$_{144}$(SR)$_{60}$ and Au$_{187}$(SR)$_{68}$ shows discrete electronic states, having 84 and 119 free electron respectively. The newly determined 329:84 formula reveals that 245 free electrons are adequate to interact with incident radiation to support surface plasmon resonance. This is of great interest in fundamental understanding of plasmon emergence, which has many applications.
Figure 6.6 (Left) Size-dependent UV-visible absorption spectra of Au nanomolecules, with \( \text{Au}_{329}(\text{SR})_{84} \) being the smallest size to support plasmon resonance (Right) UV-visible spectra of \( \text{Au}_{329}(\text{SR})_{84} \) nanomolecules synthesized using dodecanethiol (olive), phenylethanethiol (blue) and hexanethiol (red). All three spectra show the SPR peak. The 329-atom is the smallest \( \text{Au}_{n}(\text{SR})_{m} \) nanomolecule that supports a surface plasmon resonance.

6.6 Atomic structure of the \( \text{Au}_{329}(\text{SR})_{84} \)

The determination of atomic structure has revolutionized modern science in the areas of physics, chemistry, and biology.\(^{24, 122, 123}\) The lack of periodicity and crystalline lattice has contributed to the problem of determining atomic arrangements in nanomaterials, known as the ‘nanostructure problem’.\(^{47}\) The total scattering X-ray diffraction technique using synchrotron radiation, which includes both the Bragg and diffuse scattering, can provide the atomic pair distribution function (PDF).\(^{124}\) Real space modeling on atomic PDF when combined with other local and bulk spectroscopic and microscopy probes can yield structural information facilitating a solution to the complex nanostructure problem.\(^{125, 126}\) This section discussed the overall characterization of \( \text{Au}_{329}(\text{SR})_{84} \) using X-ray diffraction, STEM and SAXS as analytical tools.
6.6.1. STEM analysis

Figure 6.7 STEM micrographs of the $\text{Au}_{329}(\text{SR})_{84}$ nanomolecules. (a-b) Low resolution HAADF-STEM images (c) medium resolution image (d-f) atomic resolution images of single nanoparticle (g-h) Three dimensional atom density profile of single $\text{Au}_{329}(\text{SR})_{84}$ molecules derived from Figure 6.6f. The atom density profiles were visualized using ImageJ (version 1.46r) data analysis package.

Figure 6.7a and b shows the representative, low-resolution HAADF-STEM images of the $\text{Au}_{329}(\text{SR})_{84}$ nanoparticles. Individual particles spontaneously self-assemble over the TEM grid. Atomic resolution images in figure 6.7d-f show the single $\text{Au}_{329}(\text{SR})_{84}$ nanomolecules. It should be noted that electron beam can induce nanoparticles deformation and mass loss during image acquisition.\(^{46}\) Continuous electron beam exposure causes surface atom diffusion and particle aggregation. Therefore, the beam dose was reduced by acquiring the first pass images. Figure
6.7d-f show the individual gold atoms and it ordering as atomic columns. Closer observation shows at least nine atomic columns in the nanomolecules. STEM analysis concludes the monodisperse nature and its diameter to be $2.0 \pm 0.1$ nm.

### 6.6.2 Atomic structure determination

The atomic structure dictates the physical and chemical properties of materials and function in biological systems. This structure-property correlation necessitates structural information in order to design novel nanomaterials. Some recent breakthroughs include the crystal structure determination of $\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}$ and $\text{Au}_{102}(\text{SC}_6\text{H}_4\cdot\text{COOH})_{44}$ nanomolecules.\textsuperscript{14,24} The ‘nanostructure problem’\textsuperscript{47} is prevalent in larger nanoparticles due to the absence of crystal lattice, nanocrystallinity, large unit cells, and disorder.\textsuperscript{127} Atomic PDF analysis of powder diffraction data using synchrotron radiation provides critical structural information often intractable by other methods. Atomic PDF, $G(r)$, is the sum of inter-atomic distances between pairs of atoms in a nanoparticle.\textsuperscript{124} The structures of nanomolecules are size-dependent, ranging from polycrystalline shapes, namely icosahedra and decahedra. As the size becomes larger, the structure smoothly varies approaching bulk face centered cubic (fcc)-like, octahedral, truncated octahedral or cuboctahedral shapes.\textsuperscript{128,129}

The experimental PDF of $\text{Au}_{320}(\text{SR})_{84}$ nanomolecules is displayed in Figure 6.8. The peaks indicate the inter-atomic Au-Au distances are mainly due to intra-particle Au-Au atom correlations. This is due to the high X-ray scattering function of Au, when compared with other sulfur, carbon and hydrogen atoms.
Figure 6.8 Experimental PDF curves of \( \text{Au}_{329}(\text{SR})_{64} \) nanomolecules (top, violet). Calculated PDF curves of \( \text{Au}_{309} \) icosahedra (black), \( \text{Au}_{217} \) Marks decahedra (red), \( \text{Au}_{309} \) Inodecahedra (blue), \( \text{Au}_{260} \) truncated octahedra (olive), and \( \text{Au}_{309} \) cuboctahedra (dark yellow). Comparison of PDF shows that the fcc-like TO and CO are feasible structures. Models on the right show the top and side views of the polycrystalline and fcc-like structural models used in the PDF calculation.

The most intense peak at \( \sim 2.8 \) Å corresponds to Au-Au nearest neighbor distances. The least intense correlation or PDF peak appears at \( \sim 2.2 \) nm corresponding to the longest interatomic distance, and therefore the approximate diameter of the nanomolecules. This is in excellent agreement with size obtained from STEM results. This long range correlation, up to 2.2 nm, much beyond the 0.288 nm nearest-neighbor distances, provides nanoscale structural information. This is possible due to powder X-ray diffraction data collection over a wide range
of Q, $Q_{\text{max}} = 22\text{Å}^{-1}$, $\lambda = 0.21218 \text{ Å}$). Due to its ~2.2 nm size being located at the nanomolecules-nanoparticle crossover region, both polycrystalline and fcc-like structural models were considered as possible motifs.

Figure 6.8 also shows the comparison of experimental Au$_{329}$(SR)$_{84}$ PDF with that of the calculated PDF curves of Au$_{309}$ icosahedra, Au$_{217}$ Marks decahedra, Au$_{309}$ Ino-decahedra, Au$_{260}$ truncated octahedra, and Au$_{309}$ cuboctahedra. Due to the 329-Au atom composition, these specific Au atom cores ranging from 217 to 309 atoms were considered feasible and chosen as appropriate models. Direct comparison shows that the PDF pattern of Au$_{309}$ icosahedra does not match with experimental data with clear mis-matches at 6.5 and 10.5 Å. Compared to Au$_{309}$ icosahedra, the Marks Au$_{217}$ and Inos Au$_{309}$ decahedral structures show better match with experimental data. However, there are significant differences in the 5.8, 10.4 Å and the set of peaks in the 8–12 Å regions. Calculated PDF of fcc-based Au$_{260}$ truncated octahedral and Au$_{309}$ cuboctahedra models show much better agreement with experimental data, nicely up to 12 Å. Therefore, the atomic structure of the Au$_{329}$ metallic core proposed as fcc-like, with either a truncated octahedron (TO) or cuboctahedra (CO) arrangement. Au$_{25}$(SR)$_{18}$, Au$_{36}$(SR)$_{24}$, and Au$_{102}$(SR)$_{44}$ nanomolecules have [-SR-Au-SR-] and [-SR-Au-SR-Au-SR-] moieties protecting the surface. Considering these gold–thiol interface, the predicted atomic model could be further narrowed down to the Au$_{260}$ truncated octahedron. This is because the Au$_{309}$ cuboctahedral core model will allow only 20 Au-atoms for the ligand interface, which allows a maximum of 45 ligands, whereas the Au$_{260}$ TO model leaves 69 gold atoms, which provides enough atoms for the construction of Au-SR interface. Figure 6.9 shows the proposed four shells of Au$_{260}$ truncated octahedron core for Au$_{329}$(SR)$_{84}$. The model consists of 6, 32, 78 and 144 atoms in each shell.
The proposed Au260 model consists of 14 faces (8 hexagonal and 6 square faces), 24 vertices and 36 edges. Due to truncation from regular octahedron, it shows hexagonal shape in 2D projection (~20.1 Å at longest end and ~18.4 Å at shortest end). STEM analysis also agrees with the model dimension but diffused edges, protected ligand shell and beam induced changes also affect for the experimental HR-STEM based aspect ratio measurement.

Figure 6.9 Proposed Au260 core with truncated octahedron four shell model for the Au329(SR)84 nanomolecules.

The crystal structure of Au25(SR)18 consist only the monomeric staple motif, while Au38(SR)24 and Au102(SR)44 are protected by both mono and dimeric staple motif. Predicted Au144(SR)60 structure model fit only with monomeric staples units. Because of the larger size here, monomeric staple motif alone with Au42(SR)84 outer shell can be proposed. The remaining 27 atom may involve special bonding between the shells that add ultra-stability to the Au329(SR)84, that is bonding between hexagonal and square faces of Au260 core and Au42(SR)84 outer shell.
6.6.3 Small angle X-ray scattering analysis

Figure 6.10 SAXS data, fittings and the derived SAXS molecular envelope of Au$_{329}$(SR)$_{84}$ nanomolecules. (Left) SAXS data were collected up to 1 Å$^{-1}$. The red curve is a representative fitting in molecular envelope calculation. The molecular envelope, also known as bead model, was calculated using program DAMMIN, with SAXS data up to 0.8 Å$^{-1}$. The inset is the Guinier fit for SAXS data at q close to 0. The wide linear range indicates the monodispersity of the particle size. The radius of gyration, $R_g$, obtained from the fitting is $9.4 \pm 0.2$ Å. (Right) Pair distance distribution function, $p(r)$, derived from X-ray scattering data using program GNOM. The largest dimension, $R_{\text{max}}$, of the particle obtained from $p(r)$ is about 2.6 nm, where $p(R_{\text{max}})=0$. Inset is the final SAXS molecular envelope reconstructed from X-ray scattering up to 0.8 Å$^{-1}$. Twenty independent molecular envelope calculations were performed and the resulting envelopes were further averaged to generate the final, consensus molecular envelope.

The wide linear Guinier plot and the shape of the X-ray scattering curve with two oscillations in the q range of 0.3–1.0 Å$^{-1}$ demonstrate the very narrow size distribution or
monodisperse nature of the $\text{Au}_{329}(\text{SR})_{84}$ samples. The pair distance distribution function derived from X-ray scattering data showed that the largest dimension of the particle is about 2.6 nm. A 3D shape model (green model in the figure) was reconstructed from X-ray scattering data using program DAMMIN and it showed that the particle adopts a spheroid-like shape. The longest dimension of this 3D shape model is ~2.5 nm and the cross section diameter of the short dimension/axis is about 2.1 nm. Considering the error bar of lengths derived from scattering data is about 0.3-0.6 nm, the particle size dimensions obtained from X-ray data are consistent with STEM particle size analysis (2.0 ± 0.1 nm).

