

Surface modification of gold nanoparticles and nanoclusters

Master's thesis
University of Jyväskylä
Department of Organic Chemistry
13.04.2016
Karolina Sokołowska

ABSTRACT

Gold nanoparticles are used in many beneficial technological applications in biochemistry, medicine and electronics. Among them, monolayer protected gold nanoclusters (MPCs) have received a significant attention in the scientific community due to their well-defined atomic structure, which is important for fundamental studies of nanoparticles properties and their functionalization. These particles, with a precise number of atoms, exhibit size-dependent optical, chemical and electronic properties. The thesis focuses on the structure, preparation, characterization, and properties of MPCs.

For multifunctional applications, gold nanoparticles are an ideal class of compounds for surface functionalization reactions. Incorporating various active groups into nanoparticles' surface opens new possibilities for broad applicability. The second part of this thesis describes surface modification methods of gold nanoparticles and MPCs. Typical surface modification methods are ligand exchange, chemical conjugation, physical conjugation, and bioconjugation.

PREFACE

The work presented in the thesis was carried out at Nanoscience Centre, Department of Chemistry, University of Jyväskylä from May 2015 to November 2015.

I would like to thank my supervisors Tanja Lahtinen and Lauri Lehtovaara for entrusting me with fascinating research topic. Their experience in the field and endless new ideas in both theoretical and practical parts were conclusive for the success of the work. I would also like to thank them for their guidance and for believing in me throughout this project.

In addition, a special thank you is owed to my family and relatives for their endless support, understanding and encouragement.

Jyväskylä, April 2016.

Karolina Sokołowska

CONTENTS

ABSTRACT	i
PREFACE	ii
CONTENTS.....	iii
ABBREVIATIONS	v
I LITERATURE PART	1
1 INTRODUCTION	1
2 MONOLAYER-PROTECTED CLUSTERS	3
2.1 Synthetic methods	4
2.1.1 Turkevich method	6
2.1.2 Brust-Schiffrin method	6
2.1.3 Modification of Brust-Schiffrin method.....	7
2.1.4 Other methods	8
2.2 The Synthesis	8
2.2.1 The Synthesis of $\text{Au}_{144}(\text{SR})_{60}$	8
2.2.2 The Synthesis of $\text{Au}_{25}(\text{SR})_{18}$	10
2.2.3 The synthesis of $\text{Au}_{102}(\text{pMBA})_{44}$	11
2.2.4 Effect of the different synthetic parameters	12
2.3 Structure	14
2.3.1 Isohedral core	16
2.3.2 Decahedral core	18
2.3.3 Other structures	20
2.4 Unique properties of nanometre sized metal clusters	21
2.4.1 Size dependent optical and electronic properties	21
2.4.2 Chirality properties.....	23
2.4.3 Charge dependent properties	24
2.4.4 Charge transfer properties	25
2.4.5 Catalytic activity.....	26
2.5 Methods for detection and characterization of clusters	27
2.5.1 Stability of the clusters.....	27
2.5.2 Particle size and chemical composition	28
2.5.3 Determination of the molecular weight of clusters by ESI-MS and MALDI-MS.....	29

2.5.4 Separation and purification of clusters by polyacrylamide gel electrophoresis (PAGE)	30
2.5.5 Analysis of nanoparticle formation and morphology by nuclear magnetic resonance (NMR) spectroscopy and fourier transform infrared (FT-IR) spectroscopy	30
3 SURFACE FUNCTIONALIZATION OF NANOPARTICLES AND NANOCLUSTERS	33
3.1 Ligand exchange	36
3.1.1 Mechanism of ligand exchange	37
3.1.2 Kinetics studies for ligand exchange on nanoparticles	39
3.1.3 Effects of surface binding groups and head groups	41
3.2 Chemical conjugation of gold nanoparticles	43
3.2.1 Coupling strategies	44
3.3 Physical conjugation of gold nanoparticles	46
3.4 Bioconjugation of gold nanoparticles	49
4 CONCLUSION	53
5 REFERENCES	56

ABBREVIATIONS

AuNPs	gold nanoparticles
BPDT	biphenyl-4,4' –dithiol
DCM	dichloromethane
DFT	density functional theory
DOSY	diffusion-ordered spectroscopy
FCC	face centered cubic
FTIR	Fourier transform infrared
HAuCl ₄	chloroauric acid
HOMO	highest occupied molecular orbital
LUMO	lowest unoccupied molecular orbital
MPCs	monolayer protected gold nanoclusters
MS	mass spectrometry
NaOH	sodium hydroxide
NH ₄ OAc	ammonium acetate
NMR	nuclear magnetic resonance
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SPR	surface plasmon resonance
PET	phenylethanethiol
<i>p</i> MBA	para-mercaptobenzoic acid
TEM	transmission electron microscope
TGA	termogravimetric analysis
THF	tetrahydrofuran
TOABr	tetraoctylammonium bromide
TPDT	p-terphenyl-4,4'' –dithiol
UV	ultraviolet
Vis	visible
XPS	x-ray Photoelectron Spectrometry

I LITERATURE PART

1 INTRODUCTION

Gold nanoparticles have been known for a long time, and they have an interesting scientific history.¹ First applications of gold nanoparticles took place over two thousand years ago, when they were mainly used in aesthetic and medicine.² Their colouring properties in ceramics and fabrication of ruby glass are still utilized nowadays. The scientific approach for studying nanoparticles was introduced by Faraday in the middle of the 19th century. Faraday created a preparation of disperse gold colloids in a solution.² Since then the number of researches has increased exponentially.

Currently one of the main interest in nanoscience research are metallic nanoparticles.^{3,4} Among them, the nanometre-size gold nanoparticles are stable particles which are widely employed in contemporary nanoscience studies.³ Therefore, many methods have been developed to prepare particles with a specific size and purity.⁵ Phase solution synthesis are practical method for preparation gold nanoparticles. They are easy to scale up, and therefore a large scale production of particles is possible.⁵

Gold nanoparticles can be categorized into two size regimes. The first one is in the range of subnanometre to 2 nm, and the second from 2 nm to 100 nm.² In the early works, particles were mainly called “colloidal golds” because of their size and the arrangement of atoms.² With the rise of knowledge, of particles sizes a term “nanoparticles” was introduced, and it mainly referred to the particles in the size range of 5-20 nm. The term “clusters” refers to smaller structures with defined numbers of atoms.³ Over past few years, gold nanoparticles have attracted more and more attention due to their unique properties, which enable scaling down electronic and optical devices. Variations in electronic and optical properties hold a potential for a wide range of applications including oscillators, transistors, sensors and switches.⁶ Their ability to stabilize charge in their cores is considered an essential property for future electronics applications.² Additionally, they have shown a potential to be used in bioscience studies as effective biosensors.⁷

The unusual physical and chemical properties of NPs differ from the corresponding bulk materials and atoms; they rather behave like an intermediate of those.⁸ The

transition from bulk material to nanomaterial can lead to a number of changes in the physical properties. Typical characteristics of gold nanoparticles include size-dependent electronic, optical and chemical properties.⁵ As the particles get smaller the surface area to volume ratio increases leading, to the dominance of numbers atoms which are on the surface of the material. A main feature of nanoparticles compared to bulk properties is that they exhibit a strong visible absorption in the optical spectrum, which is known as located surface plasmon resonance.⁸ Moreover, their melting point is lower than that of bulk materials and their charging can be quantized. The intense colour of gold nanoparticles larger than 3 nm is caused by their surface plasmon resonance (SPR). The plasmon band is sensitive to the size of particles and its intensity decreases as the core size decreases, due to the loss of metallic character and the appearance of quantum size effects.⁵

A crucial aspect of gold nanoparticles is their surface functionalization for multifunctional applications.⁹ Surface modification reactions, where the bound ligand can be conjugated or exchanged by the incoming molecule, is an important aspect of gold nanoparticles. The reactions of this type are used to provide chemical functionalities to the initially non-function nanoparticles by incorporating different kinds of chemically active groups.⁹ Chemical functionality can be tailored by introducing simple chemical groups, such as carboxylic acid, or by introducing biomolecules, therapeutic molecules or other molecules of interest.⁷ In addition, most of these properties are size-dependent and can be tuned by varying the size and shape of gold nanoparticles.

Monolayer-protected clusters (MPCs) are a special type of nanoparticles that possess high stability due to their protective coating formed by organic ligands.¹⁰ These small nanoparticles significantly differ from conventional plasmonic nanoparticles. The electrons of metal atoms are cramped in molecular dimension and the discrete energy level which provides various properties and therefore, they have become a fascinating area of interest.¹¹ The structure of MPCs is well-defined down to atomic scale which allows direct comparison of theoretical and experimental work.¹² This is crucial for fundamental studies of the properties of nanoparticles and the mechanisms of their functionalization.¹³ Therefore, noble metal clusters passivated by a monolayer of thiolate ligands are the main focus of this thesis.

The next chapter of literature review discusses the area of MPCs from the physical, chemical as well as biological point of view. For the electronics application, the most interesting properties, are their unique electronic and optical properties such as molecule-like energy gaps,¹¹ high catalytic properties⁵ and strong photoluminescence³ are discussed. The preparation and characterization methods as well as the development in understanding the cluster structure are also introduced.

In the last chapter of the thesis nanoparticles functionalization, with the main focus on MPCs modification, is discussed.¹⁴ The small size, well-organised structure, in addition to highly active surface area of MPCs enable various surface functionalization reactions to tune nanoparticles' properties, providing multifunctional applications.⁹ Functionalized nanoclusters have already found a great practical interest in catalysis or biosciences as an effective drugs deliverers or sensors.^{15,16} Because of their high surface flexibility they can carry therapeutic chemical groups, immune-stabilisers or translocating peptides.¹⁷ Therefore, for future applications, a fundamental understanding of nanoparticles' properties and modification techniques is required.

2 MONOLAYER-PROTECTED CLUSTERS

Monolayer-protected gold nanoclusters are a type of metal nanoparticles, typically ranging from subnanometer to 2 nm in size, coated by a dense, monolayer of ligands (e.g. thiols, phosphines, amines) (fig.1).³ The ligand layer protects the clusters from aggregation, and influences the physical and chemical properties of the particles.⁶ The interest in these clusters is due to their most typical metal–molecule interface which turned out to be the most untypical. Additionally, the special combination of atomic and electronic structures thus making them extremely stable.³

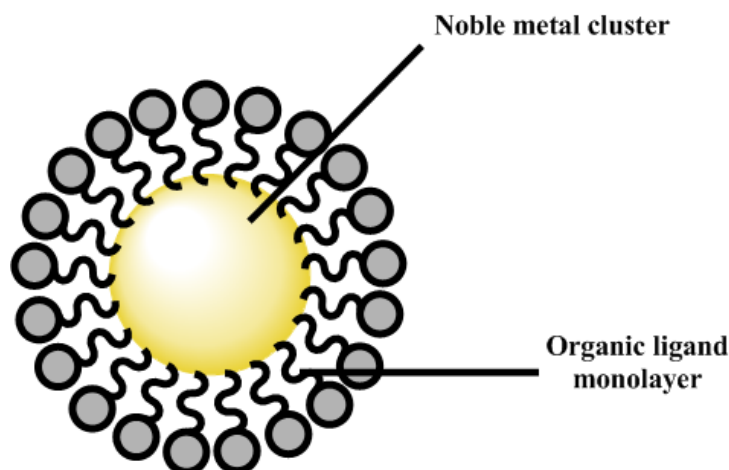


Figure 1. Schematic illustration of monolayer protected metal cluster.

In recent years among metal nanoparticles field, gold nanoclusters have become one of the most studied metal nanoparticles in the nanoscience field. The number of researches has increased exponentially, which opens up new, exciting opportunities for fundamental studies and future applications. The break-thoughts of the field of MPCs were given by Brust *et al.* in 1994.¹⁸ Their pioneering work was the preparation of stable alkanethiolate protected gold clusters. After that Murray *et al.* introduced place exchange reactions with another thiol ligands, which opened the new possibilities for surface modification chemistry.¹⁹ Ligand exchange reaction, where the MPCs surface bound thiol can be exchanged by other thiol is a powerful tool for introducing chemical functionality to AuNPs (gold nanoparticles).²⁰ Understanding kinetics and statistical nature of ligand exchange reactions give rise to the new application paths.⁹ Nanoclusters have already found a great practical interest for applications in catalysis, sensors and biochemistry.⁷ Nanoclusters are of great significance in catalysis due to their large surface to volume ratio and high number of surface atoms.⁵ Their ability to stabilize charge in the cores and to act as small capacitors is an important aspect in electronic applications.⁵

2.1 Synthetic methods

One of the main challenges in the gold nanocluster field is to develop synthetic chemistry routes which enable fabrication of monodisperse clusters.¹² Control of clusters fabrication is important in order to determine their structure and for understanding their size-dependent properties. The size dependent properties of nanoparticles require that the end product has a narrow size distribution. Therefore, it still remains as a challenge in the synthetic chemistry since the current knowledge of the

kinetics of particle growth is quite limited. Nevertheless, a lot of work has been done during past years, leading to remarkable progress in controlling the clusters with atomic precision.

The nanoparticles and sub-nanometre clusters can be synthesised through “bottom-up” and “top-down” approaches.^{21,22} For the top-down approach, the corresponding bulk matter is subdivided into smaller pieces, yielding large distribution of sizes. Bottom-up method of preparation gold nanoparticles results in defined building blocks. Generally, the synthesis usually begins with the reduction of the metal precursor to atoms and in the subsequent nucleation process metal clusters are formed.¹² The particles are coated with a stabilizing layer which inhibits the aggregation of the cluster core and terminates the growth of particles.

Most of the recently used synthetic methods of nanoparticles are based on the bottom-up synthetic strategies and are considered the best approach to produce size selected clusters.²¹ Even though the synthetic methods have a lot of important advantages obtaining a synthetic control still remains a challenge in MPC chemistry. Therefore, often some additional methodology can be applied, such as the size focusing processes.¹² Those methods enable determination of the core and surface atom rearrangement by effective control of the experimental parameters, permitting the most stable clusters to survive the size focusing process.²³ The methodologies are based on stability of the different sized clusters. Among them, etching, aging, annealing or ripening are based on “top-down” approaches. Currently all the size-focusing methods are based on procedure where the smaller clusters are formed from larger ones.¹² A lot of size focusing methods have been used to synthesis monodisperse metal clusters.

Although several methods for the preparation of hydrophilic and hydrophobic particles have been published and the number is still increasing, only a few of them have shown to be reliable and flexible to obtain a desired product. One of the most well-known syntheses is Brust-Schiffrin method.¹⁸ The pioneering work included the preparation of a stable monolayer protected cluster with alkane thiols. Another method is the older Turkevich synthesis where the gold salt is reduced in hot aqueous solution by citrate, producing water soluble particles.² This method is considered a good one because of high fraction of single size nanoparticles can be isolated from the synthesis. Therefore, Brust-Schiffrin and Turkevich methods are widely known.