**6.7 Atomically monodisperse $\text{Au}_{329-x}\text{Ag}_x(\text{SCH}_2\text{CH}_2\text{Ph})_{84}$ alloy molecules**

Compositional analysis of larger alloy nanoparticles (> 5 nm diameter) includes energy dispersive X-ray spectroscopy (EDX), X-ray photoelectron spectroscopy (XPS) and inductively coupled plasma mass spectrometry (ICP-MS). However, these methods are limited to the determination of composition as percentage or as molar fractions with certain error limits. Electrospray ionization mass spectrometry (ESI-MS) of ultra-small nanoparticles can yield composition to the atomic level yielding specific number of metal atoms and ligands.

However, in the cases of thiolated alloy nanomolecules, the MS based atomic level composition determination has been limited to the few number of nanomolecule including $\text{Au}_{25-x}\text{Ag}_x(\text{SR})_{18}$, $\text{Au}_{38-x}\text{Ag}_x(\text{SR})_{24}$ and large $\text{Au}_{144-x}\text{Ag}_x(\text{SR})_{60}$. This is due in part to challenges in the larger (>144 atom) species in terms of, (a) nanoparticle purification methodologies to isolate pure species and (b) nanoparticle characterization methods at the atomic level to determine the composition to atomic precision. It is of great scientific interest and challenge to
synthesize plasmonic alloy nanocrystals with well-defined atomic compositions to understand the emergence of SPR\textsuperscript{130,131} and its effect on silver alloying.\textsuperscript{132}

This section will discuss the synthesis and characterization of alloy nanomolecules that have precise atomic composition. The synthesis of alloy Au\textsubscript{329-x}Ag\textsubscript{x}(SR)\textsubscript{84} nanomolecules involves three steps, a crude mixture preparation, thermochemical treatment and solvent fractionation. The silver content of Au\textsubscript{329-x}Ag\textsubscript{x}(SR)\textsubscript{84} was vary by changing the HAuCl\textsubscript{4}:AgNO\textsubscript{3} molar ratios from 1:0 to 1:1 in the initial synthesis.

In contrast to the Au\textsubscript{329}(SR)\textsubscript{84} synthesis protocol, following modifications were made to yield Au\textsubscript{329–x}Ag\textsubscript{x}(SR)\textsubscript{84} alloy nanomolecule.

(1) \textit{Sequentially addition of Ag in the initial stage of synthesis}. The initial molar ratio of Au:Ag precursors were set to 1:0, 1:0.01, 1:0.05, 1:0.10, 1:0.20, 1:0.33, 1:0.50, 1:0.66, and 1:1. The reason for selecting such a broad ratio is to gain insight into silver incorporation via mass spectrometry and UV–visible spectrometry. These ratios can be further subdivided into three groups based on mass spectrometry and UV–visible spectrometric results. (a) 1:0, 1:0.01, 1:0.05, 1:0.10, 1:0.20; (b) 1:0.33 and 1:0.50; (c) 1:0.66 and 1:1.

(2) \textit{Dynamic etching protocol depends on Au:Ag incoming molar ratio}. Etching protocol were modified to improve the Au\textsubscript{329–x}Ag\textsubscript{x}(SR)\textsubscript{84} yield at high Au:Ag ratio as follow. (a) Ratio 1:0, 1:0.01, 1:0.05, 1:0.10, and 1:0.20; Nanomolecules were subjected to thermochemical treatment up to ~8 days as usual. (b) Ratio 1:0.33 and 1:0.50; subjected to thermochemical treatment for 2 days, while the sample was monitored using MALDI-MS. If required, re-etching was continued for another 2–3 days to acquire clear baseline separation (to further narrow down particle distribution) between different nanoparticles. (c) Ratio 1:0.66 and 1:1; thermochemical
treatments were limited to 3–9 h. Further thermochemical treatment led to decomposition.

(3) **Systematic separation of the AuAg alloy to yield Au$_{329-x}$Ag$_x$(SR)$_{84}$.** There were three to four types of alloy nanomolecules at 11, 30–35, 60–73, and $\sim$105 kDa mass ranges after the thermochemical treatment. However, the relative proportion of these sizes varied depending on the incoming Au:Ag ratio. Thus, other nanoparticles were systematically removed or precipitated out from the mixture to obtained Au$_{329-x}$Ag$_x$(SR)$_{84}$ in pure form. At least 10–15 solvent fractionations cycles were performed for the Au$_{329-x}$Ag$_x$(SR)$_{84}$ isolation. The first set of solvent fractionation focus on systematic removal of 11 and 30–36 kDa alloy from the polydispersed mixture by dissolving the product in toluene and precipitating by MeOH. After that, possible larger nanoparticles (>76.3 kDa) were precipitated using the same solvent mixtures, and pure Au$_{329-x}$Ag$_x$(SR)$_{84}$ was isolated.

### 6.7.1 Silver atom counting in AuAg plasmonic nanoparticle by ESI-MS

ESI-MS was used to determine the composition of the alloy, including the number of Au and Ag atoms to atomic precision. To our knowledge, this is the first report on accurate silver atom counting in silver alloys of gold plasmonic nanomolecules using mass spectrometry. Figure 6.10(left) shows the ESI-MS spectra acquired for Au$_{329-x}$Ag$_x$(SR)$_{84}$ nanomolecules synthesized using 1:0.10 incoming Au/Ag molar ratios. Spectra shows multiple charged ions at 37.7, 25.2, 19 and 15 kDa corresponding to the 2-, 3-, 4- and 5- of the intact molecular ions respectively. The 4- charge states were predominant in the ESI spectra and the inset shows the expansion of 4-ions. The spacing between each of these peaks corresponds to $\sim$89/4 m/z, which is the mass difference between gold (197 Da) and silver (108 Da). Introducing silver in the form of AgNO$_3$ into the initial synthesis lead to the formation of Au$_{320}$Ag$_9$(SR)$_{84}$ alloy nanomolecules. Although
nine silver atoms are incorporated into the nanomolecule, the total number of metal atoms still remains at 329. The mass of the molecular ions were obtained by multiplying the ESI-MS peaks with their corresponding charge. The molecular ions peak derived from 2-, 3-, 4-, and 5- charge states are in good agreement with each other, adding further validity to ESI data and the observed size distribution.

Figure 6.11 (Left) Electrospray ionization (ESI) mass spectrum of $\text{Au}_{329-x}\text{Ag}_x$(SR)$_{84}$ nanomolecules for Au:Ag precursor ratios of 1:0.10. Inset show expansion of the 4- molecular ion. The mass difference between the peaks in nanoalloys corresponds to the Au (196.97 Da) and Ag (107.87 Da) mass difference, $\Delta m=89$ Da. (Right) ESI of $\text{Au}_{329-x}\text{Ag}_x$(SR)$_{84}$ nanomolecules for different Au:Ag precursor ratios. ESI mass spectrum of $\text{Au}_{329-x}\text{Ag}_x$(SR)$_{84}$ nanomolecules for incoming Au:Ag precursor ratios of 1:0 (black), 1:0.01 (red), 1:0.05 (olive), 1:0.10 (blue) and 1:0.20 (orange).
The Au:Ag incoming mole ratio was gradually increased starting from 1:0 to 0.20 in the first set of experiments. In the next set of experiment, incoming mole ratio varies from 1:0.33 to 1:1. Figure 6.11(Right) shows the 4- ions in the ESI-MS spectra of the alloy synthesis up to 1:0.20 incoming molar ratio. The bottom black spectrum shows the Au$_{329}$(SR)$_{84}$ plasmonic nanomolecules with no silver addition. Upon silver addition in step 1, the Au$_{329}$(SR)$_{84}$ mass peak shifts to the low mass region resulting in an envelope of peaks with 89/4 mass difference due to the silver incorporation. The average silver atom incorporation was 2, 5, 9 and 26 as the incoming AgNO$_3$ ratio was increased to 0.01, 0.05, 0.10 and 0.20 respectively. The observation of these silver substituted Au$_{329-x}$Ag$_x$(SR)$_{84}$ species further validates the formula 329:84. It is interesting to note that the Au:Ag, 1:0.01 ratio shows the minor peak envelope to the right of the Au$_{329-x}$Ag$_x$(SR)$_{84}$ species, corresponding to Au$_{330-x}$Ag$_x$(SCH$_2$CH$_2$Ph)$_{86}$ and Au$_{331-x}$Ag$_x$(SCH$_2$CH$_2$Ph)$_{88}$ assignment. For smaller gold and alloy nanoparticles like Au$_{25}$(SR)$_{18}$ and Au$_{25-x}$Ag$_x$(SR)$_{18}$, there exists only one major species. However, for larger alloy nanoparticles, such as Au$_{144-x}$Ag$_x$(SR)$_{60}$, there are other closely related Au$_{144-x}$Ag$_x$(SR)$_{59}$ species$^{117}$ while the 144:60 is the dominant species.
Figure 6.12 MALDI-TOF (1+ ion on left) and electrospay ionization (ESI) mass spectra (4- ions on right) of Au$_{329-x}$Ag$_x$(SR)$_{84}$ nanomolecules for Au:Ag precursor ratios of 1:0 (black), 1:0.01 (red), 1:0.05 (olive), 1:0.10 (blue) and 1:0.20 (orange), 1:0.33 (purple) and 1:0.50 (dark yellow), 1:0.66 (brown) and 1:1 (cyan) in the starting material. The number of Au and Ag atoms of most intense peak is denoted above each distribution of peaks.

Figure 6.12 (left) shows the MALDI spectra acquired for the Au$_{329-x}$Ag$_x$(SR)$_{84}$, synthesized using different incoming molar ratio. The flat baseline indicates the purity of the nanoparticles, including the absence of 144-Au atom species and larger plasmonic particles. Until the Au:Ag incoming molar ratio reach 1:0.20, no significant mass shift was observed upon silver atom incorporation. However, nanomolecules synthesized using 1:0.20 to 1:1 show clear mass shifting to the low mass region in MALDI spectra compared to the Au$_{329}$(SR)$_{84}$. 109
Corresponding ESI spectra showed in figure 6.12(right). The envelope of peaks between 1:0.01 to 1:0.20 Au:Ag ratio nicely show the individual silver atom incorporation corresponding to the 89/4 m/z value. However, as the silver atoms increases to 1:0.20, the baseline separation is lost and only the parts of the peaks were resolved. This type of loss of baseline separation has been observed before in the case of Au$_{144-x}$Ag$_x$(SR)$_{60}$. In 1:0.33 and higher Au:Ag ratios, the peaks corresponding to Ag incorporation are not at all resolved, instead one broad peak is observed. This broad peak moves to lower mass as expected in the 1:0.50 and 1:0.66 ratios. Only a poor signal is observed for the 1:0.66 ratio. No observable signal was present for the 1:1 ratio and upon extrapolation of the ESI data, 1:1, Au:Ag atoms distribution was assumed. This intrinsic broadening of the peaks as the number of silver atoms increase is due to the presence of isotopes, as observed before. The center of the peak was used to calculate the average number of silver incorporation. This broadening might also be due to the several possible compositions, but the broadening due to silver isotope is a stronger possibility. Nanomolecules synthesized at higher Au:Ag ratio are not stable upon prolong thermochemical treatment. Bond strength within nanomolecules falls under following order, Au-Au>Ag-Ag>Au-Ag. Thus, the incorporation of larger number of Ag atoms leads to comparatively weak and unstable bonding compared to Au-Au interaction.

Figure 6.13 shows low magnification STEM images of Au$_{329-x}$Ag$_x$(SC$_2$H$_4$C$_6$H$_5$)$_{84}$ (Au:Ag 1:0.20 and 1:1 ratio). The image shows alloy nanoparticles disseminated over TEM grid. It should be noted that b unlike the monometallic Au$_{329}$(SC$_2$H$_4$C$_6$H$_5$)$_{84}$, its alloy nanomolecule seems to be more unstable for expose electron beam. Average diameter of the nanoparticles was measured to be ~2 nm. The elemental composition of the nanomolecules synthesized using
1:0.20 and 1:0.66 ratio also determined by energy dispersive X-ray spectroscopy (EDS) coupled to the STEM. STEM-EDS shows the relative elemental composition of Au\(_{303}\)Ag\(_{26}\)(SR)\(_{84}\) as Au (85%) and Ag (15%). It shows 7% deviation with respect to the ESI-MS measurement. Nanomolecules synthesized using a Au:Ag ratio of 1:0.66(Au\(_{181}\)Ag\(_{146}\)(SR)\(_{84}\) show Au(67%) and Ag(33%). We note that that alloy nanoparticle are unstable to the long STEM exposure time, leading to beam damage. This may be the reason for the deviation of the EDS and ESI-MS results. Also, ESI-MS assignments are based on the most intense peak in determination of Au and Ag in elemental composition.

Figure 6.13 Representative HAADF-STEM images of alloy nanoparticles synthesis at Au:Ag incoming molar ratio of (left) 1:0.20 [Au\(_{303}\)Ag\(_{26}\)(SR)\(_{84}\)] and (right) 1:1[Au\(_{165}\)Ag\(_{164}\)(SR)\(_{84}\)].

6.7.2 Non-linear variation of SPR intensity with increasing silver atom content

Figure 6.14 bottom spectrum (black) shows a SPR band at 490 nm observed for Au-only Au\(_{329}\)(SR)\(_{84}\) nanomolecules. No significant spectral changes were observed in the Au:Ag ratios
of 1:0 to 1:0.20. This is due to the less amount of Ag atoms substitution. The SPR band of the Au:Ag ratio at 1:0.33 and 1:0.50 containing \(\sim 115\) and \(\sim 135\) Ag atoms respectively, actually diminish compared to that of \(\text{Au}_{329}(\text{SR})_84\). This damping effect may be due to the addition of silver in a specific geometric shell in the atomic structure, which leads to the damping at the Au/Ag interface or structural modification upon alloying.\(^{27}\) The SPR band in the mole ratio of 1:0.66 resulted in an incorporation of \(\sim 148\) Ag atoms on average, 45\% of total metal atoms, which produces a narrow and more symmetric SPR peak at \(\sim 510\) nm.

For intermixed or alloy Au-Ag bimetallic nanoparticles, a single SPR band was observed, which varied between 400 to 530 nm with element composition, whereas, for core-shell nanoparticles, the SPR band red or blue shift depends on outermost shell composition and thickness.\(^{34}\) Optical properties of smaller core–shell type nanoparticles also vary depending on composition, electronic structure, size, internal structure and geometry.\(^{132}\) In a neutral charge state, \(\text{Au}_{329}(\text{SR})_84\) nanomolecules have 245 free electrons which originate from Au(6s).\(^{133}\) Upon alloying the electronic states originate from both Ag(5S) and Au(6S) states.\(^{101}\) Thus, silver atoms may enhance the degeneracy of the electron shell.\(^{101}\) Sharpening of the Au plasmonic resonance was observed upon alloying which indicate the modulation of SPR by Ag. This sharpening may be due to the contribution from the imaginary part of the dielectric constant (\(\varepsilon_i\)) of the incorporated silver atoms.\(^{27}\) Gold-silver alloy nanoparticles show blue shifting of SPR with increasing silver content.\(^{11}\)
Figure 6.14 UV–visible spectra of \( \text{Au}_{329-x}\text{Ag}_x\text{(SR)}_{84} \) nanomolecules. \( \text{Au}:\text{Ag} \) precursor ratios of 1:0 (black), 1:0.01 (red), 1:0.05 (olive), 1:0.10 (blue) and 1:0.20 (orange), 1:0.33 (purple) and 1:0.50 (dark yellow), 1:0.66 (brown) and 1:1 (dark cyan) in the starting material. Inset shows the SPR peak maximum varies with the gold mole fractions. The solid line indicates the linear fit of the maximum SPR peak values (\( \lambda_{\text{max}} \)) with the different composition. The spectra were recorded in the toluene solution. (The \( \text{Au}_{329-x}\text{Ag}_x\text{(SR)}_{84} \) spectra are offset for clarity).

In present study, large change in SPR peak position, \( \sim 100 \) nm blue shift,\(^{11} \) was not observed up to 1:1 \( \text{Au}/\text{Ag} \) ratio. On the contrary, a maximum of \( \sim 10 \) nm red shift was observed and a non-linear dependence of SPR peak intensity was observed. This suggests the synthesized plasmonic nanostructure are core/shell type structure with Au rich surface or \( \text{Au@Au}/\text{Ag@Au} \) multi-shell structure.\(^{132} \) The lack of X-ray structure thus far has impeded theoretical analysis to
fully understand the unusual behavior of this \( \text{Au}_{329-x}\text{Ag}_x(\text{SR})_{84} \) alloy nanomolecules.

### 6.8 Conclusion

Chemical composition of the 76.3 kDa plasmonic faradaurates were determined to be \( \text{Au}_{329}(\text{SR})_{84} \) based on high resolution mass spectrometric studies. This conclusion is supported by multiple independent and complementary lines of evidence including, ionization mode switching and analysis of the mass shifts caused by changing the mass of the ligand. The composition reported here will facilitate the prediction of atomic structure and other theoretical and experimental studies to understand the properties of this extraordinary stable plasmonic \( \text{Au}_{329}(\text{SR})_{84} \) nanomolecules.

The atomic structure of the plasmonic \( \text{Au}_{329}(\text{SR})_{84} \) nanomolecules were determined using a comprehensive set of tools such as mass spectrometry, high energy X-ray diffraction, X-ray scattering experiments and high resolution scanning transmission electron microscopy. STEM shows the monodisperse nature and its diameter to be \( 2.0 \pm 0.1 \) nm. X-ray scattering experiments independently suggest a good monodispersity on particle size and give a size dimension of 2.1-2.6 nm for the nanomolecules. Synchrotron based high energy X-ray PDF analysis clearly shows the \( fcc \) atomic arrangement. \( \text{Au}_{329}(\text{SR})_{84} \) nanomolecules exhibit the surface plasmon resonance at \( \sim 490 \) nm (extinction coefficient, \( 1.26 \times 10^6 \) M\(^{-1}\)cm\(^{-1}\)) which is the smallest and ultra stable thiolated gold nanomolecules that support the surface plasmonic resonance.

Synthesis of large plasmonic Au-Ag alloy composition with \( \text{Au}_{329-x}\text{Ag}_x(\text{SCH}_2\text{CH}_2\text{Ph})_{84} \) is unprecedented. Approximately 50 % of silver could be incorporate to \( \text{Au}_{329}(\text{SR})_{84} \). Surface plasmonic resonance sharpens upon silver incorporation. The result demonstrates the next
generation applications of mass spectrometry toward the determination of nanoparticles composition to the atomic level. This plasmonic behavior of the alloy nanocrystals with a molecular composition may open door for the interesting application in nano-optical devices, solar cells and catalysis.
CHAPTER 7

SUPERS-STABLE PLASMONIC NANOCRYSTALS WITH 500 AND 940 GOLD ATOMS

Part of the text and figures in this chapter are extracted from the following publication:


CHAPTER 7

SUPERS-STABLE PLASMONIC NANOCRYSTALS WITH 500 AND 940 GOLD ATOMS

7.1 Abstract

Determining the composition of plasmonic nanoparticles is challenging due to a lack of analytical tools to accurately quantify the number of atoms within the particle. Mass spectrometry plays a significant role in determining nanoparticle composition at the atomic level. Significant progress has been made in understanding ultra-small gold nanoparticles such as \( \text{Au}_{25}(\text{SR})_{18} \) and \( \text{Au}_{38}(\text{SR})_{24} \), which have Au core diameters of 0.97 and 1.3 nm, respectively. However, progress in 2-5 nm diameter small plasmonic nanoparticles is currently impeded, due in part to the challenges in synthesizing monodisperse nanoparticles. This chapter discussed the synthesis and characterizations of two plasmonic nanocrystals at 115 and 200 kDa mass ranges. These nanocrystals are highly monodisperse with unprecedentedly small size variability.

The composition of the super-stable plasmonic nanocrystals mass at 115 kDa was determined as \( \text{Au}_{500\pm10}(\text{SCH}_2\text{CH}_2\text{Ph})_{120\pm3} \). The Au-500 system, named Faradaurate-500, is one of the largest system to be characterized using high resolution electrospray (ESI) mass spectrometry. Atomic pair distribution function (PDF) data indicate that the local atomic structure is consistent with a face-centered cubic (fcc) or Marks decahedral arrangement. High resolution scanning transmission electron microscopy (STEM) images show that the monodisperse nanocrystals with diameter 2.4 ± 0.1 nm. The size and the shape of the molecular
envelope measured by small angle X-ray scattering (SAXS) confirms the STEM and PDF analysis.

Obtaining monodisperse nanocrystals, and determining its composition to the atomic level and its atomic structure is highly desirable, but is generally lacking. The second part of this chapter describe the discovery and comprehensive characterization of a ~3 nm sized plasmonic nanocrystal with a composition of Au$_{940\pm20}$(SCH$_2$CH$_2$Ph)$_{160\pm4}$, the largest mass spectrometrically characterized gold thiolate nanoparticle produced to date. The compositional assignment has been made using ESI and MALDI mass spectrometry. The MS results show an unprecedented size monodispersity, where the number of Au atoms vary by only 40 atoms (940±20). The mass spectrometrically determined size and composition are supported by STEM and synchrotron-based methods such as PDF and SAXS. Lower resolution STEM images show an ensemble of particles–1000 per frame demonstrating monodispersity. Modeling of SAXS data on statistically significant nanoparticle population, approximately $10^{12}$ individual nanoparticles shows that the diameter is 3.0±0.2 nm, supporting mass spectrometry and electron microscopy results on monodispersity. Atomic PDF based on high energy X-ray diffraction experiments show decent match with either a Marks decahedral or truncated octahedral structure. Atomic resolution STEM images of single particles and its FFT suggest face-centered cubic (fcc) arrangement. UV-visible spectroscopy data shows that the 940-atom size supports a surface plasmon resonance peak at 505 nm. These monodisperse plasmonic nanoparticles has potential application in solar cells, nano-optical devices, catalysis and drug delivery.
Author Contributions

Chanaka Kumara synthesized Au_{500±10}(SCH_2CH_2Ph)_{120±3} and Au_{940±20}(SCH_2CH_2Ph)_{160±4}. Both Dr. Amala Dass and Chanaka analyzed the PDF data. STEM image were acquired and analyzed by Dr. David Cullen at Oak Ridge National Laboratory. SAXS data were acquired and analyzed by Dr. Xiaobing Zuo at Argonne National Laboratory.
7.2 Introduction

Surface plasmon resonance (SPR) is the light-induced collective oscillation of conduction electrons, with applications in photochemistry,\textsuperscript{134} solar cells,\textsuperscript{135, 136, 137, 138} and bio-medical areas including cancer cell detection, drug delivery and chemical sensing.\textsuperscript{139} Gold shows SPR in nanoparticles as small as 2 nm diameter, at wavelengths of 500-550 nm which red shifts with increasing nanoparticle size and aspect ratio. Nanoscale gold particles can be synthesized with different sizes\textsuperscript{140} and in a variety of shapes\textsuperscript{141, 142} (e.g., prism, cubic, hexagonal, hollow), all of which can be used to tune the SPR.