2.1.1 Turkevich method

In 1951 Turkevich *et al.* introduced an experimental method which involved the reduction of tetrachloroaurate (HAuCl_4) in hot aqueous solution using sodium citrate, which acts as the reducing agent in this reaction.² The citrate's oxidation and decarboxylation products stabilize the particles by terminating the growth and preventing aggregation. The method produces water soluble particles ranging from 15-20 nm and is still commonly used. They also studied the effect of reagent concentration upon the nanoparticles' size and distribution. It was found that by decreasing the sodium citrate salt concentration and thus decreasing the number of stabilizing citrate ions the larger particles were formed upon aggregation.

Even though the citrate reduction has a great number of advantages, such as non-toxicity water solubility, inexpensive reductant and low pollution level however, this lack of stability restricts the variety of experimental conditions.² Weak bonds between citrate and gold particles make them unstable upon drying so large-scale manufacturing cannot be achieved.

2.1.2 Brust-Schiffrin method

Extended stability of the particles was achieved by Brust *et al.* in 1994.¹⁸ They investigated the method that included Faraday's two-phase fabrication for gold nanoparticles with self-assembly of thiolates on gold. Facile synthesis, simple handling, and the rapidity of the biphasic method have had a considerable impact on the field. A typical procedure involved transfer of gold ions from the aqueous phase by using tetraoctylammonium bromide (TOABr) as a fast phase transfer reagent to the toluene, and reduction of resulting polymeric gold-thiol complex with sodium borohydride (NaBH_4) in the presence of alkane thiol (fig.2). The reaction is usually completed just after the addition of the reducing agent, due to a really high concentration of hydride in the reducing agent which is typically NaBH_4 .

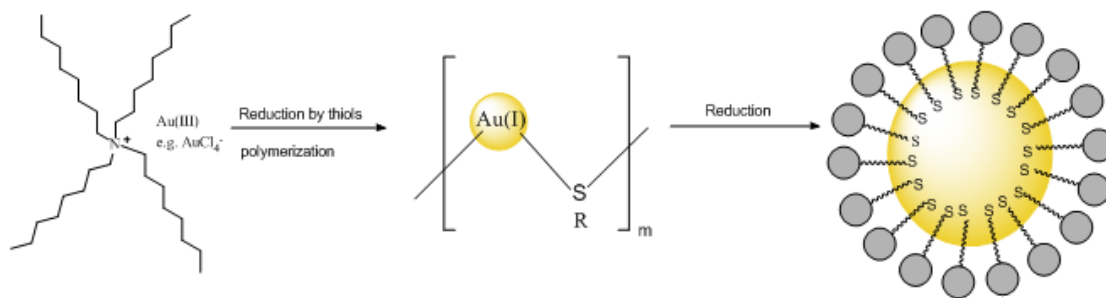


Figure 2. Synthesis of gold thiolate clusters via kinetic control.⁵

Originally, the Brust-Schiffrin method involved coating the gold with dodecanethiolate as a stabilizing ligand in an equimolar ratio.¹⁸ The monolayer-protected particles are extremely stable under drying conditions as well as in various solvents. The synthesis gives stable, easy to isolate, purified thiolate-protected gold nanoparticles with a diameter in the range of 1-3 nm. Another attractive feature of these nanoparticles is that they can be used for further synthetic manipulation, including surface functionalization.

2.1.3 Modification of Brust-Schiffrin method

Discovery by Brust and Schiffrin opened up many possibilities of preparing monodisperse nanoclusters. The method was modified, by optimizing the conditions, to prepare self-assembled monolayers of thiols on a bulk gold surface.²⁴ In 2004, Brust *et al.* extended the synthesis to *para*-mercaptophenol – stabilized AuNPs,²⁴ which rapidly grow to different synthetic methods, which can be used to stabilize a variety of functional thiols.²⁴

Nowadays, a number of different particles with a precise formula $\text{Au}_n(\text{SR})_m$ can be synthesised.¹² Revised Brust-Schiffrin syntheses based on modification of reaction conditions are currently available. The changes include ratio of thiol ligands to gold halide salt, the number of used solvents and different gold precursor molecules. For example, AuCl_3 can be substituted in the place of AuCl_4^- .

The modified one-pot synthesis is carried out in polar solvents.⁴ In general, larger thiol to gold ratios result in smaller average core sizes. The 3:1 thiol to gold ratio suggested first by Schaaff *et al.*²⁵ and verified by Goulet *et al.*²⁶ leads to formation of particles below 2 nm.²⁷ Nevertheless, in the size range below 2 nm, the control of the sizes is difficult to obtain by simply manipulating the thiol: Au ratio. Therefore, the synthesis procedure is a combination of the initial synthesis and the post-synthesis treatments,

including various size-separation methods, such as chromatography, solvent fractionation or fractional crystallization producing relatively monodisperse particles.

Based on these widely used methods, many types of synthesis have been developed. Nowadays, the clusters with a defined formula can be isolated and modified by changing the nature of ligands or reaction conditions.⁴

2.1.4 Other methods

In addition to thiol-based methods, the clusters can be synthesised with various ligands, such as amines, phosphines or sulphides.¹² The ligand plays an important role because it influences the cluster structure, solubility, and chirality. Therefore, the ligand must be chosen with respect to the desired properties.

The phosphine-stabilized cluster, known as a Schmid's cluster ($\text{Au}_{55}(\text{PPh}_3)_{12}\text{Cl}_6$), had long remained unique with a narrow size distribution (1.4 ± 0.4 nm).² The synthesis was first introduced in 1981, and it involved the reduction of Ph_3PAuCl by gaseous B_2H_6 in hot toluene or benzene. The synthesis results in which Au_{55} cluster with a stabilizing layer coordinated by PPh_3 and Cl . The phosphine stabilized particles are commercially available products can be used as bioconjugates.

Gold nanoparticles can be stabilized by other sulphur-containing ligands, including xanthates, disulphides, dithiols, trithiols, and resorcinarene tetrathiols. However, the binding affinity to the gold core is not as good as with thiols.² Recently, the impact of the presence of thiol and disulphide was studied on the size distribution of the gold nanoclusters which were obtained by Shiffrin method. The results indicated that in the presence of water, thiol is a better ligand than disulphide to produce small clusters.⁴ In contrast, disulphide is more successful in the reactions without water.⁶

2.2 The Synthesis

2.2.1 The Synthesis of $\text{Au}_{144}(\text{SR})_{60}$

Synthesis of $\text{Au}_{144}(\text{SR})_{60}$ was first reported by Huifeng Qian *et al.* in 2009.¹⁰ They developed a size focusing method without any post-synthesis treatments which turned out to be difficult.³ The two-phase method involved the preparation of truly monodisperse nanoparticles with the precise formula to be $\text{Au}_{144}(\text{SCH}_2\text{CH}_2\text{Ph})_{60}$. In this work, the first step involved the synthesis of the size focusing Au-cluster mixture by a

modified two-step Brust-Schiffrin method. High temperature and thiol concentration were used to obtain monodispersity.

Even though, the method is comparatively facile and gives high yield (20%) and avoids complicated size separation steps, the conditions of the reactions are relatively difficult to handle. First, etching requires using high concentration of thiol, producing intense odour. Second, due to the elevated temperature the method limits the use of low boiling ligands.^{10,3}

A simple and robust method was developed soon after the initial synthesis by the same group under ambient conditions.²⁸ Methanol was used as a solvent for the reaction and it turned out that the size focusing process occurs after the initial formation of Au clusters, preventing the growth of larger nanoparticles. In this one-pot synthesis, the gold salt precursor is mixed with an excess of thiol and tetraoctylammonium bromide to form Au(I)-SR polymers. Then, NaBH₄ as a reduction agent, is rapidly added leading to formation of two monodisperse sizes formation: Au₁₄₄(SR)₆₀ as a main product, and Au₂₅(SR)₁₈ as a side product.²⁸

Separation using different solvents has to be performed in order to remove the free thiol residue, and evolve the core to a specific number of Au atoms. Au(I)-SR species, which are poorly soluble, emerge as a white material and can be separated from the desired product using dichloromethane (DCM). In addition, the main product Au₁₄₄ can be easily isolated from Au₂₅, due to a large solubility difference in acetone by simple extraction.²⁸ The synthesis is more convenient and simpler in comparison with the previous two-step method. Moreover, the method's versatility and applicability enables it to be used with a wide range of thiols, including PhC₂H₄SH and various C_nH_{2n+1}SH (n= 4-8).

One-pot synthesis method of the pure Au₁₄₄(SCH₂Ph)₆₀ nanocluster was recently published by the Gao Li *et al.*²⁹ In the synthesis the product can be obtained after etching the reaction with polydispersed water-solvable Au_n(SG)_m through the combination of ligand exchange and size focusing process. First, the synthesis includes glutathione protected polydisperse cluster preparation, ranging from 400 nm to 1000 nm, by reducing Au(I)-SG in acetone. Subsequently, the size-mixed clusters react with the excess of H-SCH₂Ph ligand through ligand exchange process for 12h at 85 °C,

which leads to the polydispersed $\text{Au}_{144}(\text{SCH}_2\text{Ph})_{60}$ cluster. Then, particles are etched, resulting in stable monodisperse clusters. The structure was determined by electrospray ionization mass spectrometry (ESI-MS) and UV-vis spectroscopy.²⁹ Even though the method is not as convenient as the one described before, it still is based on ligand exchange phenomena that will be discussed later in the thesis.

2.2.2 The Synthesis of $\text{Au}_{25}(\text{SR})_{18}$

$\text{Au}_{25}(\text{SR})_{18}$ cluster is the best known in the literature and the most extensively studied MPC.^{12,3,30} Its small size and its extremely interesting properties, such as oxidation by air³, photoluminescence properties³, high stability with different ligands³ and unexpected reactivity with different types of salts³⁰ have been the main target for experimental investigation. However, the synthetic accessibility and isolation with good and monodispersity have also played an important role.¹² The identity of Au_{25} was initially mislabelled as $\text{Au}_{28}(\text{SG})_{18}$ and as $\text{Au}_{38}(\text{SCH}_2\text{CH}_2\text{Ph})_{24}$.³ The correct assignment of $\text{Au}_{25}(\text{SR})_{18}$ was labelled by Tsukuda group by electrospray ionization mass spectrometry (ESI-MS).³⁰

Water-soluble glutathione-protected $\text{Au}_{25}(\text{SG})_{18}$ nanoparticles were first synthesised by Tsukuda *et al.*³⁰ The synthesis involved mixing a gold salt precursor with glutathione ligand while adding excess of aqueous sodium borohydride. The reaction was cooled down to 0°C and conducted under vigorous stirring. The resulting polydisperse precipitate is washed with methanol and size-fractionated by polyacrylamide gel. The major drawback of the procedure is a relatively low yield, product polydispersity and lengthy fractionation.

Thiolate protected Au_{25} was also synthesised through two-phase protocols including the conversion of phosphine stabilized $\text{Au}_{11}(\text{PPh}_3)_8\text{Cl}_3$ cluster into thiolate protected $\text{Au}_{25}(\text{SG})_{18}$ via ligand exchange.¹⁴ Further improvements, used a modified version of Brust-Shiffrin reaction for preparing functionalized thiol-capped Au_{25} nanocluster.³² The size-focusing was used in the growth process to evolve into a desired size of the core. The method was also based on one-pot synthesis, which eliminated the phase-transfer agent and allows synthesis of Au_{25} nanoclusters with different capping thiols, such as water-soluble and long chain thiols as well as thiols bearing a polymerizable group.³²

Low temperature and slow stirring conditions lead to a direct formation of Au₂₅, thus eliminating the formation of larger clusters.³³ Moreover, it was observed that the careful control of Au(I)-SR formation influences the product's monodispersity. Surprisingly, Au₂₅ core framework is independent of surface thiolate ligands^{34,35}. As an example, 2-phenylethanethiol (-SCH₂ CH₂Ph), 1-dodecanethiol, 3-mercapto-2-butanol and 6-mercapto-hexane (-SC₁₂H₂₅, -SC₄ H₁₀O, -SC₆H₁₃), and bulky glutathione (glutathione, N-acetyl-L-cystine, N-formyl-glutathione and N-acetyl-glutathione) produce the same structure.^{34,35} The fluorescence properties of the MPC core, come not only from the metal core but also from the protecting ligands. Therefore the ligands with electron rich atoms such as -COOH or NH₂ can considerably enhance fluorescence.

2.2.3 The synthesis of Au₁₀₂(pMBA)₄₄

The structure of Au₁₀₂(pMBA)₄₄ was first reported in 2007.³⁶ However, the preparation of Au₁₀₂(pMBA)₄₄ was obtained before as a minor component of the mixture; for the first time the Kornberg group provided essentially pure material with a good yield. The synthesis was based on a careful control of the ratio between the mixed water and methanol in the presence of NaOH.³⁷ The size control was achieved by the fractional precipitation of clusters.

The preparation of water soluble Au₁₀₂(pMBA)₄₄ cluster is similar to the Brust-Schiffrin method except that the phase transfer TOA⁺ ions are not needed because the particles can be prepared in a water/methanol mixture.³⁷ Three-to-one ratio of *p*-MBA to gold is combined in water and 47% methanol resulting in the final gold concentration of 3 mM. Following the procedure, the reduction agent NaBH₄ was added in two to one ratio of BH₄⁻ to gold and the reduction was allowed to proceed for five hours minimum to as long as overnight. The monodispersity of the cluster was obtained by fractional purification with methanol.³⁷ Various analytical methods such as mass spectrometry (MS), UV-vis spectroscopy, Thermogravimetric analysis (TGA) and X-ray Photoelectron Spectrometry (XPS) gave a consistent size with the X-ray crystal structure measured for Au₁₀₂(pMBA)₄₄.²⁴

Later, Salorinne *et al.* synthesised water soluble clusters with the core size of Au₁₀₂ atoms, protected by *p*-MBA ligand.³⁸ Using DOSY (Diffusion-ordered spectroscopy), they studied, the hydrodynamic size of the cluster and found that the size of the cluster depends on the size and nature of the counter ion of the deprotonated *p*-MBA ligand.

The experimental results were proven theoretically by DFT calculation which has shown that the size and the choice of the counter ion affect the surface chemistry.