Characterization of citrate-protected colloidal gold nanoparticles (<5 nm), where molecule-to-plasmonic transitions occur, is limited by sample polydispersity and instability, with particle aggregation often observed on short time scales. Ultra-stable gold nanoparticles have previously been prepared by protecting the nanoparticle core with thiols using the Brust-Schirrfin method.\textsuperscript{17} The dispersity of the Brust-Schirrfin product can be enhanced by a thermochemical treatment.\textsuperscript{20} This produces atomically monodisperse Au-SR molecules (SR=thiolate), like \(\text{Au}_{25}(\text{SR})_{18}, \text{Au}_{38}(\text{SR})_{24}, \text{and Au}_{144}(\text{SR})_{60}\), which are stable in air, at high temperature (~80 °C), and under humid conditions. Concurrently, advances in instrumental methods have developed mass spectrometry (MS) as a valuable tool to identify nanoparticles via compositional analysis.\textsuperscript{38} This is because transmission electron microscopy (TEM) characterization remains a challenge at smaller nanoparticle sizes (<3 nm diameter) due to rapid electron beam-induced damage, though limited success is achievable. Specifically, progress in electrospray (ESI) mass spectrometry with multiply charged molecular ions and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) with DCTB matrix\textsuperscript{3} enabled the compositional assignment of these
ultra-small nanoparticles.\textsuperscript{71, 89} This has enabled successful identification of various gold,\textsuperscript{85} silver\textsuperscript{31} and alloy\textsuperscript{6, 69} nanomolecules using mass spectrometry. However, evaluating the composition of larger plasmonic nanoparticles (> 2 nm diameter, > 300 atoms) is still impeded by limitations in ionization and detection of molecular ions.

For ultra-small thiol protected gold nanoparticles, which can be isolated as single crystals, fundamental understanding has been facilitated by structural analysis using X-ray crystallography. For such ultra-small systems, the small particle size and particle uniformity are critical to isolating single crystals. Thiol-protected nanoparticles as large as \( \text{Au}_{102}(\text{SPh-COOH})_{44} \) have been crystallographically-characterized\textsuperscript{24} with several other Au nanomolecules crystal structures containing less than 102 gold atoms have also been reported.\textsuperscript{14, 73, 143} However, atomic structural analysis of plasmonic nanoparticles remains challenging, with the larger particle size and increased polydispersity often preventing crystallization. In such cases, atomic pair distribution function (PDF) analysis of high energy synchrotron based X-ray total scattering data can provide atomic scale structural information, with crystallographic resolution independent of the sample crystallinity. In a preliminary report on the 76.3 kDa nanomolecules mentioned that other stable sizes exists at 115 and 207 kDa mass range.\textsuperscript{16} This chapter describes the MALDI and ESI mass spectrometry at unprecedented high mass for nanoparticle characterization at 115 and 200 kDa, respectively. Further, Synchrotron X-ray and high resolution electron microscopy were employed to study the atomic structure.

Highly monodisperse plasmonic nanoparticles at 115 kDa containing \( \sim 500 \) (±2%) Au atoms. The \( \text{Au}_{500\pm10}\text{SR}_{120\pm3} \) system named as “Faradaurate-500”, in honor of Michael Faraday.\textsuperscript{16} Compared to similarly size colloidal plasmonic nanoparticles, where the term “monodisperse” is
generally applied to a system with ca. 10% standard deviation, the ±10 Au atom variability reported here is unmatched for systems of this size. High-energy X-ray scattering-based atomic PDF analysis shows that the atoms are in a cubic close packed (fcc) or Marks decahedral arrangement. Aberration-corrected high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) images of the particles shows the size, shape, and core morphology of thiol protected plasmonic gold nanoparticles with atomic resolution. Low-magnification STEM images presenting an ensemble of particles show a uniform size distribution in the 2.4 ± 0.1 nm range, and self-assembled regions indicating size uniformity. Furthermore, small angle X-ray scattering data yield a diameter of 2.6 ± 0.2 nm. SAXS data also shows an oscillatory behavior indicating high monodispersity. A variety of complementary characterization techniques yield information about composition, size, size distribution and atomic structure.

Second part of the chapter 7 discussed the synthesis, isolation and characterization of plasmonic nanoparticles whose composition is Au$_{940}$(SR)$_{160}$. This is the largest mass spectrometrically characterized thiolated gold nanoparticles, to date. The monodispersity and size distribution shown by mass spectrometry was supported by other standard nanoparticle characterization methods, including electron microscopy and SAXS, which is generally lacking in research on nanomolecules. The HAADF-STEM studies is made possible due in part to the enhanced stability due to the larger size of the 940-atom species, when compared with the <329 atom nanomolecules. Atomic resolution STEM images and its FFT pattern suggest that the particle has a cubic closed packed fcc atomic arrangement. This is distinct from the non-crystallographic icosahedral and Marks decahedral cores of smaller than 329-atom
nanomolecules protected by phenylethane thiol ligand. Not only can such highly monodisperse plasmonic nanocrystals facilitate the fundamental understanding of evolution of atomic and electronic structure with size, but these also have diverse potential applications.

7.3 Method

7.3.1: Synthesis

Synthesis involves three steps. Firstly, a crude product containing polydisperse Au nanoparticles was prepared in a modification of a reported process. Then, thermochemical treatment of the crude product with excess thiol was applied to eliminate meta-stable nanoparticles. Finally, solvent fractionation was used to isolate pure monodisperse nanocrystals.

Step 1. Aqueous solution (30 mL) containing HAuCl₄ (0.1772 g/0.45 mmol) was mixed with toluene solution (30 mL) of tetraoctylammonium bromide, TOABr (0.55 mmol). After stirring for 30 min, the organic phase was separated and phenylethane thiol (0.0622 mL/0.225 mmol) was added and further stirred for 30 min at room temperature. For Au₃₅00(SR)₁₂₀ synthesis, gold to thiol molar ratio was set to 1:0.50 and for Au₉₄₀(SR)₁₆₀ ratio further bring down to 1:0.25. Then, mixture was cooled in an ice bath for 30 min. An aqueous solution of NaBH₄ (10 mmol, 20 mL) cooled to 0°C, was rapidly added to the reaction mixture under vigorous stirring. After 2 hours, the organic layer was separated and evaporated to dryness. The product was washed with methanol to remove other byproducts. The residual mixture was extracted with toluene.

Step 2: ~100 mg of crude product was dissolved in 0.50 mL of toluene and subjected to thermochemical treatment with excess phenylethane thiol (0.50 mL) at 80°C under stirring,
while monitoring with MALDI-MS. After meta-stable clusters disappeared (~4 to 7 days), the product was isolated, washed with methanol several times and extracted with toluene.

*Step 3:* There were several types of nanomolecules in the thermochemically treated product, 36 (major fraction), 76.3, 115 and 200 kDa sized nanoparticles. The solvent fractionation used to isolate the nanoparticles observed at 115 kDa and 200 kDa mass regions.

![Scheme 7.1 Synthesis and isolation of plasmonic nanocrystals](image)

Scheme 7.1 Synthesis and isolation of plasmonic nanocrystals: The synthesis was carried out in three steps. First, crude nanoparticles mixtures were synthesized. Then, thermochemical treatment was conducted to narrow down the size distribution and remove the meta-stable nanoparticles. Finally, solvent fractionation was continued to isolate the pure plasmonic nanocrystals.
7.3.2 Mass spectrometry

MALDI mass spectrometry was undertaken using Bruker AutoFlex 1 and using DCTB matrix.\textsuperscript{3} ESI-MS was measured using Waters Synapt HDMS instrument and sample mixed with 50:50 toluene:CH$_3$CN or 50:50 THF:CH$_3$CN solvent system. Au$_{25}$(SCH$_2$CH$_2$Ph)$_{18}$ and Au$_{144}$(SCH$_2$CH$_2$Ph)$_{60}$ were used for calibration checks. Reactions were monitor using MALDI mass spectrometry.

![Figure 7.1 MALDI-MS of (a) crude product, (b) product after thermochemical treatment for ~4 days, and isolation of (c) Faradaurate-329 [Au$_{329}$(SR)$_{84}$] (d) Faradaurate-500 [Au$_{500}$(SR)$_{120}$], and (e) Faradaurate-940 [Au$_{940}$(SR)$_{160}$] by solvent fractionation. Asterisk indicates 2+ charge states.](image-url)
Figure 7.1a shows the mass spectrum of the crude product obtained in step 1. Figure 7.1b shows the mass spectrum of the product after the thermochemical treatment in step 2. This spectra shows how the sample with the broad mass spectrum is reduced to a few major, highly stable species, including the 329, ~500, and ~940 atom species. Figure 7.1c-e shows the products obtained during the solvent fractionation and purification process described in step 3 and scheme 7.1. The interesting aspect of this figure 7.1b is the presence of ultra-small Au\textsubscript{38} nanomolecules, and then the middle mass range contains Au\textsubscript{130} and Au\textsubscript{144} nanomolecules, followed by the plasmonic 76.3, 115 and 200 kDa nanoparticles all in one synthesis batch.

7.3.3 STEM analysis

Samples were prepared for STEM analysis by drop casting a suspension of particles in toluene onto lacey carbon films supported on 3 mm Cu grids. Nanoparticles were imaged in a JEOL JEM-2200FS with a CEOS aberration corrector on the probe-forming system. Because the particles proved to be sensitive to the electron beam, images were recorded with a probe current of 7 pA and dwell time of 10 µs to limit beam dose. At higher magnification, neighboring particles were prone to rapid necking and coalescence. Thus, for particle counting analysis, areas of well-separated particles were chosen, and coalesced particles were excluded from the counting statistics. While cooling the particles in a cryogenic holder slightly improves particle stability, this was not routinely used to obtain images for particle counts.