2.2.4 Effect of the different synthetic parameters

One of the most interesting aspects of metal clusters are their unique properties which can be easily tailored, by using different thiol ligands with various chemical groups.²⁷ Ligand plays an important role in MPC. It has a strong impact on nucleation and chemical properties which directly influence the final size of the particles and the solubility properties. The chemical group capped at the opposite end of the thiol ligand can make the particle either hydrophilic or hydrophobic.²⁷ The protecting ligand layer keeps the particles from aggregation with each other, this enhances the stability of clusters, which is strictly correlated with the surface charge. Because all thiols have nearly the same affinity towards gold, it is worth mentioning that a place-exchange reaction, where one protecting ligand is exchanged to another, is extremely important for tuning the particles characteristics.²⁷

The effect of size of the ligands on the nanoparticles' core was studied by Tsukuda *et al.*²⁷ They proved that bulky glutathione ligand is effective for the synthesis of a wide range of small clusters such as Au₁₀, Au₁₅, Au₁₈, Au₂₂ or Au₂₅.³⁰ Tsukuda *et al.* demonstrated that if extremely bulky thiolate was applied it gave rise to the new surface protecting motifs resulting in other Au_n(SR)_m sizes.³⁹ Kauffman *et al.* approved this finding by showing that under similar conditions the size of atomically precise cluster was decreasing with increasing hindrance of methyl group.⁴⁰

The recent studies have shown that all-thiolate capped Au₂₅ cluster preserve the same structure independent of the ligand type.³⁵ The glutathione capped Au₂₅ clusters were studied by NMR and mass spectrometry and the results showed that the structure was the same with the phenylethanethiolate protected Au₂₅ clusters.³⁵

The ligand effect occurred when Azubel *et al.*⁴¹ performed synthesis with 3-mercaptopbenzoic acid (3-MBA). The well-known thiolate ligand in Au₁₀₂(SR)₄₄ was *para*-mercaptopbenzoic acid (*p*MBA). The small change of substituent position into 3-MBA resulted in different size of uniform, water soluble, Au₆₈ particles. Moreover, the structure of the particle was determined, and it turned out to differ significantly from that of Au₁₀₂ species.⁴¹

Recently, a new approach was developed to induce size and structure transformation and obtain new $\text{Au}_n(\text{SR})_m$ clusters.⁴² This approach utilizes ligand-exchange reactions which enable control of size and structure under thermal conditions with addition of large excess of thiol molecules. The transformation of $\text{Au}_{25}(\text{SR})_{18}$ to $\text{Au}_{28}(\text{SR}')_{20}$, $\text{Au}_{38}(\text{SR})_{24}$ to $\text{Au}_{36}(\text{SR}')_{24}$, and $\text{Au}_{144}(\text{SR})_{60}$ to $\text{Au}_{133}(\text{SR}')_{52}$ was achieved. Moreover, they confirmed that the incoming ligand is a key point in transformation chemistry, and it should be significantly different than the original thiolate to induce the transformation.⁴²

Despite protecting group and its surface modification properties, the ratio between the gold-ligand affect the final size of the gold core.²³ The experimental observations have shown that specific types of the ligands seem to be more effective in preparation of certain core size. The final size seems to be affected by the amount of thiol that was used. In a related work, Murray and co-workers synthesised different sizes of AuNPs stabilized by hexanethiolate ligands by simply changing the mole ratio between ligand and gold salt.^{43,44} Generally, they observed that when higher amounts of thiol were used it gave smaller average core sizes.⁴⁴ For instance, a thiol to gold ratio of 1:6 forms 4.4 nm diameter particles, whereas increasing the ratio of thiol to 3:1 leads to below 2 nm sizes.

Schaaff *et al.*²⁵ in their structural characterization studies generalized that low temperatures, fast reductant addition and short reaction times give smaller size particles.²⁵ Subsequently, the nucleation and the growth process are likewise influenced by the solution temperature which is directly correlated with the final size of the nanoparticles. However, Sardar *et al.*²³ investigated that the size can be reduced by increasing temperature and thus reducing the reaction time. The rapid formation of nuclei at higher temperature favours the nucleation and growth process leading to very small 1.5 nm particles.²³ On a note, different thiol protected clusters show different thermal stability.²³ The longer carbon chain indicates slightly higher stability. Identification of the changes in reaction parameters and control of structural characteristics at different stages of nanoparticles formation process, by manipulating conditions to favour specific stages may provide an important insight into the stages of nucleation and growth.

The two phase Brust-Shiffrin method of gold nanocluster synthesis opened new possibilities of studying the mechanism of cluster formation. Even though the mechanisms of the MPC synthesis has been studied widely, the pathways to the formation of gold thiolate complexes from gold (III) chloride are not exactly understood.²⁷ Shortly, the synthesis including reduction of gold salt precursor (III) to insoluble polymeric gold-thiol complex is accomplished by adding a specific amount of thiol, followed by the reduction of the polymer and nanoparticles' formation.²⁸

Murray, in his studies assumed that the precursor species of the reaction was polymer $[\text{Au}_I\text{-SR}]_n$ which was formed upon the reduction of Au(III) to Au(I).¹ Recently, Goulet and Lenox²⁶ showed, based on quantitative ^1H NMR analyses of the two phase synthesis, that the Au-(I) thiolate polymer is not the precursor of the reaction instead the metal(I)-tetraoctylammonium complex halide is the relevant Au species under the reduction with NaBH_4 . It was assumed that TOA^+ ions affect the initial Au(I)-SR polymer structure and modify the polymeric structure suitable for the formation of Au clusters. Therefore, changing the reaction conditions, such as varying the ratio of thiol to tetrachloroaurate, has an impact on ligand substitution causing changes in the core size and structure.

More recently, Lauren *et al.* applied NMR techniques to study the noble metal nanoparticles. They suggested that there are fundamental differences between the formation pathways in the one-phase synthesis and the two-phase method.⁴⁵ It was experimentally shown that in the two-pot synthesis after the reduction of Au(III) to Au(I) there was no evidence of metal-sulphur bond formation before addition of NaBH_4 , instead $\text{TOA-}[\text{AuX}_2]^-$ species were formed. Oppositely, one phase synthesis which involved the same reagents, with the exception of phase transfer agent, the metal-sulphur bond was observed before the introduction of NaBH_4 indicating Au(I)-thiolate formation which was consistent with the Murray results.¹

2.3 Structure

It is widely known that the large metal nanocrystals have face centred cubic (fcc) structures.⁴⁶ The ligand packing and how the atoms are arranged in the metal core have been intensively studied.⁴⁶⁻⁵¹ In the MPCs X-ray and neutron diffraction techniques are typical experimental methods to determine crystal structures of metal nanoparticles.⁵² The stability and chemical nature of clusters depends on cluster size and is associated

with the number of total valence electrons.⁵³ In small clusters, the cluster is particularly stable if the shell is fully filled with electrons.⁵³ As the size increases the geometry of the clusters becomes more relevant than the electron shell.⁵³ The complete crystal structure of the $\text{Au}_n(\text{SR})_m$ permits to better understand fundamental properties of the cluster. Understanding the detailed information about how gold atoms and ligands interact and are arranged in the cluster is highly crucial for future applications, including signal transmittance properties, such as electron transport and electronic excitations.⁴⁶ The determination of the exact structure via X-ray crystallography requires growing single crystals which is challenging. Besides experimental determination of the structure, DFT calculations have been commonly used to obtain more information about clusters' structures.⁵⁴ It is worth pointing out that the electronic structure calculations have been shown to estimate the structure successfully.⁵⁵

Recently, significant progress has been achieved in the synthesis, crystal structure determination and in the studies of physio-chemical properties of thiolate monolayer-protected gold nanoclusters. Due to high purity synthetic methods, a number of monodisperse clusters have been obtained, including Au_{25} , Au_{36} , Au_{38} , Au_{102} and Au_{144} . The seminal step in understanding the structure of thiolate-protected gold clusters was the structure determination geometry of *p*-mercaptobenzoic acid protected, $\text{Au}_{102}(\text{SR})_{44}$.²⁴ After that, the structures of Au_{25} for two redox states, $\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}^-$ and $\text{Au}_{25}(\text{SC}_2\text{H}_4\text{Ph})_{18}^0$, have also been determined.^{49,51} The crystal structure was also supported experimentally with DFT calculations. In the recent experimental structure determination, Qian *et al.*⁵⁶ and Lopez-Acevedo *et al.*⁵⁷ obtained the x-ray structure of a phenylethanethiolate-protected $\text{Au}_{38}(\text{SR})_{24}$ which was additionally supported earlier by theoretical predictions by Jiang *et al.*⁵⁸ and by Pei *et al.*⁵⁹ The nuances of these crystal structures led to theoretical prediction on the structure of other nanoparticles including $\text{Au}_{40}(\text{SR})_{24}$.

The abundance of different clusters indicated that certain sizes of clusters have unique and exceptional stability. This unusual stability comes from the structure and it is associated with the electronic shell structure.⁵³ The shell structure is determined by the numbers electrons. The identification of number of electrons corresponding to closed shell in small clusters of sodium was done by Knight *et al.* in 1984.⁵³

The structure of the Au core can be described as polyhedral geometry. Small MPCs have icosahedral and decahedral cores.¹¹ Both symmetries show a five-fold symmetry axis and are constructed by regular polygonal faces. Icosahedral core is regular polyhedron consisting of twelve vertices within each there of twenty triangular faces, each for one vertex. The decahedral forms the junction of twelve regular pentagonal faces and twenty vertices. Both structures are considered the most compact, symmetric cores with complete steric protection.¹¹

The structures of clusters differs from gold thiolate polymers made up of linear S-Au-S bonds.⁵⁰ The surface of gold atoms can bind two, one or zero sulphur atoms. The shorter monomer units RS-Au-SR protect the $\text{Au}_{144}(\text{SR})_{60}$ structure and longer dimer units RS-(Au-SR)₂ protect the $\text{Au}_{25}(\text{SR})_{18}$. The protecting units are an important driving force to understand the stability, chemistry and symmetry of clusters.⁵⁰

2.3.1 Isohedral core

The $\text{Au}_{25}(\text{SR})_{18}$ cluster has been the most extensively studied due to the availability of high purity synthesis. After Zhu *et al.*³³ group reported a high yield synthesis of Au_{25} clusters through kinetic control, the total structure of $\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}$ was solved.⁴⁹ The structure of Au_{25} is built up with icosahedral Au_{13} core which consists of one central gold atom and twelve atoms on the vertices. The rest of the gold atoms form six -S-Au-S-Au-S- units surrounding the Au_{13} core in the octahedral arrangement (fig. 3(1)). The external gold atoms from the core were found to be bound to the sulphurs. The structure exhibits unique bonding arrangement between eighteen thiolate ligands and the 24 gold atoms (fig. 3(2)). The external gold atoms form six oligomers of -S-Au-S-Au-S- that are capped by -SR ligands bridging between the gold atoms.⁴⁹

Surprisingly, Jin *et al.*⁴ found that the structure of Au_{25} turned out to be independent of the surface thiolate ligand. All types of thiolate ligands exhibit the same UV-vis spectra, indicating no changes in the core size. The second crystal structure of Au_{25} was published by Murray *et al.*⁵¹ The crystal structure of the ionic form exhibits distortions which are not observed in a neutral form. The distortions come from different motifs bending of ligand and the ligands orientation. These structural differences are not only caused by negative charge at the core cluster resulting from the presence of the TOA^+ counter-ion. In the later work, positively charged $\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}^+$ was also obtained.^{46,11} The anionic form can be easily oxidized to $\text{Au}_{25}(\text{PET})_{18}^0$ and

$\text{Au}_{25}(\text{PET})_{18}^{+1}$. During the chemical oxidation or when the cluster is exposed to air, the negative charge in Au_{13} core disappears without causing any destabilization in the clusters. The most reasonable explanation comes with the fact that HOMO orbitals of Au_{25} are located in the Au_{13} core and not in the surface Au-SR bonds.^{51,49}

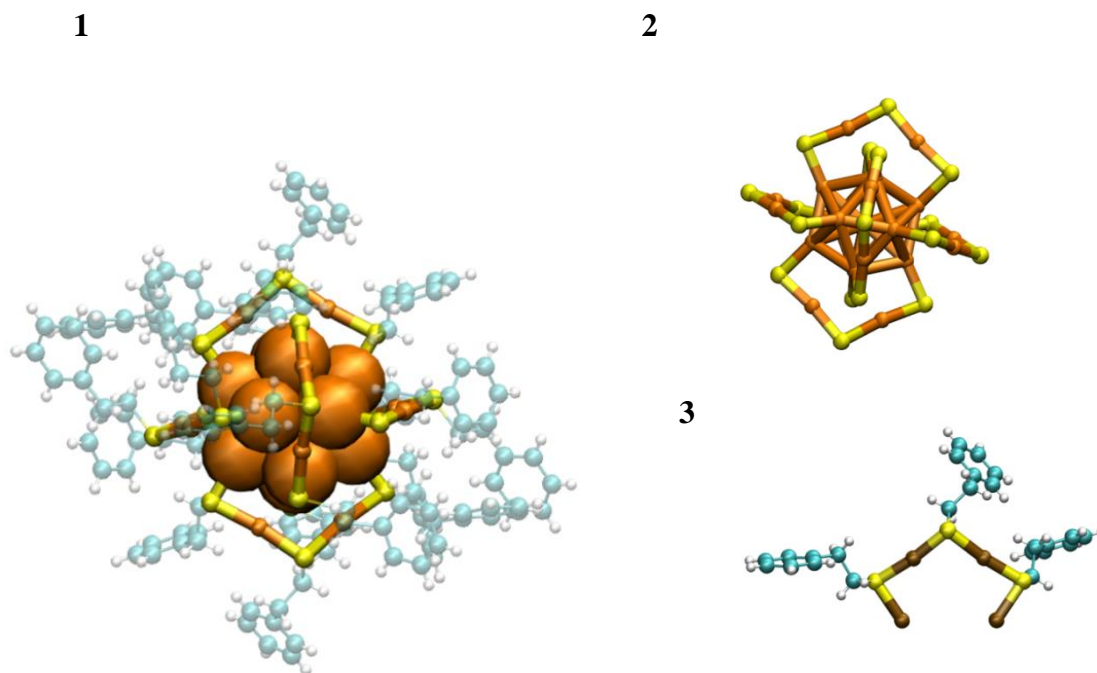


Figure 3. Core-shell structure of the $\text{Au}_{25}(\text{PET})_{18}$ (1) space filling representation of $\text{Au}_{25}(\text{PET})_{18}$ nanoparticles. Au, orange; S, yellow; C, blue; H, white. (2) The view of the Au_{13} core with six protecting $\text{RS}-(\text{AuSR})_2$ units (3) Close-up of the protecting $\text{RS}-(\text{AuSR})_2$ unit.

$\text{Au}_{144}(\text{SR})_{60}$ has unique structure and electronic properties, which can provide an explanation for the stability and other properties.⁵⁰ In 1996 Whetten and co-workers identified the core cluster to be approximately Au-140 by laser desorption ionization (LDI) mass spectrometry. Due to the fragmentation, the determination of the exact molecular formula remained challenging. Tsukuda's group by using the same characterization technique determined the cluster to be $\text{Au}_{144}(\text{SR})_{59}$. After that the formula was redetermined by Murray *et al.* as $\text{Au}_{144}(\text{SR})_{60}$.⁴⁶ The one ligand difference between those two formulas is perhaps due to the oxidation pre-treatment. In the Murray's work the cluster ionization was performed by formation of Cs^+ adducts.⁴⁶

Electronic structure calculations of $\text{Au}_{144}(\text{SR})_{60}$ indicated that it was composed of icosahedral Au_{144} core arranged into three concentric shells of 12, 42 and 60 atoms (fig. 4).⁵⁰ The core's atom is surrounded by 30 equivalent RS-Au-SR units. The energy binding of single unit to the core was calculated to be 2 eV. The two first shells of the core consist of 54 atoms forming an icosahedron and 20 triangular faces. The third shell is filled by three atoms in a bulk packing order in each of 20 triangular faces. An interesting feature of this cluster is that it can appear in two enantiomeric isomers due to the arrangement of the RS-Au-SR units.⁵⁰ The crystal structure of $\text{Au}_{144}(\text{SR})_{60}$ remains to be determined and will play a critical role for the future understanding of optical properties of MPCs.

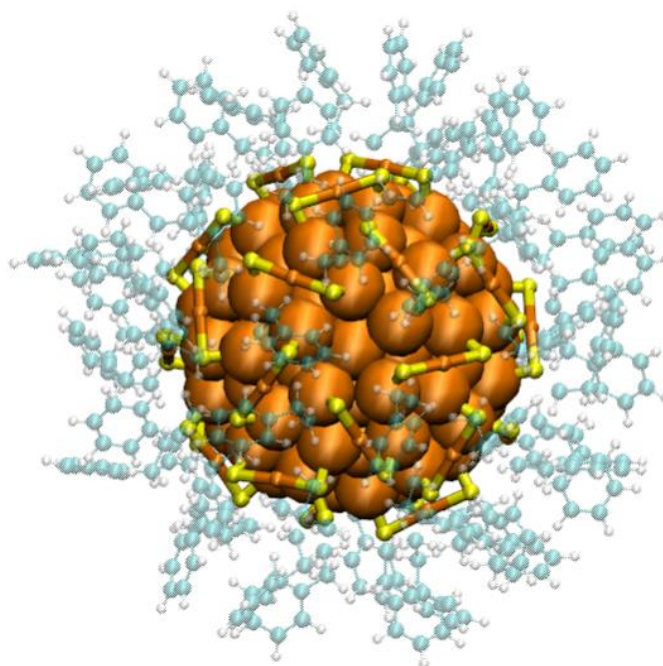


Figure 4. Core-shell structure of the $\text{Au}_{144}(\text{PET})_{60}$. Au, orange; S, yellow; C, blue; H, white.

2.3.2 Decahedral core

$\text{Au}_{102}(\text{p-MBA})_{44}$ was the first reported structure for thiolate-capped nanoclusters by Jadzinsky *et al.* in 2007.³⁶ The arrangement of the atoms is similar to the $\text{Au}_{144}(\text{SR})_{60}$ structure, however, it differed significantly from the standard model of geometries.⁵⁰ The distances between surface atoms of the gold core are considered identical, which was also observed in case of Au_{144} .⁵⁰ The protective layers of oligomers consist of short units $\text{Au}(\text{SR})_2$ and long one $\text{Au}_2(\text{SR})_3$. The core consists of Au_{79} and the shell composed of a protective layer with composition $\text{Au}_{23}(\text{pMBA})_{44}$ (fig. 5(1)). The central gold atoms packed in a Marks decahedron are in a metallic state surrounded by 23

oxidized gold atoms. The 23 gold atoms belong to nineteen $\text{Au}(\text{SR})_2$ and two $\text{Au}_2(\text{SR})_3$ oligomers, which are bound directly to the gold core by the thiols at both ends of the oligomer (fig. 5(2,3)). The characteristic features of Au_{102} structure arise from the “double anchoring” phenomena. Two gold atoms with two Au-S bonds are located at the core-mantle interface. The arrangement of the atoms exhibits chirality arising from the structure of the equatorial gold atoms and linked thiolates on the surface.^{36,11}

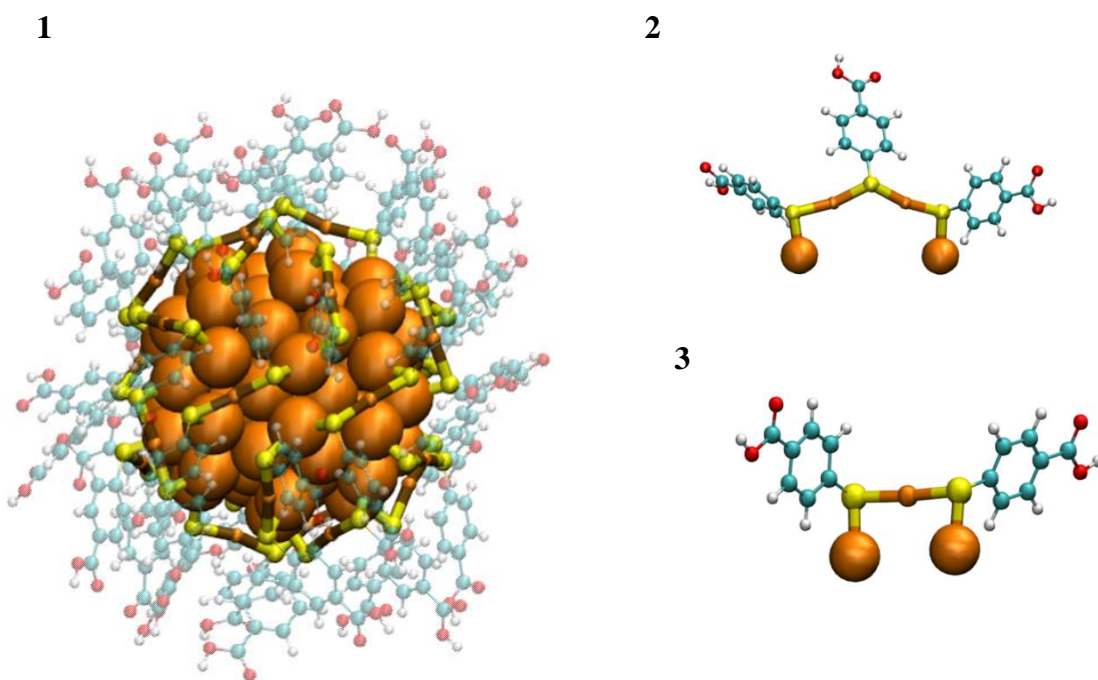


Figure 5. Core-shell structure of the $\text{Au}_{102}(\text{pMBA})_{44}$ (1) space filling representation of $\text{Au}_{102}(\text{pMBA})_{44}$ nanoparticle. Au, orange; S, yellow; C, blue; O, red; H, white. The view of the Au_{79} core to nineteen $\text{Au}(\text{SR})_2$ and two $\text{Au}_2(\text{SR})_3$ oligomers (2,3) Close-up of the protecting $\text{Au}_2(\text{SR})_3$ and $\text{Au}(\text{SR})_2$ oligomers.

Neqishi *et al.* has recently reported dedecanethiolate-protected Au_{130} nanocluster synthesis following the modified Brust-Shiffrin method.³ However, the crystal structure has not yet been obtained. The group proposed an elongated decahedral structure and the chemical composition was revealed by the mass spectrometry studies. The X-ray diffraction pattern of $\text{Au}_{130}(\text{SC}_{12}\text{H}_{25})_{50}$ indicated that the core of $\text{Au}_{102}(\text{SR})_{44}$ has similar geometric structures. It was reported that the Au_{130} central core contains an additional layer on Marks decahedral and consists of 105 gold atoms which are covered by 25 $\text{Au}(\text{SR})_2$ oligomers.³

2.3.3 Other structures

The high yielding synthesis⁶⁰ of $\text{Au}_{38}(\text{SCH}_2\text{CH}_2\text{Ph})_{24}$ has led to a successful crystallization⁶¹ and structure determination⁵⁶. The structure of Au_{38} significantly deviates from a spherical and it is chiral due to the pair of enantiomeric clusters. Each isomer contains a biicosahedral Au_{23} core and $\text{Au}_{15}(\text{SR})_{24}$ shell. The shell consists of three monomeric $\text{Au}(\text{SR})_2$ and six dimeric $\text{Au}_2(\text{SR})_3$ oligomers.⁵⁶ The arrangement of the dimeric units on the bottom icosahedron is rotated relative to the top one making the entire structure chiral. The DFT calculations by Lopez- Acevedo *et al.*⁵⁷ showed good agreement between the powder x-ray diffraction measurements. The structure of $\text{Au}_{40}(\text{SR})_{24}$ was first found in the size focusing intermediates of the $\text{Au}_{38}(\text{SCH}_2\text{CH}_2\text{Ph})_{24}$ synthesis and separated by size exclusion chromatography. The structure hasn't been determined, either experimentally or by theoretical calculations.³ Surprisingly, $\text{Au}_{40}(\text{SR})_{24}$ does not exhibit as pronounced absorption peaks as $\text{Au}_{38}(\text{SR})_{24}$ and their optical spectra are significantly different. The differences are probably due to the different structure assembly.

Li *et al.* synthesised $\text{Au}_{99}(\text{SPh})_{42}$ through size-focusing method, and precise cluster mass assignment and formula was obtained using ESI-MS.³ The same cluster was synthesised with a SPh-Me ligand and consistent mass was obtained. Additional confirmation was obtained by thermogravimetric analysis (TGA). The UV-Vis spectrum of $\text{Au}_{99}(\text{SR})_{42}$ indicated the absence of plasmon resonance band, therefore the cluster still remains in the non-metallic regime.³

Zhu *et al.*⁶² first observed a 20-gold atom cluster protected by phenylethylthiolate (PET) ligand in a size-controlled synthesis in 2009. The ultra-small structure of tert-butylbenzenethiolate protected Au_{20} , $\text{Au}_{20}(\text{SPh-}t\text{Bu})_{16}$ was recently solved.⁶³ The structure features a vertex-sharing bitetrahedral Au_7 kernel. Surprisingly, an octameric ring $\text{Au}_8(\text{SR})_8$ circles the Au_7 kernel and interacts between each other through $\text{Au}_{\text{ring}}-\text{Au}_{\text{kernel}}$. The surface protecting octamer ring was observed for the first time in nanoclusters and it might be common in smaller gold nanoclusters, such as $\text{Au}_{18}(\text{SR})_{14}$ and $\text{Au}_{15}(\text{SR})_{13}$. The interactions between the ring and the kernel₇ make the structure interesting compared with previously reported geometries. The gold atoms in the kernel are not bonded to thiolate ligands from the ring therefore no covalent bonding interaction $\text{Au}_{\text{kernel}}-\text{S}$ occurs. Additionally, the kernel is further protected by trimeric staple $-\text{SR}-\text{Au}-\text{SR}-\text{Au}-\text{SR}-\text{Au}-\text{SR}$ and two $-\text{SR}-\text{Au}-\text{SR}-$ monomerics. However,

the theoretical structure prediction of $\text{Au}_{20}(\text{SC}_2\text{H}_4\text{Ph})_{16}$ and $\text{Au}_{20}(\text{SCH}_2\text{Ph})_{16}$ by Jiang *et al.*⁶⁴ and Zeng *et al.*⁶³ differs from the experimentally determined crystal structure of $\text{Au}_{20}(\text{SPh-}^t\text{Bu})_{16}$. In the future, it remains to be found, whether the differences are caused by ligands or by two isomeric forms of the core.³

2.4 Unique properties of nanometre sized metal clusters

The properties of nanoparticles dramatically change with decreasing core size.⁴⁶ The sub-nanometre gold nanoparticles exhibit discrete electronic structure which directly influence on their unique optical and electronic properties which are different from large nanoparticles. Below, the most important properties of clusters, including optical, catalytic, magnetic and capacitance charging energies are summarized.

2.4.1 Size dependent optical and electronic properties

A significant aspect of a smaller particles is their unique stability, which comes from geometry effects and electronic properties of the clusters.⁶⁵ The unusual stability and abundance of clusters, derived from the geometric packing, number of electrons called “magic numbers”, which indicate a stable cluster size. Based on the theory, the lowest energy superatom orbital are mainly derived from gold 6s orbitals. If the numbers of valence electrons correspond to the number of electron required to fill an electron shell is 2, 8, 18, 34, 58, 92 or 138, then the cluster is considered as stable.⁵³ This model is often used to predict the stability of clusters.

The stability of thiol protected clusters is affected by the ligand layer and the electron withdrawing nature of the thiol ligands.⁶⁵ The divide and protect model may be used to describe the stability of these clusters.¹¹ However, it was also observed that for some of them the rule couldn't be applied, indicating that the geometric effects were more important.⁶⁶ Moreover, the size and geometry can be affected by the choice of the ligand molecule.⁴⁰

The optical and electronic properties of gold nanoparticles change dramatically as function of size. The particles have a critical size for electronic state energy quantization. The first size range is subnanometer particles with discrete electronic orbitals and HOMO-LUMO energy gaps. The gaps can be quite large, for example, 1.3 eV for $\text{Au}_{25}(\text{SR})_{18}$ and 0.9 eV for $\text{Au}_{38}(\text{SR})_{24}$. The second size range refers to larger particles with surface plasmon resonance (SPR).¹¹ The smaller clusters (>2 nm) do not

exhibit plasmon resonance due to the quantum effect. Therefore, the ultra-small clusters display spectacular optical behaviour significantly different from the large plasmonic particles.¹¹

Molecular-like optical transition depends on the number of atoms forming the cluster. The absorption spectra of very small metal clusters show individual peaks that give information about their electronic states. For example, $\text{Au}_{25}(\text{SR})_{18}$ and $\text{Au}_{38}(\text{SR})_{24}$ exhibit highly structured absorption bands. Absorption bands are due to a single electron transition between quantized electronic stages.^{14,15,67}

Recently, Mustalahti *et al.* studied the photodynamic properties of $\text{Au}_{102}(\text{pMBA})_{44}$ by using ultrafast time-resolved mid-IR spectroscopy and density functional theory calculations in order to distinguish between molecular and metallic behavior.⁶⁸ Interestingly, it was found that the cluster containing 102 atoms behaved like a small molecule, which turned out to be in striking contrast to the $\text{Au}_{144}(\text{SR})_{60}$ which showed relaxation typical for metallic particles.