7.3.4 High-energy X-ray and pair distribution function (PDF) analysis

The total scattering data suitable for PDF analysis were collected at beam line 11-ID-B at the Advanced Photon Source at Argonne National Laboratory. Samples were loaded in polyimide tubing of ~1 mm diameter. A large area detector (Perkin-Elmer amorphous silicon)
was combined with high energy X-rays ($\lambda = 0.2128$ Å, E ~58 keV) to collect data to high values of momentum transfer ($Q$). The two-dimensional images were reduced to one-dimensional diffraction data as a function of $Q$ within fit2d. The data were corrected for background and Compton scattering within pdfgetX2 as described previously.$^{49,48}$ Fourier transform of the data to $Q_{\text{max}} = 22$ Å$^{-1}$ gave the PDFs, $G(r)$. The calculated atomic PDF and PDF fitting was performed using PDFgui.$^{36}$

7.3.5 Small angle X-ray Scattering (SAXS) analysis

The SAXS measurements were performed at the 12-ID-B beam line of APS using 12 keV X-ray energy. The SAXS data were collected with Pilatus 2M detector (DECTRIS Ltd.) cut-off energy was set as 10 keV to eliminate possible fluorescence background. The beam size with 0.05 x 0.2 mm$^2$ and exposure times of 1s used for the measurement. The data were fully corrected, reduced to intensity vs scattering vector ($q$) profiles, and background subtracted, using the software package at the beamline.

7.4 Super stable Au$^{\sim500}$ (SR)$^{\sim120}$ nanocrystals

7.4.1 Mass spectrometric identification

The composition of the Faradaurate-500 was determined using MALDI and ESI mass spectrometry. The red and blue curves in figure 7.2 show the MALDI and ESI-MS spectra of the purified nanoparticles, featuring a mass at 115 kDa. MALDI shows broad peaks at 115 and 67 kDa which are the singly and doubly charged ions of the Faradaurate-500. The breadth of these features is associated with the resolution limits of the MALDI-TOF (time-of-flight) tube at high mass range and also fragmentation/ligand loss in the laser beam. The MALDI does show the purity of isolated nanocrystals.
Figure 7.2 Mass Spectrometry. MALDI (red) and ESI (blue) mass spectra of ‘115 kDa’ nanoparticles yielding a composition of Au$_{500±10}$(SCH$_2$CH$_2$Ph)$_{120±3}$. MALDI-MS shows 1+ and 2+ ions, while ESI yields 3-, 4-, 5- and 6- ions. Deconvolution of the six charged ions is consistent with a 115 kDa molecular ion. The inset shows the deconvoluted spectra of Au$_{500}$ protected by –SCH$_2$CH$_2$Ph and –SC$_6$H$_{13}$ ligands, where the 3-, 4- and 5- ESI peaks are multiplied by 3, 4 and 5 to yield the 1- molecular ion, at 114.8 kDa. From the mass difference, the total number of ligand was calculated to be 120.

High resolution mass analysis was performed using ESI-MS for composition assignment. Electrospray ionization of Faradaurate-500 shows multiply charged peaks at 38.23, 28.62, and 22.97 kDa corresponding to the 3-, 4-, and 5- intact ions, respectively. The ESI peaks exhibit broad features as shown in the figure 7.2. This is due to the presence of several stable species and/or gold thiol combinations. The observed mass peaks suggest the existence of at least 20
intact species in the 115 kDa mass range, and the composition of the Faradaurates was assigned to be \( \text{Au}_{500}(\text{SCH}_2\text{CH}_2\text{Ph})_{120} \). The composition was confirmed by the mass difference found when preparing the nanoparticles using another ligand (HSC\(_6\)H\(_{13}\)). In this case, ESI-MS of nanoparticles with two ligands of varying mass yields the number of ligand molecules. Based on the number of ligand molecules, the total number of metal atoms could be calculated. All of the above evidence supports a composition of \( \text{Au}_{500}(\text{SR})_{120} \). The width of the peak was analyzed to get an estimate of the number of species present, yielding a composition of \( \text{Au}_{500\pm10}(\text{SCH}_2\text{CH}_2\text{Ph})_{120\pm3} \), as discussed later.

### 7.4.3 Scanning transmission electron microscopy analysis

Direct imaging of the gold nanoparticles of less than 5 nm diameter is challenging due to the electron beam induced instability, dynamic aggregation and sintering.\(^{46}\) This may lead to changes in the atomic structure and can limit the analysis of the degree of size homogeneity, nanoparticle shape, and individual particle size. Figure 7.3 show low-magnification and high-resolution HAADF-STEM images of the \( \text{Au}_{500}(\text{SR})_{120} \) particles, to study both the size dispersity and the atomic structure. The low-magnification images in figure 7.3a, 3b present representative ensembles of particles, including regions of self-assembly (see the expansion in Figure 7.3c). The self-assembled particles demonstrate the size-selected and monodisperse nature of the sample. The other regions show isolated particles that are well separated from each other.
Figure 7.3 HAADF-STEM images of Au$_{500}$(SR)$_{120}$. (a-c) Low-magnification images of dispersed Au$_{500}$. (d-h) High-resolution images of individual particles.

Figure 7.3d-3h show the high resolution aberration corrected HAADF-STEM images of the Au$_{500}$(SR)$_{120}$ nanoparticles. These images illustrate the arrays of surface atoms on the particles, in addition to their size and shape. By observing single particles at different orientations one can, in principle, study the surface morphology of the individual Au$_{500}$(SR)$_{120}$ nanoparticles. This is because the intensity of the HAADF-STEM image is dependent on both atomic number and number of atoms in the each atomic column. However, rapid surface atom diffusion or loss, and reconstruction, is often observed under the high dose rate required for high-resolution imaging. The electron beam can also induce a change in particle size and shape. Therefore, care was taken to try to minimize dose ($<1.0 \times 10^8$ e$^-$/Å$^2$) and only first-pass images were used in the analysis.
7.4.4 High energy X-ray scattering and PDF analysis

Figure 7.4 Experimental atomic PDF of Au_{500±10(SR)}_{120±3} in comparison with calculated PDF patterns of icosahedral Au_{309}, Marks Dh Au_{389} and 100-nm fcc (bulk-like) patterns. PDF patterns of Au_{500} show decent match with Marks Dh and fcc patterns.

High energy X-ray total scattering measurements include both Bragg and diffuse contributions to the scattering intensity. Fourier transformation of the background corrected data to real space yields the PDF. The atomic PDF provides a histogram of all the atom-atom distances in a material. The PDF method has significant advantages over other local structural probes, such as X-ray absorption spectroscopy (XAS) and there is negligible radiation damage of the sample as the monochromatic high-energy X-rays (>60 keV) are weakly absorbed. PDF
data is obtained at longer length scales (>10 Å) compared to XAS, yielding insights into size, shape and atomic arrangement; the inter-atomic distances measured are absolute and can be compared with the PDFs calculated for structural models.

Figure 7.4 shows the atomic PDF patterns of the Faradaurate-500 compound. To evaluate the structure, experimental data were compared with the PDF of icosahedral, decahedral and fcc shapes. The Au$_{309}$ icosahedron and Au$_{389}$ Marks decahedron were selected as their size is close to that of the metallic core with a shell of Au adatoms in the form of -SR-Au-SR- units. The 100 nm Au nanoparticle purchased from Sigma-Aldrich was chosen to represent the cubic closed pack system, which has a bulk-like fcc structure. Comparing the experiment PDF of Au$_{-500}$ with various models in Figure 7.4 shows that there is a poor match with Au$_{309}$ icosahedron. However, the experimental PDF of Au$_{-500}$ equally matches both the Au$_{389}$ Marks decahedron (Dh) model and the 100 nm Au NP with a bulk-like fcc structure.

In addition to atomic structure insights via PDF, information about the nanoparticle size can also be obtained by modeling how the intensity of features in the PDF at longer distances attenuates. The longest interatomic distances observed in the PDF, at ~2.4 nm, correspond to the size/diameter of the particles. This is in agreement with the size measurements from HAADF-STEM images shown above. The PDFs were dominated by atom-atom correlations associated with Au-Au interactions; Au-S and other correlations involving more weakly scattering carbon and hydrogen atoms did not contribute significantly to the measured PDFs. Features in the PDF of the Au$_{-500}$ sample extended to 25 Å, while longer range features are observed in 100-nm particles. Considering the total number of Au atoms and assuming monomeric [-SR-Au-SR-] arrangement for the interface, the Au$_{405}$ and Au$_{389}$ core can be proposed for the Au$_{-500}$(SR)$_{-120}$
particles, which contain the 5-shell structure (4-shells of core and an outermost shell with [-SR-Au-SR-] bonding).

Figure 7.5 PDF fits. (Right) Truncated octahedral Au$_{405}$ model and PDF fitting of Au$_{405}$ TO model with that of the experimental PDF of Au$_{500}$(SR)$_{120}$ particles (R$_w$ = 37%). (Left) Marks decahedral Au$_{389}$ model and PDF fitting of Au$_{389}$ Marks Dh model with that of the experimental PDF of Au$_{500}$(SR)$_{120}$ particles (R$_w$ = 23%).

STEM images in figure 7.3d,g, and h seem to suggest an fcc shape, while images in Figure 7.3e and f are ambiguous and may have a decahedral shape. Different particle shapes were observed, due to the random orientation of the Au$_{500}$(SR)$_{120}$ nanocrystals on the substrate. PDF fitting was performed with Au$_{405}$ truncated octahedral and Au$_{389}$ Marks decahedral model.
using PDFgui. Based on PDF fits and HAADF-STEM analysis, both Au$_{405}$ truncated octahedra and Au$_{389}$ Marks decahedron models are the most probable structures for the inner core of the Au$_{500}$ nanoparticle.

7.4.5 Small angle X-ray scattering (SAXS)

Figure 7.6 shows the SAXS data for Au$_{500}$. SAXS provides independent, statistically representative, ensemble measurements of the size and conformation of nanoparticles. The radius of gyration, R$_g$, of the nanoparticles obtained from the SAXS Guinier fit, i.e., Ln[I(q)] = Ln[I(q=0)] – R$_g^2q^2/3$ at q close to zero, is 1.05 ± 0.05 nm. The wide range of linear Guinier behavior (Ln[I(q)] vs. q$^2$, Fig. 7.6a inset) and the scattering oscillation features at a q of 0.3-0.8 Å$^{-1}$ strongly suggest that the Au$_{500}$ nanoparticles are nearly monodisperse in solution. However, a simple spherical model, including a size distribution, was unable to fit the data. A three-dimensional molecular envelope were reconstructed from the SAXS data using an ab initio program DAMMIN$^{51}$ and this SAXS envelope adopts an ellipsoid-like shape with dimensions in the range of 2.2-3.0 nm, displayed in Figure 7.6b. This SAXS molecular envelope is consistent with the HAADF-STEM images, in terms of shape and size, and interestingly, it also exhibits facet features. It should be pointed out that since the electron density of the SR ligand is much less than that of the Au-cluster core, the SAXS data and the molecular envelope are dominated by the contribution from the Au$_{500}$ nanoparticle core.
Figure 7.6 SAXS data, data fits, and the derived SAXS molecular envelope for Au$_{500}$ nanoparticles. (a) SAXS data (black solid circle with error bar) were collected up to 0.8 Å$^{-1}$. The red curve is a representative fit for the molecular envelope calculation. The molecular envelope, also known as a bead model, was calculated using the program DAMMIN, with SAXS data up to 0.6 Å$^{-1}$. The data points in the red curve beyond 0.6 Å$^{-1}$ were calculated from the resultant molecular envelope, matching well with the experimental data. The inset is the Guinier fit for SAXS data at q close to 0. $R_g$ obtained from the data fit is 1.05 ± 0.05 nm. (b) The two views of the final SAXS molecular envelope with dimensions. Twenty independent molecular envelope calculations were performed and the resulting envelopes were further averaged to generate the final, consensus molecular envelope. The error bar of the dimensions is expected as 0.1-0.2 nm.
7.4.5 UV-visible spectra

Figure 7.7 UV-visible spectroscopy of Au$_{\sim500}$SR$_{\sim120}$ (red) showing the surface plasmon resonance peak at ~498 nm compared to the Au$_{329}$SR$_{84}$ (black solid). Au$_{144}$SR$_{60}$ (dashed) shows no surface plasmon resonance and Au$_{38}$SR$_{24}$ (dotted) show molecule-like transitions.