$\text{Au}_{144}(\text{SR})_{60}$ is the smallest cluster to develop plasmonic response.⁶⁹ Malola *et al.* studied the optical properties and found out that the spectrum of the Au_{144} cluster is rather featureless. Very weak but characteristic bands of $\text{Au}_{144}(\text{SR})_{60}$ were observed at around 540, 600 and 700 nm, appeared in the calculated spectrum as well.⁶⁹ On the contrary Weissker *et al.*⁷⁰ demonstrated that the thiolate monolayer-protected gold nanoclusters exhibit a broad spectrum of bands that were visible over the entire near-IR, VIS and near UV-regions. The content of the spectra gave the information of the quantum size effects, which helped to distinguish from bulk materials.⁷⁰

Au_{25} exhibits interesting optical absorbance and fluorescence properties.^{34,67,8} The luminescence properties of metal nanocluster come from the electronic transition between unoccupied d bands. The absorption spectra of Au_{25} exhibit three transition maxima at 670 nm, 450 nm and 400 nm called intraband, interband, and mix of intraband and interband respectively. Intraband transition (sp->sp) is an excitation of valence electron near the Fermi energy, which is rather low cost excitation. The interband excitation occurs from d band to sp band.⁸ The electronic transition at 670 nm corresponds to the HOMO-LUMO transition. According to the study, the absorption bands are influenced by geometric and electronic interactions between the core and the

ligand resulting in a complex spectrum. Taken together, all three types of electron transitions affect the optical absorption properties of the clusters, however in order to fully understand the electron dynamic and properties more studies are needed on size-discrete clusters.⁴⁶

Similarly, fluorescence properties come from the metal core or the interaction between metal core and surface ligands. The surface ligands with capability of donating electrons to the metal core, enhance the fluorescence intensity.^{46,67} Recently, Hulkko *et al.* studied spectroscopic properties of the $\text{Au}_{102}(\text{pMBA})_{44}$.⁷¹ They found out the existence of electronic band gap of 0.5 eV for Au_{102} which might indicate a possibility for luminescence in IR region. Other photoluminescence observation has shown that increasing electro-positivity of the metal core of Au_{25} cluster leads to a strong fluorescence enhancement.⁴⁶ Different charge states of Au_{25} nanoclusters display various fluorescence contributions. Other studies were performed by Jin's group suggested that protecting ligand effects the fluorescence intensity.⁴⁶ The charge donating capability of ligands largely enhances the fluorescence of nanoclusters.

Therefore, the optical properties of subnanometre clusters can be tuned by controlling the core size, charge state and the use of different stabilizing ligand layers. Decreasing the core size of nanoclusters, the percentage of fraction of surface atoms increases, affecting the optical properties of gold nanoclusters.⁷

2.4.2 Chirality properties

The optical properties of clusters were first observed in glutathione (GSH) protected Au_{25} nanoclusters.^{4,5} The distinct circular dichroism properties were predicted to originate from the inherent properties of the cluster or the ligand-core interactions. Later, the same gold structure capped with different types of thiolate ligands was studied by Wu and co-workers. Surprisingly, the obtained 1D and 2D NMR spectra indicated no chirality from $\text{Au}_{25}(\text{SCH}_2\text{CH}_2\text{Ph})_{18}$ structure.⁶⁷ The results suggested that the chirality of metal nanoclusters arise directly from the glutathione induced chiral field, contrary to previous expectations about metal core and the ligands themselves. The recently reported structure of $\text{Au}_{102}(\text{pMBA})_{44}$ nanoclusters also exhibit chirality.⁴

Chirality can be achieved either by using chiral molecules as a protecting ligands directly in the synthesis of metal clusters or by surface functionalization methods such

as ligand exchange.⁵ The enantiomeric studies on different sizes of optically active D- and L-penicillamine-capped gold nanoclusters pointed out that the optical activity increases with the core size decreasing. Further, the studies indicated that gold nanoclusters also have well-defined stereostructures, which is similar to chiral molecules.⁵ The strong optical activity of the nanoclusters were explained by the chiral core model.⁵

2.4.3 Charge dependent properties

Besides electronic structure, the stability of thiolate protected gold clusters is correlated with the thiolate ligand layer, preventing it from aggregation. Generally, there are two factors that can influence clusters stability: electronic shell closing and geometric shell closing. However, Tsukuda *et al.* concluded that electronic shell closing is not a main issue in cluster stability.⁵ For example, the $[\text{Au}_{25}(\text{SC}_6)_{18}]^q$ ($q = 1, 0, 1+$) clusters charge states can be easily changed by the redox reactions. Therefore, it was concluded that the cluster stability is attributed with geometric structure. On the other hand, the structure of Au_{25} is an open structure, having incomplete second shell and the number of valence electron of anionic Au_{25} cluster seems to fit with the superatom model, which can explain the stability.

The core of the particles can either possess excess or deficiency of the electrons. The charging may come from the ligand and its charged groups, such as carboxylic acid or amine functionalities.^{1,5} The charged particles suspended in the electrolyte solution are surrounded by an ionic cloud termed the electrical double layer.

The stability of water soluble charged stabilized particles protected by carboxylic acid or amine terminated ligands depends on their structure and pH of the system.³ Longer ligand chains have a significantly higher stability than the shorter ones, preventing them from aggregation. The electrolyte enhances stability and enables modification of the system. It was observed that citrate protected clusters were detached when the solvent was removed, leading to irreversible aggregation.² At low pH, carboxylic acid-stabilised nanoparticles agglomerate due to protonation and hydrogen bonding, making them soluble in basic conditions. Whereas at high pH, the carboxylic acid groups deprotonate and stabilise the particle dispersion through electrostatic repulsion. The electrical double layers surrounding the particles prevent them from approaching each other and agglomerating by attractive van der Waals forces.

Among the charge states of Au_{25} the magnetic properties were also found.^{5,67} The magnetic properties were studied by Negishi group, however the unknown structure of cluster made impossible to develop studies further. Nowadays, the magnetic properties of Au_{25} can be easily tuned by controlling the charge state of the particles.⁶⁷ The charge neutral $\text{Au}_{25}(\text{SR})_{18}^0$ cluster exhibits magnetism arising from the unpaired HOMO electron. The oxidation mechanism of $\text{Au}_{25}(\text{SR})_{18}^-$ is a one electron transfer mechanism whereas cluster reduction is composed of several steps. Reduction mechanism is followed by a reversible desorption of thiolate anion after which the neutral cluster is capable of accepting another electron.¹³ The desorption of thiolate anion is known as a one electron transfer reaction. Findings are significantly different compared to the previous ones that showed the magnetism of gold originates from the particle surface via charge transfer in the Au-S bond.⁶⁷

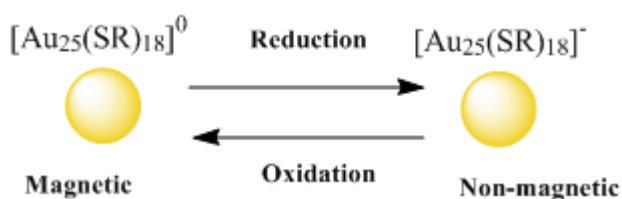


Figure 6. Reversible conversion between the neutral and anionic $\text{Au}_{25}(\text{SR})_{18}$ nanoclusters.⁵

2.4.4 Charge transfer properties

Physical properties of nanoparticles strongly depend on the particle size, shape and the nature of the surrounding ligand.⁶⁹ The thiolate-stabilized cluster can act as an electron donor or acceptor. The addition or removal of an electron creates an energy barrier that needs to be overcome before the second electron is added or removed.⁵

A quantization effects appear as the particles size approaches molecular behaviour, resulting in a HOMO-LUMO gap between the valence band and the conduction band.⁵ As the size is reduced the metal-like electron band converts to discrete energy levels analogous to molecular orbitals. In electrochemical experiments the gap between highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) is seen as a voltage gap between the first electron addition and removal. The spacing between energy level results from the capacitive charging. Each cluster has its

own charge state which changes with the core size and strongly depends on monodispersity of the sample. Ramakrishna *et al.* studied two photon absorption of $\text{Au}_{25}(\text{SR})_{18}$ cluster.³ The fast electron transfer was observed in both neutral and anionic form of the cluster. The photooxidation occurs near the HOMO-LUMO gap and was assigned to the core-to-surface relaxation.

Thiolate stabilized clusters are commonly used for electrochemical studies of gold nanoparticles because their size significantly affect the capacitance of the cluster. For highly polar ligands discrete charging cannot be resolved due to too large dielectric constant of the ligand. In water, the increase in ionic strength would collapse the charge, making the electrostatically stabilised MPCs more susceptible to changes in salt concentration.

2.4.5 Catalytic activity

Among noble metals, gold has rather poor activity for reactive molecule adsorption.^{4,5,72} The filled d band of gold leads to higher activation barriers compared to other transition metals with half-filled d bands. It was investigated that the clean bulk gold surface is quite inert for the O_2 adsorption.^{4,5} However, this changes when the dimensions of the gold catalyst approach nanoscale. Gold clusters were found to be reactive with oxygen at the room temperature.⁴ The unusual catalytic properties of gold nanoparticles arise from the fact that oxygen binds more strongly at the defects, such as steps and edges on the particle surface, which consequently increase as the size of particle decreases.⁷² Another factor is the Au-interface which may induce strain influencing the surface reactivity.^{4,5} Last years, gold nanoclusters have been studied as an active catalyst for oxygen electro reduction and CO oxidation.

Herzing *et al.* found that the catalytic activity for CO oxidation came from ten atom gold nanoclusters, which clearly suggested that metal clusters represent good class of materials for catalysis.⁴ Further theoretical studies proved that the smaller clusters are more favourable for O_2 adsorption onto cluster surface, and therefore, more reactive.⁴ The studies demonstrated that ~1.5 nm gold clusters surrounded by Polyvinylpyrrolidone (PVP) showed higher catalytic activity than larger clusters stabilized by poly(allylamine) (PAA). Chen *et al.* also studied the size effect on the catalytic activity.^{4,5} They prepared a wide range of gold clusters ranging from 11 to 140 gold atoms and evaluated the size effect on the electrocatalytic activity for oxygen

reduction. Again, the results showed that electrocatalytic activity increases as the core size decreases. More recently, Gao *et al.* studied the catalytic activity for 12 different clusters from 16 to 35 gold atoms and concluded that the anionic clusters can adsorb O₂ and CO more strongly than a neutral one.⁴

2.5 Methods for detection and characterization of clusters

Because of the electronic, optical and catalytic activities of metal nanoclusters depend on their size, morphology, composition and surface properties, various techniques have been applied to characterize them. A detailed structural characterization enables to understand the structure-property relationships which provide the basis for cluster structural optimization for different applications. For instance, it was found that the size of the gold core has an influence on the electrocatalytic activities of particles.⁷² The main aim of nanoparticle characterization is the determination of particle size and monodispersity.

Nanoparticle characterization is based on the determination of particle mass, diameter or the analysis of the exact cluster composition. To evolve the core to a specific number of Au atoms post-synthesis treatments may be applied.² Fractions with different sizes may be separated via centrifugation methods with different rotational speeds and times.⁷³ Currently used purification methods of nanocluster samples are focused on removing unreacted reducing agents and free ligands. The impurities may be removed by washings, centrifugation, extraction or reprecipitation.⁷³ Nevertheless, the purification of distribution of crude metal nanoparticles may be more challenging and other techniques need to be utilized. Separation using different solvents, high pressure liquid chromatography (HPLC) or polyacrylamide gel electrophoresis (PAGE) are commonly used techniques to improve monodispersity.⁷ Purification techniques of clusters enable easier characterization with respect to mass, diameter, composition and structure.

2.5.1 Stability of the clusters

To examine the stability of clusters, UV-vis spectroscopy is a powerful tool. UV-vis absorption involves electronic transitions from valence-orbital of the clusters. The technique enables to monitor changes of the metallic core, including oxidation state and the number of metal atoms in the cluster.^{7,74} Therefore, UV-Vis absorption spectroscopy is a powerful tool to identify clusters size. Moreover, as the method can be used to detect changes in the core, therefore can be used to examine stability of clusters over the

time. Gold clusters are rather stable in a solution or in a powder form, indicating no changes in the absorbance peaks. The UV-vis spectroscopy was also used to confirm the stability of $\text{Au}_n(\text{SG})_m$ clusters when exposed to air.⁷⁵ For metal nanoparticles, position and absorption of peaks are well-defined by Mie theory, which is used to define polydispersity and concentration of particles.⁶ However, in more rigorous analysis particles shape and capping ligands should be considered. The choice of the medium can cause shifts in the spectra. Spectrum of large gold nanoparticles are dominated by plasmon peaks at around 420 nm, 520 nm and 600 nm, whereas the subnanometre nanoparticles exhibit molecular-like optical transitions with absorbance bands due to the quasi-continuous electronic band structure.⁴⁶ UV-vis absorption spectroscopy has been a convenient and powerful tool to study size-dependent optical properties and the electronic state structure of gold nanoparticles.⁷⁴

2.5.2 Particle size and chemical composition

Particle diameter measurements can be done using transmission electron microscopy (TEM) and x-ray spectroscopic techniques, including x-ray absorption spectroscopy (XAS) and x-ray photoelectron spectroscopy (XPS). Thermogravimetric analysis (TGA) and elemental analysis (EA) can be used to obtain the ratio between metal part and organic thiolate layer part.⁵² The x-ray diffraction analysis of crystals resulted in a determination of a particle with 102 gold atoms and 44 *p*-mercaptobenzoic acids (*p*MBA).³⁶ This finding was very important because was the first crystal structure of thiolate-protected nanoclusters which was solved. The mentioned techniques are the best known analytical methods to gain chemical composition and cluster size.

The transmission electron microscope (TEM) enables seeing nanoparticle morphology and precise size.^{23,75} Nowadays, TEM analyses with a nanometre resolution are used in measuring particle sizes larger than 1 nm, and studying the morphology of particles. Due to the large scattering cross section of the metal atoms the high quality images can be taken. However, performing TEM measurements sometimes can be challenging because migration of particles and coalescence of the particles may occur.⁷³ It is worth noting that TEM measurements have usually small uncertainty. Damages of particles may influence the imaging due to the electron beam heating. TEM can usually confirm that the particles are smaller than 2 nm. However, for the particles around 1 nm it is hard to distinguish the cluster size, even by phase-contrast high-resolution TEM (HR-TEM).³¹ However, HR-TEM is often used as a complementary tool to evaluate the crystalline

structure and the size of particles. TEM sample preparation of gold nanoclusters requires diluted solution to avoid cluster aggregation and consequently provide to imaging difficulties. Densely distributed metal clusters can quickly agglomerate under electron beam exposition. Besides HR-TEM, scanning transmission electron microscopy (STEM), atomic force microscopy (AFM) and scanning tunnelling microscopy (STM) have been used to study metal nanoclusters.⁷³

X-ray absorption spectroscopy (XAS) is highly correlated with the regular UV-vis measurements. The main difference lies in transitions' level. XAS involved core-level electronic transition to unoccupied valence states thus making it useful for the studies of electronic properties of absorbing atoms.⁵² Zhang⁵² was studied X-ray absorption spectroscopy of Au₁₄₄, Au₃₈, Au₃₆, Au₂₅, Au₂₄Pt, and Au₁₉ clusters. He reported that in order to understand their-structure relationship X-ray structure methods play an important role. The spectra can also provide reliable information about local structure of absorbing atoms and size information which is directly related to the number of neighbouring atoms. The quantitative information can be obtained by fitting with theoretical framework. X-ray photoelectron spectroscopy (XPS) provides complementary information to the XAS technique. This analytical method found broader applications in material characterization. It gives straightforward information about the chemical composition of materials from the peak position and shapes.

2.5.3 Determination of the molecular weight of clusters by ESI-MS and MALDI-MS

As described above, it has been challenging to determine the exact size of clusters only by using microscopic methods due to the inaccuracy of measurements. Weis *et al.* realized that mass spectrometry methods provide reliable information of clusters, including the number of metal atoms and the surface protecting ligands.⁷⁶ They were the first to use laser-desorption/ionization and time-of-flight MS to analyze gold nanoclusters. Nowadays, the molecular weight of monolayer protected clusters can be determined by choosing a suitable mass spectrometer.⁷³ A variety of mass spectrometry methods have been applied to the study of nanoparticles. For example, Laser desorption ionization (LDI) mass spectrometry analysis were used to confirm the same structure of Au₂₅(SR)₁₈ cluster, regardless of the types of thiols.³⁵ The molecular formula of Au₆₈(SR)₃₄ was assigned by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. The research reported that the stability of the cluster

was attributed to the 34-electron count shell closure.⁷⁷ Nevertheless, the analyses are often challenging due to fragmentation of clusters and their high molecular weight. The problems can be overcome by using soft ionization techniques such as electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) techniques. Both techniques are in favor of the analysis of such systems because they result in no or little fragmentation. Additionally, ESI-MS allows the mass determination of intact clusters and can produce multiply charged ions which enable the high molecular weight determination.

2.5.4 Separation and purification of clusters by polyacrylamide gel electrophoresis (PAGE)

Despite all the available syntheses of monodisperse sub-nanometre metal clusters, still purification of core sizes remain challenging. One of purification method, polyacrylamide gel electrophoresis (PAGE), has been widely used to separate different core sizes, even smaller than 2 nm.^{78,79} PAGE is based on the migration of charged molecules and separating them due to the mass difference. The charged molecules migrate in response to electric field created between a cathode and an anode. Rate of migration depends on the charge, shape and size of the molecule and also on the properties of the medium where the molecules are moving. The sample is run in a polyacrylamide matrix, which is the most common matrix to separate molecules by size. Porous structure of the matrix acts as a sieve by retarding the movement of large macromolecules. The method is used to separate and purify water-soluble gold nanoclusters protected by various ligands, including glutathione or pMBA. The method turned out to be a convenient separation technique of gold nanoparticles due to their colour which made them directly visible by the eye. The separation of Au:SG glutathione clusters was reported by Negishi *et al.*⁸⁰ They succeeded in separating series of cluster sizes into the fractions by high-resolution PAGE analysis. Of a note, the preparation of polyacrylamide is a crucial step in separation of clusters. The concentration affects the separation process.

2.5.5 Analysis of nanoparticle formation and morphology by nuclear magnetic resonance (NMR) spectroscopy and fourier transform infrared (FT-IR) spectroscopy

Nuclear magnetic resonance (NMR) is a characterization tool that requires little perturbation of the analysed system, giving information and details about the chemical environment of the nuclei.^{81,45} NMR has been widely used in analysis of chemical

structure, reactions and dynamics. Cluster properties can be investigated by proton (^1H) and carbon (^{13}C) NMR, which provide information about the properties of cluster and surface structure.⁴⁵ On the other hand, diffusion-ordered NMR spectroscopy (DOSY) was recently used to study hydrodynamic size of $\text{Au}_{102}(\text{pMBA})_{44}$ cluster.³⁸ The versatility of the method and chemical resolution enable characterization of formation of noble metal nanoparticles. Different chemical shifts of free ligands and ones bound to the gold core, enable verification of purity of the synthesised nanoclusters.⁸¹ Studies showed that when protecting ligands were bound to the nanoparticle's surface, spectral broadening occurred. The reason of this broadening is probably ligand-core interface which results in rapid spin relaxation by dipolar interactions.³⁸ Another factor that may cause the spectral changes is associated with different surface sites for ligand binding. Recently, Lauren *et al.* has used NMR techniques and reported that NMR provides crucial insight into nanoparticles formation, morphology and properties.⁴⁵ They focused on using NMR techniques that are generally accessible to the synthetic nanochemistry community. Following a formation of nanoparticles, requires a method that can capture the chemical and physical transformation in real-time with sufficient resolution. Broad range of NMR techniques, as well as their combination with other analytical methods, can be used to study nanoparticle formation and structure. The chirality of $\text{Au}_{38}(\text{SR})_{24}$ and $\text{Au}_{25}(\text{SR})_{18}$ was also studied by ^1H NMR. For $\text{Au}_{38}(\text{SCH}_2\text{CH}_2\text{Ph})_{24}$ clusters, especially for $\alpha\text{-CH}_2$, the chirality shift was observed for each terminal proton, indicating that Au_{38} has chiral properties, whereas for Au_{25} cluster no chirality was observed.⁶⁴ Moreover, the crystal structure of Au_{25} protected by different ligands was studied using 1D and 2D NMR, and other analyses.⁴⁶ On the other hand, the technique was used to study the mechanism of metal nanoclusters. Contrary to the previous assumptions, it was concluded that Au(I) – and Au(III) –tetraalkylammonium complexes are the relevant species in the reaction mechanism.⁴⁵

Another technique to study the surface structure of synthesised metal nanoclusters is detecting molecular vibrations by FTIR spectroscopy.⁸² The formation mechanism of metal clusters can be studied by comparing the metal particle and the free organic protecting ligand. Thiol protected nanoparticles are formed through Au-S bond, therefore the peak associated with S-H vibrational stretching disappears when the S-H group is attached to the metal cluster surface, indicating the formation of metal-sulphur bond. The FTIR spectroscopy was used to study the differences between Au_{25} , Au_{38} and Au_{144} 2-phenylethylthiol (PET) protected gold clusters. The studies were performed

in order to elucidate how the ligand binds to the metal core and what are the differences between clusters. It turned out that besides small perturbation, the IR spectra were very similar when comparing to bare ligand.⁸²

3 SURFACE FUNCTIONALIZATION OF NANOPARTICLES AND NANOCLUSTERS

The chemical properties of the gold nanoparticles mainly depend on the ligands that are present on the external and internal structure.²⁰ The modification of ligands enables possible applications by providing a new functionality by improving their inherent characteristics. Modification methods can control physico-chemical properties of nanoparticles, such as preventing aggregation, improving stability and making them compatible with biological environment.²⁰ This can be done to avoid compatibility problems between two insoluble phases and to expand the mechanical properties. Furthermore, nanoparticles can accommodate multiple functional groups which tune their properties nearly endlessly.⁷ The synthesis methods have been continuously evolving, leading to a control of the shape and size improvements.

There are three primary methods to introduce a functional groups to the surface of noble nanoparticles (fig.7).^{20,83} The first one, direct synthesis, is based on introducing the whole functional ligand in one step (method 1). The bifunctional organic compound reacts with the metal particle by attaching one of its functional groups to the nanoparticle surface, generating a gold nanoparticle surrounded by dense protecting ligand layer with a desired functionality. The second method is the ligand exchange method, where the initial ligands undergo substitution by incoming ligand bearing desired functionality. The last method is based on conjugation where, the bifunctional compound reacts with a metal particle, while, the other group acts as a coupling site (method 3). The next step is based on chemical converting of the coupling site to gain the final functionality. The main drawback of the first method may be the incompatibility of functional groups, which can react with the surface.²⁰

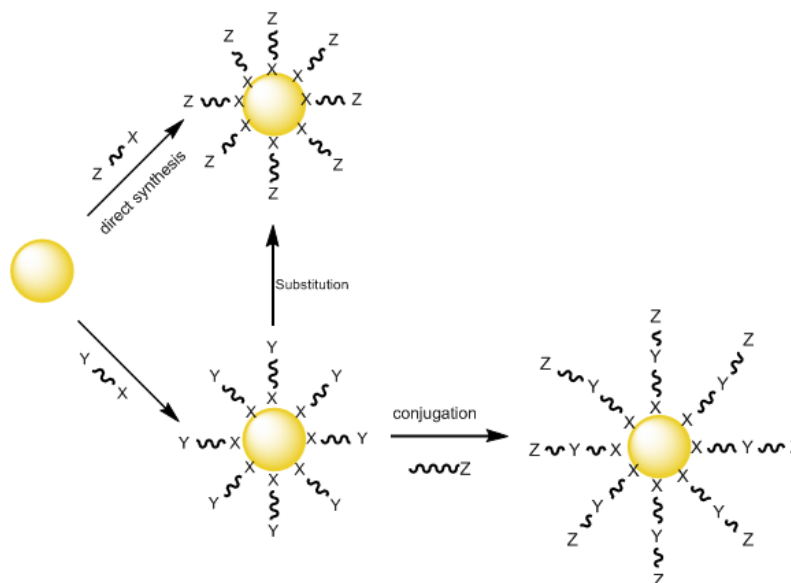


Figure 7. Three ways to functionalize particles. Method 1 (*top*) Nanoparticle reacts directly with the ligands with the Z functionality; method 2: substitution (exchange) of initial ligands in order to obtain desired functionality; method 3 (*bottom*): a ligand with a Y functionality reacts directly with the nanoparticle and acts as a coupling site in the second step to obtain another functionality Z.²⁰

The choice of stabilizing ligand mainly depends on the size of particle and the solvent. The capping ligands that permit introducing various functional groups are usually thiols.^{20,84} It was found that thiols form dense packing ligand layer which stabilizes the core of particles. Water soluble particles, containing polar ligand molecules were found to stabilize the particles for a longer time.⁸³ The most common groups that are used to functionalize metal nanoparticles are: COOH, NH₂, OH, and long alkyl chains.²⁰ They can ensure compatibility and stability with the environment of the nanoparticles, and can be used as a base for further chemical reactions once attached to the particle surface.²⁰ Functionalized trialkoxysilanes groups are commonly used as coupling sites. Many groups can be attached to alkoxy silanes, such as epoxy, amino, vinyl, sulphur-containing, and phosphonic acid groups. Chlorohydrogenosilanes, (H(CH₃)₂SiCl) are also powerful agents, which are reactive towards alkynes, alkenes and triazonium salts.²⁰

The main drawback of the direct synthesis is the limitation of suitable types of capping ligands and functional groups. Ligand exchange reactions and modification methods have become necessary in order to adapt these materials for different applications.⁷

Metal nanoparticles can be modified in various ways by ligand exchange, chemical, physical and bio-conjugation reactions.⁷ The strategy to functionalize and modify ligands depends on the structure of particle surfaces and their interactions with ligands. To overcome some of the synthetic difficulties, ligand exchange can be applied.¹⁴ Recently, alkane thiols used as capping ligands have been the ligands of choice for exchange reactions.^{81,14,85} However, gold nanoparticles can also be modified by disulphides, amines, nitriles, phosphines and carboxylic acids.

Many chemical approaches have been used to conjugate nanometre sized gold clusters with biomolecules of interest. Proteins, nucleic acids, lipids, biologically active small molecules, therapeutic molecules, targeting moieties, and contrast agents can be modified as example for biological applications. A main aim of conjugation strategies is to bind a targeting moiety without losing its functionality after attachment to the surface of nanoparticle.⁸⁶ Conjugation of multifunctional groups on the particles surface allow constructing of nanomaterials which can be used for detection of the biological structures, and functioning of biomolecules and organelles, and also for targeted imaging and treatment of cancers. The functional group at the end of the ligands can be easily tailored, enabling the nanoparticles to interact with organic molecules, other nanoparticles, and polymers. Consequently, the possibilities of surface modification synthesis are endless and are extremely important for future applications.⁷

Traditionally, there are three chemical approaches for conjugating the molecules into gold nanoparticles.⁸⁷ The conjugation methods can be categorized based on the nature of the interaction between the biomolecule and gold particles. The molecules can be covalently attached, adsorbed, or conjugated with biomolecules to the surface. The first approach, where chemical conjugation, is based on covalent bonding of the molecule to the nanoparticle surface. Another approach called encapsulation is based on weak non-covalent interactions. Encapsulation is non-specific bonding, based on electrostatic interactions effected by medium.⁸⁷

The conjugation of nanoparticles with the biomolecules of interest is another strategy that has been developed.^{7,15} Biological interactions are based on both chemical and physical interactions which make them highly selective. Therefore, combination of interactions with gold nanoparticles, makes them extremely useful as sensors for detecting biological molecules.⁸⁸ Various functional groups on nanoparticle's surface

allow conjugation with peptides, ligands, antibodies, genes, drugs, and therefore constructing multifunctional nanomaterials. Due to their unique properties, nanoclusters are of considerable interest because of their unlimited variety of potential applications in various fields, such as optics, biosensing, electronics, nanotechnology, catalysis and DNA or drug delivery.⁷ Consequently, the studies of chemical surface modification have become an important issue towards nanotechnology applications.

3.1 Ligand exchange

Ligand exchange reactions are versatile methods to control the composition of organic layer on the nanoparticle surface. The concept of ligand exchange reaction is very simple and includes mixing nanoparticles with the free ligands, resulting in the replacement of outgoing ligands with an incoming one (fig. 8).¹³ Such an exchange is usually applied to transfer the NPs from aqueous solution to organic phase, providing new properties and stability. Typically, the hydrophobic ligands are replaced by hydrophilic ones that bind strongly to surface of nanoparticles.⁸³ After the exchange of ligands they may be conjugated with various biomolecules and used as catalyst, biosensor, or for electronics application.⁷

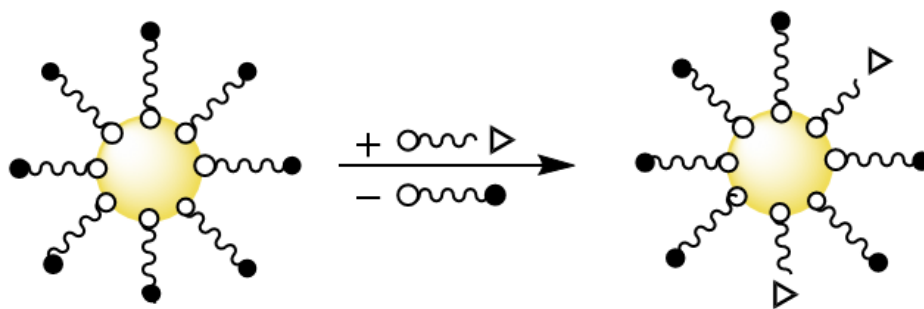


Figure 8. Schematic diagram of a ligand exchange reactions of nanoparticles.