Figure 7.7 presents the UV-visible of the Au$_{\sim500}$SR$_{\sim120}$ in comparison with other reported nanomolecules with 38- and 144-atoms, as well as the plasmonic Au$_{329}$SR$_{84}$. The bottom spectrum shows the molecule-like transition of Au$_{38}$SR$_{24}$ nanomolecules. The optical spectrum of Au$_{144}$SR$_{60}$ shows the discrete electronic states of the nanoparticles, but due to the overlap of various electronic transitions, it appears featureless. However, recent studies show that the origin of localized surface plasmon resonance of the Au$_{144}$SR$_{60}$ is confined to the metallic core.$^{146, 147}$ The Au$_{329}$SR$_{84}$ nanomolecules support a distinct surface plasmon
resonance (SPR) peak at ~490 nm. This is due to the collective oscillation of the conduction electrons. \( \text{Au}_{-500}(\text{SR})_{-120} \) also show the surface plasmon resonance at ~498 nm. However, this peak is not prominent compared to the \( \text{Au}_{329}(\text{SR})_{84} \). This is due to the presence of several overlapping stable species, which show SPR in the 480-510 nm range.

7.4.6 Size dispersity and transition from fixed-composition to polydisperse nature

Comparing the mass spectra of a mixture of \( \text{Au}_{329}(\text{SR})_{84} \) and \( \text{Au}_{-500} \) species in figure 7.8(top) shows that there is a large difference in the peak width and full width at half maximum (fwhm). Figure 7.8(bottom) shows the expanded version of the 5- of the \( \text{Au}_{-500} \) and the 3- of the \( \text{Au}_{329}(\text{SR})_{84} \). The width of the 5- of the \( \text{Au}_{-500} \) peak at the baseline is approximately 800 m/z. Deconvolution of this peak to the molecular 1- ion leads to a width of 4000 Da. In the extreme case, where the entire 4000 Da difference is due to Au atoms, a variation of 20 Au atoms would be observed. If the entire mass is due to ligand, then it would lead to a variation of 29 ligand. Nevertheless, a more accurate prediction is to calculate the percent variation of Au atoms (20/500=4%) and apply it to number of ligand. This would lead to a reasonable variation of (120×0.04=5) five ligands. Also, note that the expanded 5- peaks show ~ 30 partially resolved individual peaks within the envelope. Thus, composition of the compound can be deduced as \( \text{Au}_{-500\pm10}(\text{SR})_{-120\pm3} \) with over 30 individual \( \text{Au}_m(\text{SR})_n \) species present.
Figure 7.8 (a) ESI-MS of \( \text{Au}_{329}(\text{SR})_{84} \) and \( \text{Au}_{-500} \) nanoparticles that were intentionally mixed to study the peak width and size dispersity. Note the sharp peaks for \( \text{Au}_{329}(\text{SR})_{84} \) when compared with the \( \text{Au}_{-500} \) peaks. (b) Expansion of the 5- peak of \( \text{Au}_{-500} \) and the 3- peak of the \( \text{Au}_{329}(\text{SR})_{84} \). The fwhm of the 3- peak of \( \text{Au}_{329}(\text{SR})_{84} \) is 23 m/z suggesting that it is a fixed composition, with \( \pm 0 \) atom variation. The \( \text{Au}_{-500} \) nanoparticle is broad (fwhm of 800 m/z) and shows overlap of several peaks, suggesting a size dispersity of approximately 20 Au atoms.

The width of the \( \text{Au}_{-500}(\text{SR})_{-120} \) mass spectra was analyzed to determine the size dispersity. For this purpose, \( \text{Au}_{329}(\text{SR})_{84} \) were intentionally mixed with that of the \( \text{Au}_{-500} \) nanoparticle. \( \text{Au}_{329}(\text{SR})_{84} \) has one fixed composition, with a \( \text{Au}_{329}(\text{SR})_{84} \) formula, with no variation in the number of Au atoms and ligand.\(^{148} \)
Nanomolecules protected by aliphatic thiols or phenylethane thiol include Au\textsubscript{25}(SR\textsubscript{18}, Au\textsubscript{38}(SR\textsubscript{24}, Au\textsubscript{67}(SR\textsubscript{35}, Au\textsubscript{130}(SR\textsubscript{50}, Au\textsubscript{144}(SR\textsubscript{60}, and Au\textsubscript{329}(SR\textsubscript{84}. These have a molecular definition with \( \pm 0 \) metal atom and ligand variation. The only ultra-small, < 2 nm sized phenylethanethiol-protected nanoparticle which does not have a molecular definition is the Au\textsubscript{103-105}(SR\textsubscript{45-46} species. However, this species, upon thermochemical treatment at 80°C, is unstable, resulting in a core size conversion that produces a new core size, Au\textsubscript{40}(SR\textsubscript{24}.\textsuperscript{39} Nanomolecules that are stable under 80 °C thermochemical excess thiol treatment include the 25-, 38-, 130-, 144- and 329- atom species. The Faradaurate-500 species is also highly stable because its synthesis involves a 80°C thermochemical treatment for 4-7 days. However, the highly stable Faradaurate-500 does not contain a single species with fixed composition. It contains at least 30 different nanoparticle compositions. Several control experiments of known nanomolecules performed under similar conditions were free to fragmentation. Results in Figure 7.8 shows that the important transition from a fixed composition to a polydisperse composition occurs within the 329- and 500-atom range.

7.4.7 Exemplary stability

Note that these sizes are extremely stable in air and under thermochemical treatment at 80 °C in excess thiol. Nevertheless, the Au\textsubscript{500} nanoparticle, which underwent 80°C thermochemical treatment for several days, still remains polydisperse with a composition of Au\textsubscript{500±10}(SR)\textsubscript{120±3}. The superatom model\textsuperscript{150} relies on electronic stability due to closed electronic shells. There is not a singular fixed composition indicate that the Au\textsubscript{500} is not stabilized due to electronic considerations. This would suggest that geometric stability\textsuperscript{104} plays a more significant role in the stability of Faradaurate-500. Au\textsubscript{25}(SR)\textsubscript{18} and Au\textsubscript{102}(SPh-COOH)\textsubscript{44} are stabilized by two factors:
(a) electronic factors exhibiting 8 and 58 electron shell closing, respectively (b) geometric factors exhibiting a 13 atom icosahedral core and a 49 atom Marks decahedral core respectively. Note that Au$_{144}$(SR)$_{60}$ and Au$_{329}$(SR)$_{84}$ has 84 and 245 electrons, respectively. So none of the species in the series, namely 144-, 329- and ~500-atom species exhibit electronic shell closings. This suggests that as the nanoparticle size increases, the geometry plays a larger role in the nanoparticle stability. However, as the size increases, there are apparently various possible combinations that would lead to a stable nanoparticle.

7.4.8 Conclusions

The composition of the plasmonic Faradaurate at 115 kDa determined as Au$_{-500}$(SR)$_{-120}$. HAADF-STEM imaging shows that the particles are monodisperse and the diameter is 2.4 ±0.1nm. SAXS results support the monodispersity and the particle diameter was determined to be 2.6 nm, in agreement with the STEM analysis. High-energy X-ray PDF analysis suggests that the atomic structure of Faradaurate-500 is consistent with both fcc or a Marks decahedral atomic arrangement. Finally, plasmonic peaks for these particles appear at ~498 nm. The extinction coefficient was determined to be $1.42 \times 10^6$ M$^{-1}$cm$^{-1}$ which is supported by ~380 free electrons. These plasmonic nanoparticles can be used in plasmonic application such as optical sensors, drug delivery, and cancer cell detection and also in catalytic applications.
7.5 Super stable Au-940(SR)-160 nanocrystals

7.5.1 Mass spectrometry

Figure. 7.9 Mass Spectrometry. MALDI (red) and ESI (blue) mass spectra of 200 kDa nanoparticles synthesized using HSCH$_2$CH$_2$Ph thiol. MALDI-MS with DCTB matrix shows 1+ ions, while ESI-MS yields 4-, 5-, and 6- multiply charged ions. The theoretical values for charge states 1, 2, 3, 4, 5, and 6 are shown by dotted lines at the top. The inset shows the deconvoluted ESI mass spectra of 940-atom nanoparticles protected by –SC$_6$H$_{13}$ and –SCH$_2$CH$_2$Ph. The deconvolution of the multiply charged ions (4-, 5- and 6- ESI peaks are multiplied by 4, 5 and 6 respectively, to yield the 1- molecular ion at 200 kDa) of the multiply charged ions. This mass difference was used to calculate the number of ligands. The composition of the nanoparticles was determined to be Au$_{940±20}$(SR)$_{160±4}$. 