The main challenges of nanoparticle synthesis are restrictions of medium which the particle remains stable, and the lack of functionalities of the ligands.¹³ The end group of ligands, such as thiols, amines or phosphines are electron donating groups which can undergo several binding and unbinding processes that may lead to aggregation. Therefore, the molecules bound to the nanoparticle's surface are not only responsible of the growth control but also prevent them from aggregation.⁸³ The ligands with two functional head groups can be used to ensure strong binding affinity and to induce

stability and dispersion of particles in both aqueous and organic solution. Exchanged ligands can be used for targeting agents, drug deliveries, and to make possible to modify nanoparticles with ligands that cannot be introduced during nanoparticles synthesis.¹³

Although the concept of ligand exchange is simple the researches has proved that the mechanism is extremely variable and it strongly depends on bonding strength, binding sites, nanoparticle size, and the ligand chain length.¹³ For this reason it remains particularly challenging to obtain uniform results and compare them from one experiment to another, as the nanoparticle systems and ligands are all different. For the ligand exchange reaction, the ratio of incoming to outgoing ligands is extremely important and too high concentration may decompose the nanocluster, whereas too low results in incomplete exchange.⁴ Despite the significant progress that has been made towards surface functionalization of gold clusters, most of the synthetic methods still struggle with the wide size distributions and low yield of functional nanoparticles. Recently, Qian *et al.* reported a large scale synthesis of monodisperse Au₃₈ clusters.⁶¹ The water soluble glutathione stabilized clusters were mixed with organic phase of 1-dodecanethiol (C₁₂SH), resulting in Au₃₈(SC₁₂H₂₅)₂₄ nanoclusters. The ligand exchange process lead to etching of Au glutathione clusters. Transfer to organic phase indicated that the gold cores were capped with a hydrophobic monolayer.⁴

3.1.1 Mechanism of ligand exchange

As mentioned before, the atomic structure of nanoparticles plays a crucial role in ligand exchange synthesis. Ackerson *et al.* studied the structure of Au₂₅(SC₂H₄Ph)₁₆(p-BBT)₂ and Au₁₀₂(p-MBA)₄₀(p-BBT)₄ clusters (p-BBT = p-bromobenzenethiol, p-MBA = p-mercaptobenzoic acid) and calculated the sites where the reaction is more likely to take place.^{14,85} Recently, Millstone and co-workers performed a quantitative analysis of thiolated ligand exchange on gold nanoparticles by H¹NMR spectroscopy.⁸¹ They found that the ligand addition mechanism is influenced not only by the functional group interacting with nanoparticle's surface, but also by intermolecular interactions within the ligand shell. More densely packed ligands which are formed by molecules containing strong intermolecular interactions were found to be passive on ligand exchange modification.

Depending on size of the core of the particle, solvent type, and the ligand molecule, different attractive interactions have impact on stability of particles.⁸³ The ligands

molecule can be bound to the nanoparticles' surface by electrostatic attraction, chemisorption, or hydrophobic interaction. Typically, these interactions localized to the head group of the ligand molecule.⁸³ In organic solvents, the hydrophobic ligand molecule bound to nanoparticle surface undergo dynamic binding and unbinding processes, leading to aggregation. In the aqueous solution, the interactions are similar, however, the particles are additionally stabilized by electrostatic repulsion.

General distinction of ligand exchange processes was first suggested by Langford and Gray. Ligand exchange process with similar to associative (S_N2), similar to dissociative (S_N1) and a interchange (S_N2/S_N1) mechanisms.⁸⁹ In the associative one, the ligand exchange is driven by the introduced free ligand, while in the dissociative the exchange is controlled by detachment of ligand already bound to the nanoparticles. The kinetics of ligand exchange can be classified in a three steps process. The first step includes rapid exchange, followed by slower ligand exchange and concluding with ligand rearrangement on the particle surface.

Ligand exchange reaction on thiol monolayer-protected gold nanoparticles was first introduced by Murray *et al.*⁸⁸ Many studies have been done to try to fully understand the reaction mechanism.^{90,91} Murray *et al.* in their work used nuclear magnetic resonance and mass spectrometry which provided information on the rates of ligand exchange reaction and the chemical composition of thiol functionalized Au₃₈ and Au₁₄₀ with *p*-substituted arylthiols.^{90,91} They found that molecules exchange first at defects in the ligand shell or at corners and edges of the core crystal. Moreover, the exchange kinetics on the surface was described as an “ S_N2 type” mechanism where the initially bound ligands are replaced with the incoming one. This means that the ligand exchange is an associative mechanism, depending upon the incoming ligand. The method has been further extended to the preparation of water soluble gold nanoparticles with the functional groups including carboxyl acids, alkyl halides, amines, azides, maleimides, phenols, alcohols, carbohydrates, amphiphilic polymers, amino acids, nucleic acids, peptides and proteins.

Caragheorghopol and Chechik conducted a series of experiments under strictly controlled reaction condition and concluded that the kinetics of thiol exchange with thiol protected gold nanoparticles strongly depend on their morphology.¹³ The surface of nanoparticles consists of two sites (two defect and one non-defect site). The vertexes

and edges are classified as defect sites, whereas terraces are classified as non-defect sites (fig. 9). Each of the binding sites possesses a different electron density and steric accessibility leading to different exchange kinetics. It is assumed that the vertexes and edges have a higher reactivity than terrace sites, and are thus responsible for initial rapid exchange. The rate of ligand exchange is dependent on the ratio between different binding sites. Further, the reactivity of nanoparticles depends on the surface curvature and the reaction rate may differ with differently sized particles.^{84,91}

Recently, kinetic study on the adsorption process of dodecanethiol ligands of gold nanocrystals surface was reported.⁸⁴ The study concluded that the adsorption process occurred via two-steps process. At the beginning of ligand exchange thiol molecules are adsorbed at corner and edges sites.⁸⁴ After that, the reaction rate is slowed significantly. They explained, that during the second step the surface structure may be reorganised into more ordered ligand shell, which resulted in slow ligand adsorption.⁸⁴

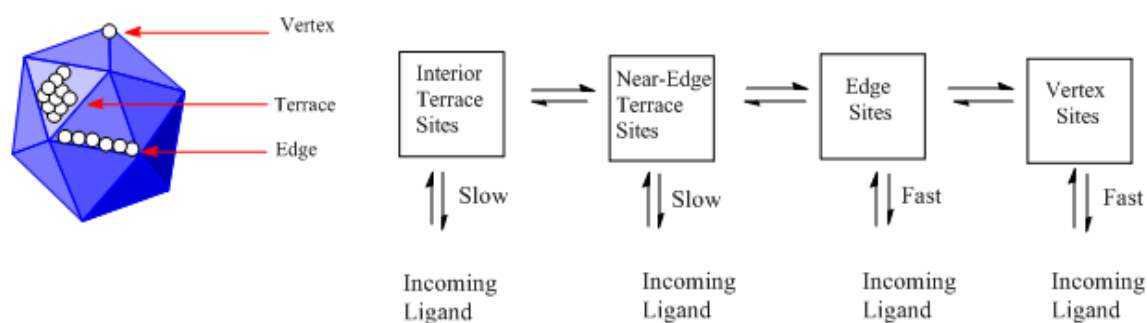


Figure 9. Schematic diagram representing the nanoparticles' surface sites.⁹²

3.1.2 Kinetics studies for ligand exchange on nanoparticles

Understanding the mechanism of ligand exchange is crucial in order to modify the ligand shell and its kinetics.⁸⁴ Chemical reaction kinetics determine the rate of chemical processes which are usually broken down into several steps yielding the products. Additionally, kinetics gives information about the reaction mechanism, with variables such as pressure, temperature, activation energy, surface area and reactant concentration which directly influence reaction rate. In the past, ligand exchange kinetics of aromatic thiolate ligands on gold nanoparticles were investigated by using NMR whereas the kinetics of short-chain thiolates, amines, and disulfides were studied by EPR spectroscopy.⁹³ Highly reactive ligand thiols require faster kinetics approaches such as fluorescence or optical spectroscopies.⁹⁴

The kinetics of the exchange of 2-phenylethanethiolate on the Au₃₈ and Au₄₀ by chiral BINAS (1,1'-binaphthyl-2,2'-dithiol) were studied by using circular dichroism (CD) spectroscopy.⁹⁵ It was shown that the ligands were only partially exchanged by BINAS, and each BINAS ligand replaces two PhCH₂CH₂S. The reason was assumed to be the nature of BINAS ligand, and its steric hindrance, as well as the binding sites in gold clusters. They explained that the changes in reactivity between Au₃₈ and Au₄₀ are caused by the different ratio of defect to non-defect sites. The comparison of both exchange reactions shows that Au₄₀ undergoes the exchange much faster and to a higher extent.⁹⁵ However, the kinetics of both reactions slow drastically after a few hours even in a huge excess of incoming molecules.⁹⁶ More recently, the studies on absorption kinetics of dodecanethiol ligands on cluster's surface were reported.⁵ The most dramatic changes were observed within the first reaction minutes, indicating that the reaction occurred initially more preferably on the edge and corner sites.

Furthermore, Murray *et al.* studied kinetics of the exchange of phenylethanethiolate ligands of monolayer-protected gold cluster of Au₃₈(SC₂Ph)₂₄ and Au₁₄₀(SC₂Ph)₅₃ by para-substituted arylthiols.⁹¹ They assumed that the reactivity of clusters at the initial stage is almost independent of their size. The places for initial ligand exchange such as vertices and edges, are common for these species. However, the later stages of ligand exchange are much slower because larger terraces are present with the increasing size. While several authors observed an increase in the ligand exchange rate of gold nanoparticles with a decreasing particle size^{5,91}, the other results were quite contrar.⁹⁷ Further, the kinetics were followed during the period of rapid exchange and the data indicated that the mechanism occurred via first-order-rate. However, the reaction started varying linearly with an incoming arylthiol concentration indicating a second-order reaction. The results were consistent with ligand exchange being an associative process.

Further studies on ligand exchange reaction, using a high resolution separation method indicated that the reaction started to occur preferably at thiolates rather than disulfides or diselenides.⁹ The studies have shown that disulphides exchange at a much lower rate than thiol ligands.¹³ However, density density theory structure investigation showed that the framework structure of the selenolate protected gold cluster is similar to the thiolate gold cluster. The studies also indicated that the formation of selenolate protected clusters is thermodynamically more favorable than thiolate protected clusters.⁸⁹

Similar results were obtained by Graf *et al.*, who studied the effect of multivalence and nanoparticles' size on the binding kinetics of thiol ligands on gold nanoparticles.⁹⁴ The monovalent, divalent and trivalent ligands were explored and the results showed that with the increasing valence, the rate of reaction was decreased. The particle size dependence was also studied by the same group, and it was shown that the exchange rate increases with the particle size.⁵⁴ In addition, the effect of the length of ligand chain was investigated.⁹⁸ It was concluded that longer chains as well as the bulkiness indicate much slower reaction rate comparing to shorter ligands.⁹⁸ Interestingly, it was found that charged ligands require shorter exchange time than uncharged ones. This may be explained by the increased solubility of the particle in the aqueous layer. Furthermore, the exchange reaction of phenylethanethiolate ligands on gold clusters by para-substituted aryl thiols was studied.⁹¹ At the initial stage the rate of reaction increased rapidly and after the equilibrium was reached it slowed down. They concluded that thiols with an electron donating substituent at the end group of the ligand usually increase the rate.

3.1.3 Effects of surface binding groups and head groups

One of the major factors of surface modification reactions is the ligand being used. The properties and stability of nanoparticles can be enhanced by the use of different functionalized ligands.⁹⁹

Various ligands have a different binding affinity on the surface of gold.⁸⁴ The gold surface composition and structure of particles are crucial for the selection of a new ligand. Metal nanoparticles can be modified by thiols, disulfides, amines, nitriles, carboxylic acids and phosphines. Most of the studies involve thiol or phosphine as headgroups.⁴ In 2005, Hutchison *et al.* exchanged all the phosphine ligands by using variable alkyl or arylthiols -functionalized ligands. The synthesis turned out to be easy to prepare a large variety of functional groups. Chen *et al.* found out that the surface ligand field exhibits a strong effect on the electronic energy structure of nanoparticles. They exchanged phosphine stabilized ligands with dodecanethiol protected Au₁₁ clusters and observed photoluminescence from the thiol protected clusters.⁴

Modification of metal nanoparticles by using organosulfur compounds is a well-known method for introducing ligands into the core.⁸⁴ Sulphur groups have strong affinity to various metals, including gold. The modification reaction usually takes place rather

quickly because the sulphur group often adsorbs spontaneously. In addition to the compounds with only one thiol group, the compounds with more than one thiol group are often used. Recently, multi-thiols compounds have been of particular interest because of the enhanced bond strength to metal cations. Disulfides also result in better stabilization comparing to their monovalent derivatives. The first functional group facilitates the surface binding, whereas the other initiates the designed chemical reactions. The thiol-thiol exchange reactions mostly take place in excess of incoming ligand. The exchange may occur completely or partially depending on the reaction time, amount of ligand and the ligand itself. The partial exchanged gold's surface is composed of both the previous and the incoming ligand in a certain ratio.²⁰ The weak Van der Waals interactions are the main driving force in the surface modification reactions thus the stronger binding is necessary between gold and the ligand. Therefore, gold particles usually require sulphur as a binding group due to its strong affinity towards gold.