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Figure 7.9 shows the MALDI and ESI-MS of the 200 kDa nanoparticles synthesized using phenylethanethiol. MALDI-MS (Red curve) obtained using a DCTB matrix shows a single peak at ~200 kDa, showing the purity of the nanoparticles. The broad peak in MALDI with a full width at half maximum, fwhm, of 6 kDa is due to the resolution limit of the time-of-flight (TOF) tube and fragmentation due to the incident laser energy. Therefore, ESI-MS, a softer technique when compared with MALDI-MS, was employed to determine the composition without fragmentation. Figure 7.9 (Blue) shows the electrospray ionization mass spectrometry with multiply charge peaks at 51.8, 41.6 and 34.6 kDa which correspond to the highest points of the 4-, 5- and 6- molecular ions. The deconvolution of these multiply charge peaks (multiplying m/z values of the 4-, 5- and 6- peaks with 4, 5 and 6 respectively) resulted in peaks all centering at 207.5 kDa as shown in the inset. This suggests that the three ESI peaks all correspond to the same MALDI peak at ~200 kDa, but are present with different number of charges. The initial compositional assignment was made by ESI-MS to be Au\textsubscript{940}(SR)\textsubscript{160}. However, further compositional verification was made by the synthesizing the Au\textsubscript{940}(SR)\textsubscript{160} with a second thiol (hexanethiol) using the same synthetic protocol. The molecular weight of phenylethane thiol, C\textsubscript{6}H\textsubscript{5}CH\textsubscript{2}CH\textsubscript{2}SH and hexanethiol, C\textsubscript{6}H\textsubscript{13}SH is 138 and 118 Da respectively. The difference in the mass of the ligand, 20 Da was first used to assign the total number of ligand, 160. From the ligand count, the number of gold atoms was back calculated. Assigning the composition of a newly synthesized nanoparticle by the mass difference of nanoparticles prepared using two different ligand, was successfully used by several research groups. Deconvoluting the multiply charge peaks to the molecular 1- ion leads to a width of 8250 Da. The extreme case, where the entire 8250 Da difference corresponds to Au atoms, would lead to a variation of 40 Au
atoms or 940±20. If the entire mass is due to ligands, then it would lead to a variation of 60 C₆H₅CH₂CH₂SH ligands. The percent variation of Au atoms were calculated (40/940=4.2%) and apply it to the ligand to account for ligand variation. This would lead to a reasonable variation of (160×0.042=6.8) seven ligands. Finally, the composition of the 200 kDa determined to be Au₉₄₀±₂₀(SR)₁₆₀±₄. Control experiments were performed to ensure that fragmentation was minimized. For example, under identical conditions the multiple charge peaks Au₃₂₉(SR)₈₄ and Au₁₄₄(SR)₆₀ in the 30-40 kDa/z range yields sharp peaks, with fwhm of 30 Da suggesting that these 329- and 144-atom species contain one peak. The broad peak–fwhm of 8250 Da for the deconvoluted 1- peak, raises the question if the electrosprayed Faradaurate-940 ions are accompanied by solvent molecules. The desolvation temperature in the ESI source was 200 °C. Control experiments using Au₁₄₄(SR)₆₀ and Au₃₂₉(SR)₈₄ under identical conditions show no additional broadening of the peaks suggesting absence of solvent molecules.

7.5.2 Scanning transmission electron microscopy analysis

Figure 7.10a and b shows the low-magnification STEM images of ensemble of particles (1000’s per frame), displaying the monodispersity of the particles. Furthermore, the atomic structure was studied in high-resolution STEM images. Atomic resolution high-angle annular dark-field (HAADF) STEM images show the fcc-like, 2.9 ± 0.1 nm nanoparticles. Figure 7.10c-i show the representative atomic resolution images of the Au₉₄₀(SR)₁₆₀. Figure 7.10j shows the fast fourier transform of the particle in i. The measured lattice spacing are very close to that of bulk fcc Au. Faceting is also regularly observed in the atomic resolution images, as would be expected based on the PDF analysis, which indicated fcc truncated octahedra or Marks decahedra as possible structures for the Au₉₄₀(SR)₁₆₀ nanocrystals.
Figure 7.10  Electron Microscopy: STEM images of the Au\textsubscript{940(SR)}\textsubscript{160} nanoparticles. (a,b) Low-magnification STEM images (c-i) High resolution HAADF-STEM image of the individual nanoparticles.(j) Fast fourier transform of (i) showing lattice spots similar to bulk fcc Au oriented along the [0-11] zone axis

Further, HAADF-STEM images of the Au\textsubscript{940(SR)}\textsubscript{160} nanocrystals presented here are consistent with fcc truncated octahedral structure\textsuperscript{151,152} and the model presented in Figure 7.12. Thus, STEM imaging strongly suggests a fcc truncated octahedral structure, while also confirming the monodispersity of the sample.

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7.5.3 Atomic pair distribution function (PDF) analysis

Figure 7.11 (Left) Atomic pair distribution function (PDF) of Au$^{940}$(SCH$_2$CH$_2$Ph)$_{160}$ in comparison with naked commercially available 100 nm fcc-like nanoparticles. The calculated PDF of icosahedral Au$_{923}$, Marks decahedral Au$_{831}$ and truncated octahedral Au$_{807}$ are also included. Based on atomic PDF alone it is not possible to distinguish between decahedral versus fcc atomic structure. (Right) (a) Proposed six-shell Au$_{809}$ atomic model for the metal-core only portion of Au$^{940}$(SR)$_{160}$ nanoparticles. (b) PDF fit of the Au$_{807}$ TO model with that of the experimental PDF of Au$^{940}$(SR)$_{160}$.

Atomic pair distribution function is the sum of the inter-atomic distances associated with each pair of atoms within the nanoparticle.$^{153,125,126,154}$ However, the Au-Au atom correlations
are dominant due to the higher scattering function associated with Au atom, when compared with sulfur or carbon atoms. Figure 7.11 shows the synchrotron high energy X-ray based pair distribution function analysis of Au-940(SR)-160 nanoparticles, in comparison with commercially available fcc-like 100 nm gold nanoparticles. The calculated atomic PDF of other structure models of related size, such as the icosahedral Au$_{923}$ Marks decahedral Au$_{831}$ and truncated octahedral Au$_{807}$ are also included.\textsuperscript{128} Clearly the icosahedral model PDF patterns are significantly different than that of the experimental PDF pattern, suggesting that the core of Faradaurate-940 does not have an icosahedral arrangement. However, the PDF of Marks decahedron, truncated octahedral, and the 100-nm fcc-like naked Au nanoparticle (Sigma-Aldrich) all are closely related.

Based on PDF and STEM data, the Au$_{807}$ truncated octahedra model were propose as the most probable structure for the metal core of the Au-940 nanoparticles. The Au$_{923}$ cuboctahedral core model will allow only 17 Au-atoms for the ligand interface, which can interact with a maximum of 34 ligand. However, the proposed Au$_{807}$ TO model leaves 133 gold atoms, a reasonable number, to form a complete Au-SR interface, supporting 160 thiolate ligands. Figure 7.11 (Right) shows the proposed six shells of Au$_{807}$ truncated octahedron core for Au$_{940}$(SR)$_{160}$. The model consists of 13, 42, 92, 162, 252 and 246 atoms in each shell. using PDFgui,\textsuperscript{49} PDF fitting was performed for the Au$_{807}$ TO model with that of the experimental data of Au-940(SR)-160. The residual Rw value of 44\% is high. But this can in part be explained by the fact that we only considered the metal core of Au$_{807}$ and the remaining ~140 atoms in the interface were omitted.
7.5.4 Small angle X-ray scattering

Figure 7.12  SAXS data, fittings and the derived SAXS molecular envelope of Au$_{940}$ nanoparticle. (a) SAXS data (black open circle) were collected up to 0.6 Å$^{-1}$. The red curve is a representative fitting in molecular envelope calculation. The molecular envelope, also known as bead model, was calculated using program DAMMIN, with SAXS data up to 0.6 Å$^{-1}$. The inset is the Guinier fit for SAXS data at q close to 0. $R_g$ obtained from the fitting is $1.32 \pm 0.07$ nm. (b) Pair distance distribution function, p(r), derived from SAXS data in (a) using program GNOM. (c) The two views of the final SAXS molecular envelope. The length of the white bar is 1.0 nm. Twenty independent molecular envelope calculations were performed and the resulting envelopes were further averaged to generate the final, consensus molecular envelope.
Figure 7.12 presents the SAXS data of Au\textsubscript{940}(SR)\textsubscript{160}. Since the molecular weight is roughly proportional to the cube of molecular size, the narrow molecular weight dispersion (\(<\pm2\%\)) measured for Au\textsubscript{940} implies that its size falls in an even narrower dispersion, i.e., \(<\pm1\%). This nearly mono-dispersion of size was further supported by the wide range of linear Guinier behavior (\(\ln[I(q)]\) vs \(q^2\), Fig. 7.11 inset) and the strong oscillating features in the \(q\) range of 0.2-0.6 Å\(^{-1}\). The SAXS Guinier fit, i.e., \(\ln[I(q)] = \ln[I(q=0)] – R_g^2q^2/3\) at \(q\) close to zero, provides the radius of gyration, \(R_g\), of 1.32±0.07 nm. The pair distance distribution function that is the inverse Fourier transform of the SAXS profile and essentially a weighted population histogram of atom-pair distances, displays a Gaussian shape (Figure 7.12b), indicating the particle adopts a globular form.\(^{155}\)

The three-dimensional molecular envelope derived from the SAXS data using an \textit{ab initio} program DAMMIN\(^{51}\) exhibits an ellipsoid-like shape, and its length is about 3.3 nm and the cross-section about 2.5-2.8 nm, displayed in Fig. 7.12b. It should be pointed out that since the electron density of SR ligand is much less than that of the Au-cluster core, the SAXS data and the molecular envelope are dominated by the contribution from the Au\textsubscript{940} nanoparticle core. This SAXS molecular envelope is consistent with the STEM images, in term of shape and size, and interestingly, it also shows facet features. In contrast to SAXS measuring the overall particle size, PDF only probes the size of the ordered domain within the particle, which is about 2.4 nm for Au\textsubscript{940} showed in Figure 7.11. The size difference from SAXS and PDF measurements reflects the amount of disorder in the particle, most likely in the regions close to surface. Compared to STEM measurements, the SAXS represent an average of contributions from a statistically significant population of nanoparticles. For example, current SAXS data measured ca \(10^{12}\)
individual nanoparticles in the beam path.

7.5.5 Conclusion

Composition of 200 kDa nanocrystals were determined to be \( \text{Au}_{940\pm20}(\text{SCH}_2\text{CH}_2\text{Ph})_{160\pm4} \). This is the first instance in which ESI-MS was used to determine composition of thiolated gold nanoparticles to the atomic level, at such high mass, namely the 200 kDa mass range. HAADF-STEM (2.90 ± 0.1 nm) and SAXS (3.00 ± 0.2 nm) size measurement show good agreement and both shows monodisperse \( \text{Au}_{-940}(\text{SR})_{-160} \) nanoparticles. The plasmonic behavior of the nanocrystals with a molecular composition may open door for potential applications in sensors, solar cells, catalytic and nano-optical devices.
CHAPTER 8

CONTRIBUTIONS AND FUTURE DIRECTIONS
CHAPTER 8
CONTRIBUTIONS AND FUTURE DIRECTIONS

This chapter describes the contribution of this dissertation toward the plasmonic and alloy nanomolecules research and their future directions. Thiolated gold nanomolecules and nanocrystals have precise number of gold atoms and protecting organic thiolate ligands. These compounds process high stability, size dependent electrochemical and optical properties which make them promising in a variety of applications. This dissertation studied several areas of nanomolecules research in detail including synthesis and characterization.

We were able to established a robust synthesis protocol for atomically precise alloy nanoparticle synthesis for \( \text{Au}_{38-x}\text{Ag}_x\text{(SR)}_{24} \), \( \text{Au}_{144-x}\text{Ag}_x\text{(SR)}_{60} \) and \( \text{Au}_{329-x}\text{Ag}_x\text{(SR)}_{84} \). In 2014, we were able to crystallized silver alloy of \( \text{Au}_{25}\text{(SR)}_{18} \) nanomolecules. The crystal structure reveals that the gold core favor the hetero-atom incorporation except the central gold atom. This was the first crystal structure of the gold based thiolated alloy nanomolecules. This structural study will pave the way to fundamental understanding of the optical, electrochemical and catalytic properties of thiolated 25 metal atom Au-Ag alloy by knowing the active silver atom incorporation sites. According to the Thomson Reuters this report has been highlighted as one of the highly cited (top 1%) paper in year 2014 among the scientific community.

\( \text{Au}_{38}\text{(SR)}_{24} \) is one of the mostly studied nanomolecules due to the high stability, single-crystal X-ray structure, unique spectroscopic features and intrinsic chirality. In 2011, we
introduced series of $\text{Au}_{38-x}\text{Ag}_x(SR)_{24}$ alloy nanomolecules for the well established $\text{Au}_{38}(SR)_{24}$ nanomolecule system for the first time. Few years later, we were able to produce diffraction quality single crystals of $\text{Au}_{38-x}\text{Ag}_x(SR)_{24}$ for that lead to the structural investigation. We found important structural information of the alloy nanomolecules system including the possible silver atom doping sites and how these selected sites contribute for the stability. This is the first crystal structure of the $\text{Au}_{38}(SR)_{24}$ alloy family.

$\text{Au}_{144}(SR)_{60}$ is one of the extremely stable nanomolecules upon thermochemical treatment. Unlike the $\text{Au}_{25}(SR)_{18}$ and $\text{Au}_{38}(SR)_{24}$ system, $\text{Au}_{144}(SR)_{60}$ shows featureless optical spectrum. We were able to modulate this monotonous spectrum to enhanced optical spectrum by alloying with silver. Following our report, several computational and experimental research groups have been working on $\text{Au}_{144-x}\text{Ag}_x(SR)_{60}$ system based on their research interest including catalytic activity, electron microscopy and optical spectroscopy studies.

Synthesis and detailed analysis of several super-stable nanocrystals were introduced to the nanoparticle research community, including $\text{Au}_{329}(SR)_{84}$, $\text{Au}_{-500}(SR)_{-120}$ and $\text{Au}_{-940}(SR)_{-160}$. Total synthesis, isolation and comprehensive characterization using different analytical tools were discussed. These super-stable nanoparticles have great potential towards the optoelectronic and plasmonic applications. Further, solvent fractionation technique was established to systematically isolate the large plasmonic nanocrystals. The protocol involved, solvent mixture of toluene and acetonitrile and it lead to the successful mass spectrometric characterization with minimal interferences from other nanoparticle impurities.

Composition of the first plasmonic nanomolecules was determined using mass spectrometry to atomic precision. A comprehensive compositional determination using mass
spectrometric analysis with three different ligands, in positive and negative mode and Cs+ adduction, leads to a conclusive composition of $\text{Au}_{329}(\text{SR})_{84}$. Fundamental understanding of the ultra-small thiolated gold nanoparticles has been facilitated by structural analysis using X-ray crystallography. Such an in depth analysis of plasmonic nanoparticles remains a challenge due to the larger particle size and increased polydispersity. In such cases alternative techniques can be used for the structural analysis.

We used atomic pair distribution function (PDF) analysis of synchrotron-based X-ray total scattering data to obtained atomic-scale structural information for gold nanocrystal systems. Composition, size and shape determined the structure and properties of the nanoparticles. We introduced SAXS data along with pair distance distribution function analysis to the thiolated gold nanoparticle research, which is typically used in protein analysis. Low resolution atomic models were reconstructed from the SAXS measurements using an ab-initio calculations. All the synchrotron based X-ray experiment were conducted in collaboration with Argonne National Laboratory.

Previously, gold nanoparticles research was based on electron transmission microscopic studies to determine the size, shape and size dispersity. However, it is extremely difficult to image the nanoparticles at ultra-small scale due to the electron beam induced aggregation and sintering. We were able to capture the low and high resolution HAADF images of gold nanoparticles at 2-3 nm scale developing the STEM image acquisition strategy in collaborations with Oak Ridge National Laboratory. STEM experiments were conducted by applying minimal electron dose and taking only first-pass images for the analysis.

These developments and achievements in synthesis and characterization protocols will
lead to the synthesis of multi-metallic alloy nanoparticles system. Elemental properties of Pd, Pt and Ag can be combined into a single thiol protected gold nanomolecule. Heteroatom incorporation can be determined by using mass spectrometry and growing the diffraction quality single crystals. Finally, these findings will lead to the fundamental understanding of heteroatom distribution and their effect on stability, electronic structure and physicochemical properties of gold nanomolecules. Also future achievements in synthesis methodology coupled with analytical techniques will push forward the boundaries of the existing mass spectrometry window of plasmonic nanocrystals.


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HIGHLIGHTS

- Research and technical experience in chemistry/material science. Organometallic nanocrystals synthesis, physicochemical characterization and structure investigation.
- Creativity, multi-tasking and communication skills: 18 publications in peer reviewed journals (including ACSNano, JACS, and Analytical Chemistry and over 400 citations) and several presentations. One of our recent articles on nanomaterial analysis (J. Phys. Chem. Lett., 2014, 5,461-466) has been highlighted as “Highly Cited Paper (top 1%) in 2014” by Thomson Reuters.
- Strong experience in analytical instruments: mass spectrometry, TEM, XRD, AAS, FTIR, TGA, Chromatography and optical spectroscopy.
- Expertise on atomically monodisperse gold and alloy nanocrystals (±0 atom variation) synthesis.
- Team work skills: collaborative research with local, international institutes and working with top national laboratories such as Oak Ridge, Argonne and Lawrence Berkeley.

EDUCATION

- 2010-2016: Ph.D. in Analytical Chemistry
  University of Mississippi, MS, USA.
- 2006-2009: M. Phil. in Chemical Science
  Post Graduate Institute of Science, University of Peradeniya, Sri Lanka.
  Dissertation: Gibbsite Nanoparticles Based Arsenic Removal Methodology Development: Mechanistic and Molecular Modeling Investigations of Interfacial Processes.
- 2001-2005: B.Sc. [Honors] in Chemistry
  University of Peradeniya, Sri Lanka,
  Minor subjects: Biology and Computer Science
  Research project: Structural Study of Mycosorb, a Mycotoxin binder.
PROFESSIONAL EXPERIENCES

Graduate Research Assistant: University of Mississippi  2010-2016
- Method Development: Synthesized atomically precise (± 0 atom uncertainty) plasmonic gold and alloy nanocrystals at 1-4 nm range. Atomic composition were determined quantitatively
- X-ray studies: Applied powder and single crystal X-ray technique to understand the total structure and atomic arrangements of the metal in the nanostructure.
- Mass spectrometric analysis: Method development for mass spectrometric analysis of small nanomolecules using MALDI-TOF-MS and ESI-MS-QTOF
- STEM analysis: Examined the particle morphology, shapes, size, size dispersity and atomic structure
- Element analysis: Used high resolution mass spectrometry for quantitative analysis of gold and alloy nanoparticles. Utilized ICP-MS and EDX for further verification
- Separation and isolation: Method development to isolate nanoparticles in high purity. Applied various solvent systems and size exclusive chromatography for separation
- Thermal analysis: Applied thermo-gravimetric analysis(TGA) to study the composition and thermal stability of the nanomaterials

Research Assistant: Chemical and environmental modeling research group, Institute of fundamental studies  2006-2010
- Developed method for synthetic nano-clay material synthesis
- Determined the toxic metals (such as As, Hg, Cd and Pd) adsorption to nano-clay qualitatively and quantitatively using AAS.
- Examined the mineral-solution interfacial interactions using FTIR
- Investigated the thermal induced transformation of nano-clay polymorphs by TGA/DTA and XRD

TECHNICAL SKILLS
- Mass Spectrometry: Analyzed gold and alloy nanomolecules by MALDI-MS (Bruker Autoflex I and II) and high resolution ESI-MS (Waters Synapt HDMS instrument) (Both electro-spray and nano-spray mode of operation).
- XRD: Prepared Powder and single crystal X-ray samples for data collection (Bruker D8-Focus and Bruker Apex2), interpretation and structure determination.
- FTIR Spectroscopy: Experienced with Thermo Nicolet-6700 FTIR for surface sites and functional group characterization using Transmission, ATR and DRIFT mode of operation and interpretation skills
- Atomic Adsorption Spectroscopy (AAS): Hands on experience with GBC933 AAS, Flame, Flameless and Hydride Generation mode of operation.
- Thermo gravimetric analysis (TGA): Determined the organic content and formula for nanomaterials
- **UV-Visible-NIR**: Examined the electronic structure and optical properties of molecules including the low temperature measurement (78 to 298 K)
- **Chromatography**: Utilized size exclusive chromatography, HPLC, Gas Chromatography for analysis
- **Electron microscopy**: Used JEOL 2100 High resolution TEM for nanomaterial characterization
- **Electrochemistry**: Conducted potentiometric titration for surface sites characterization, cyclic voltametric (CV) and differential plus volumetric (DVP) analysis
- **NMR**: Utilized of Bruker 500 MHz and 300 MHz instruments for nanomaterial characterization.
- **Experience on Windows, Office package, Origin, Olex2, Crystal maker and ChemDraw**

**AWARDS AND HONORS**

- 2015: Graduate School Dissertation Fellowship, University of Mississippi
- 2015: Honorable Mention, Outstanding Student Manuscript Award, Mississippi Academic of Science
- 2014/11: Won second prize in the poster competition: American Chemical Society and National Center for Natural Products Research at University of Mississippi
- 2013-2014: Graduate Student Research Award, Local section of American Chemical Society

**International travels and scientific visits**

- 2013/11: Visited University of Geneva, Switzerland as part of collaborative research
- 2013/12: Visited University of Birmingham, United Kingdom as part of collaborative research
- 2012-2014: Travel and collaborative research with Oak Ridge National Laboratory, TN, USA
- 2012-Today: Travel and collaborative research with Argonne National Laboratory, IL, USA

**OUTREACH**

- Worked as a Teaching Assistant in undergraduate chemistry labs for seven semesters. The responsibilities include mentoring the labs and assess their performance.
- High School program: Actively involved in setting up chemistry laboratories at North Panola High School (NPHS), Sardis, Mississippi, USA.
- Involved in summer research (K-12 program) mentoring the high school students at university. Performed chemistry demonstration/outreach activates for the high school student and children.
PEER REVIEWED PUBLICATIONS


16. Nimmala, P. R. Theivendran, S. Barcaro, G.; Sementa, L. Kumara, C. Jupally, V. R. Apra, E. Stener, M. Fortunelli, A. Dass, A. Transformation of \(\text{Au}_{144}(\text{SCH}_2\text{CH}_2\text{Ph})_{60}\) to \(\text{Au}_{133}(\text{SPh-}t\text{Bu})_{52}\) Nanomolecules: Theoretical and Experimental Study. *J. Phys. Chem. Lett.* 2015, 2134-2139.

17. Liu, J., Krishna, S., Kumara, C., Chattopadhyay, S., Shibata, T., Dass, A., Kumar, S., Understanding \(\text{Au}_{98}\text{Ag}_{46}(\text{SR})_{60}\) nanoclusters through investigation of their electronic and local structure by X-ray absorption fine structure, *RSC Adv.* 2016, 6, 25368-25374


Abstracts and Presentations (selected)