The surface of particles can be modified by the use of amines.⁸³ The interaction between amino group and metal surface is weaker than that of thiolate groups, which has an impact on their size. The adsorption of long chain ammonium ions is commonly used because of their amphiphilic properties. The hydrophobic particles can be tuned into hydrophilic which is useful for bio-applications. Shorter chain ammonium ions have been used to stabilize transition metal nanoparticles.²⁰ Other molecules including fluorescent dyes, drugs, protein, peptides, antibodies or other molecules can also be attached to amine/carboxyl groups. The advantage of using biomolecules such peptides over other surface ligands is that they offer solubilisation and biofunctionalization simultaneously. Other studies used amines to functionalize gold structure because of their weak covalent bonding with gold. They reported that the amine-gold interaction is much weaker comparing to the sulphur-gold interaction. They also claimed that the alcohol group does not exhibit any interaction with the Au⁰ surface.¹⁰⁰

Even though the phosphine stabilized metal nanoparticles have been widely studied, the synthesis process involves several steps, in addition to a challenging purification.⁴ Moreover, phosphine interaction with nanoparticles is very weak, which makes them unstable, with a tendency to decompose even at ambient conditions. Due to their high exchange probability such an exchange usually proceeds completely in a way that every phosphine ligand becomes exchanged. Phosphine ligands can be exchanged with thiols

resulting in increased stability of particles. Hutchison *et al.* replaced triphenylphosphines protecting Au_{11} cluster through the exchange reaction and obtained an alkanethiol protected cluster.⁷¹ Recently, Shischiu *et al.* also used a phosphine-stabilized Au_{11} clusters to obtain glutathione (GSH) protected Au_{25} nanoclusters under optimized conditions.⁴ Amine protected clusters can be synthesised by ligand exchange reaction with the original phosphine stabilized ligands. Nevertheless, the results showed that less stable particles were obtained. Interestingly, the growth of particles was observed after the exchange reaction.²⁰

Nanoparticles can be modified with organic or inorganic molecules depending on their solubility. The hydroxyl group appears to be commonly used capping group because it can easily react with the carboxyl group or with various silane groups.¹⁰¹ Organic soluble particles have to be first modified with functional groups such as mercapto or hydroxyl groups for further conjugation reactions. A great number of ligand exchange reactions on alkane thiol protected gold nanoparticles have been introduced by Murray *et al.*⁹³ After that the reactions were extended to the preparation of water-soluble particles containing various functional groups. Small molecules with functional head groups such as thiols, carboxyl acids, alkyl halides, amines, azides, maleimides, phenols, alcohols, amino acids, nucleic acids, peptides and phosphine groups have been reported as a good candidates for generating water soluble particles. Nevertheless, electrostatic interaction in solution stabilizes nanoparticles capped with small molecules, hence they become dependent on solution conditions, sometimes causing aggregation. Therefore, polymeric ligands are an alternative way to overcome poor stability of nanoparticles. Among them, poly(ethylene glycol) (PEG) offers good stability and water solubility due to the hydrogen bonding of ether group in the backbone of PEG chain.⁷

3.2 Chemical conjugation of gold nanoparticles

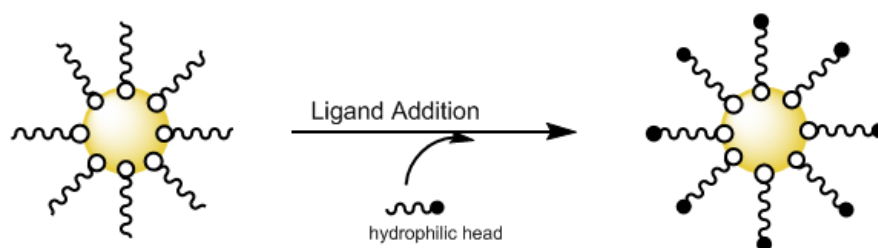


Figure 10. Chemical conjugation of gold nanoparticles.¹⁰²

Chemical conjugation is based on the ligand modification strategy, where the ligand molecule stabilizing the particle is modified (fig. 10). For example, the polarity of hydrophilic nanoparticles stabilized by mercaptobenzoic acid (*p*MBA) can be changed by modification with hydrophobic molecule by using carboxylic terminal group, by formation of complex on nanoparticles surface or by covalent attachment of ligand.⁸³ Ligand addition may be efficient phase transfer concept, however, some of the systems may not be compatible with each other, leading to restrictions.

In the chemical conjugations covalent bonding is the most widely used interaction, yielding a stable and specific conjugation of molecules with NPs.^{7,83} The molecules with the functional groups capable of covalent bonding result in higher stability. The functional groups on nanoparticles surface such as amides, thiols and carboxylic acids can be terminated with molecules through various coupling methods. Generally, covalent gold-molecule coupling ensure a good control of the particle size distribution, stability, solubility, and stable linkage. On the contrary, covalent bonding is challenging in producing site specific linkage and controlling stoichiometry of conjugated nanoparticles and molecules.

3.2.1 Coupling strategies

The most common coupling method, for immobilizing molecules with various functionalized head groups, is the amine group ($-NH_2$) because its stability.^{20,83} Therefore, the amide linkages are highly attractive for covalent coupling conjugation strategies. Generally, any primary or secondary amino group can be coupled using carbodiimide coupling chemistry. For example, amine-terminated nucleic acids, small molecules with amine groups, and various proteins (enzymes and antibodies) can be coupled to carboxylic group functionalized NPs. In addition, amine-functionalized gold nanoparticles can be conjugated to a carboxylic acid bearing molecule or material by using the same method. In addition, the nucleophile character of the amine group allows reacting with some other functional groups such as aldehydes, thiols, isocyanates and epoxides. In addition, nanoparticles stabilized by amine group can be conjugated by amide bond with various crosslinker molecules such as SMCC (succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate). The group from linker molecule can be converted to maleimides that are reactive towards thiols.⁸³

Commonly used conjugation methods are based on thiolate groups of cysteine amino residue which coordinate proteins or peptides to noble metal nanoparticles.^{20,83} The thiol group selectively conjugates with primary amine groups, which provides various conjugation possibilities with multiple types of thiolated biomolecules, peptides and proteins with free or reduced cysteine and residue thiol-terminated DNA. Other typical thiol-reactive functional groups include maleimides, iodoacetamides and disulfides.⁷

Thiolates undergo Michael addition reaction with maleimides to form succinimidyl thioethers. At high concentration the selectivity of the reagents of iodoacetamides and maleimides is relatively low. In contrast, disulphide reagents react selectively with thiols. Disulphides, are susceptible to reduction by a biological reducing agents, hence they are used for reversible coupling of NPs.¹⁰²

Esterification method can be also used for functionalization and bioconjugation of gold nanoparticles.⁷ Typically, the reaction takes place between alkyl halogen-functionalized nanoparticles with phenols or carboxylic acids in a mild environments. Gold nanoparticles functionalized with carboxylic acids can also form ester linkage by reacting with phenols or molecules with hydroxyl functionalized molecules. This approach enables to conjugate various carbohydrates and polymers groups to the surface of gold nanoparticles.⁷

Conjugation of monodisperse nanoparticles to large bionanomolecules is a new tool for tracking and bioimaging biological systems.⁷ Conjugation methods with biological materials can be used to study pathogenesis of virus infection by tracking the virus in tissues and cells. Recently, Marjomäki *et al.* described a procedure of site-specific covalent conjugation of monodisperse gold nanocluster to viral surface (fig. 11).¹⁵ Water soluble $\text{Au}_{102}(\text{pMBA})_{44}$ clusters were functionalized with maleimide linkers that bind covalently via an ester bond. The functionalized gold nanoclusters were conjugated to target cysteine of viral capsid proteins via Michel addition reaction. Additionally, it was also confirmed that the labelling procedure didn't compromise the infectivity of the virus.

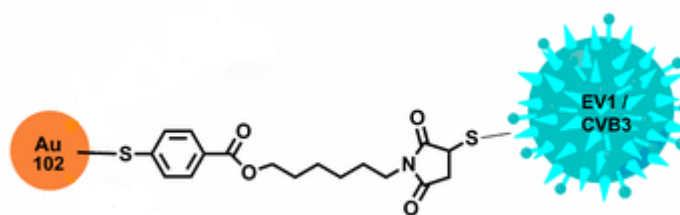


Figure 11. The maleimide functionalized $\text{Au}_{102}(\text{pMBA})_{44}$ clusters and site-specific conjugation to enteroviruses. Adapted from Ref. 15.¹⁵

Click chemistry is another highly versatile and practical approach for conjugation of gold nanoparticles. Typically, brominated alkane thiol capped gold nanoparticles upon treatment with sodium azide give an azide 1,2,3 triazole ring which can be conjugated by click chemistry to any molecules with the alkyne group.^{7,103} This conjugation chemistry gives the possibility to introduce multiple functional groups into NPs. A consequence of a click chemistry strategy is that it requires a special modification and preparation of alkyne-functional bioactive species and an azide, often resulting in low yield.²⁰

3.3 Physical conjugation of gold nanoparticles

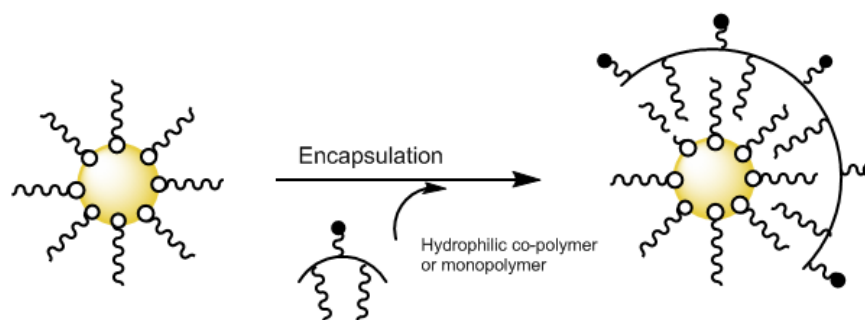


Figure 12. Physical conjugation of gold nanoparticles.¹⁰⁴

In contrast to various phase-transfer ligand exchange and modification approaches addition of amphiphilic coating layer that adsorbs by hydrophobic interaction to the hydrophobic ligand molecules of the nanoparticles does not depend on the core material and ligand type.⁸³ Addition of the molecular layer on the particles' surface is an addition of an external surface of the NPs shell without causing any changes of the initial ligands (fig. 12).⁸³ The idea is based on the addition of a hydrophobic layer of polymer and encapsulating it into the initial shell of NPs. This approach enables to transfer hydrophobic nanoparticles to water, and particles from aqueous phase to organic

solvents.⁸³ Taking advantage of using the polymer coating method, various NPs can be transferred into water with one amphiphilic polymer. One of the most known surfactant are quaternary ammonium salts.⁸³ This molecule can transfer hydrophilic nanoparticles to the organic phase by adsorbing electrostatically onto the negatively charged surface of the nanoparticles. On the contrary, CTAB can be used to transfer hydrophobic particles to the aqueous phase by hydrophobic interactions. Additionally, they appear to be stable over wide pH range and high salt concentration.

Physical conjugation strategies use noncovalent binding in a combination of hydrophobic and electrostatic interactions of the gold surface and molecules.⁹⁹ Examples of physical conjugation include adsorption of proteins on nanoparticles surface¹⁰⁵ or assembly of therapeutic agents onto nanoparticles.⁸⁶ Nanoparticles coated with hydrophobic layer can adsorb hydrophobic anticancer drugs and the drugs may then be released inside cells.⁸⁶ Even though this type of conjugation has several advantages, such as the lack of modification step and simple rapid binding, it is extremely difficult to control the orientation of the bound ligand. Therefore, this binding it is non-specific and is not appropriate for immobilizing targeting moieties.

Gold nanoparticles were successfully adapted as powerful sensor based on electrostatic properties of single and double-stranded DNA.¹⁰⁶ DNA sequencing and sensing is of great importance for pathogen detection and biomedical fields. It was found that single and double stranded oligonucleotides have a different affinity towards the nanoparticle's surface. By adsorption of single stranded DNA, the nanoparticles are stabilized, which prevents them from aggregation. The colour of nanoparticles depends on SPR and their aggregation state, therefore electrostatic properties of single-stranded DNA and double-stranded DNA can be used to design a simple colorimetric hybridization assay. More interestingly, the studies indicated that the various sizes and shapes of gold particles are essential for enhancing the sensitivity of the SPR biosensor applications.¹⁰⁶

Flexible surface chemistry, rather simple synthesis, and a large surface area of nanoparticles makes them ideal candidates for the intracellular delivery of genes, antibacterial drugs, or particular anticancer drugs. These molecules can be either covalently or non-covalently conjugated. The release of covalently bonded molecule from nanoparticle's surface can be accomplished by photo-uncaging or redox reactions.⁵ In contrast to covalent conjugations, non-covalent ones are released as the solution

condition changes or by the photothermal effect. The functionalization of polycationic molecules such as polylysines, polyarginines, polyaminoesters, polyethyleneamines or amphiphilic thiols on nanoparticle's surface enables them to attach negatively charged DNA or RNA molecules to the surface of nanoparticles and also renders intracellular delivery.

Adsorption and self-assembly are straight forward of surface modification of nanoparticles, providing good hydrophilicity and stability in suspension. Direct adsorption is considered a relatively strong, non-covalent interaction of certain biological molecules to NPs.⁸⁸ Small molecules or even polymers can attach to the surface of particles through adsorption or exchange with original ligands under mild conditions. Nevertheless, a lot of effort has been applied to direct the self-assembly on nanoparticles, especially with biomolecules or other templating agents.

Recently, Ackerson *et al.* reported the assembly, of gold nanoclusters mediated by gold-ligand interactions with nonthiolate ligands.¹⁰⁷ They investigated the dynamics and the nature of $\text{Au}_{20}(\text{SC}_2\text{H}_4\text{Ph})_{15}$ -diglyme into $\text{Au}_{20}(\text{SC}_2\text{H}_4\text{Ph})_{15}$ -diglyme- $\text{Au}_{20}(\text{SC}_2\text{H}_4\text{Ph})_{15}$ assembly and tried to understand the generality of the electron sharing among neighbouring particles. The assembly arises from the attractive forces between the gold nanoclusters and diethylene glycol dimethyl ether (diglyme), only in the presence of diglyme as a cosolvent. Similar assembly of nanoclusters was observed for *p*-mercaptobenzoic acid and glutathione protected gold nanoclusters. The results indicated that synthesis of nanoclusters in the presence of diglyme result in a system that merges the solvent and clusters together. Understanding the nature and dynamic of these assemblies may open a avenue to their fundamental properties which may find applications in sensing.¹⁰⁷

Breaking the interaction symmetry in gold nanoparticles by placing a known number of other molecules in their ligand shell would open the avenue for potential applications. Stellacci *et al.* reported creative method to obtain anisotropic assembly of gold nanoparticles.¹⁰⁸ The topological nature of monolayer protected gold nanoparticles was used to functionalize them at two diametrically opposed points. The synthesised particles worked as divalent building blocks which could produce self-standing films, by reacting with complementary divalent molecules. The carried out tests confirmed that van der Waals interactions between ligand shell of the particles and interchain

molecules morphology were stable and mechanically strong.¹⁰⁸ Generally, self-assembly of metal nanoparticles driven by the interaction of surface ligands such as proteins, DNA or multivalent thiolates, is a method capable of making one, two or three dimensional nanoparticle structures. The major driving forces for self-assembly include electrostatic interactions, capillary forces, surface tension, hydrophobic interaction, and bio-specific recognition. Host-guest interactions are common for biological systems, however, the non-biological molecules can be also stabilized by these weak interactions.

Biomolecules and different chemicals can be adsorbed to nanoparticle's surface via an electrostatic interaction.²⁰ The negatively charged molecules such as nucleic acids can be attached to the positively charged surface of nanoparticles. Further, the proteins which have a natural positive or negative charge domains can be adsorbed to NPs with negative or positive charges.¹⁰⁹ As the electrostatic interaction is the main driving force of the conjugation, the attachment is simply dependent on the surface charge domain. There are, however, lots of drawbacks which need to be considered before testing this approach. Due to a high degree of non-specific binding the target biomolecules can lose their biological function.¹⁶ Therefore, poor control over the site of modification, sensitiveness to pH and salt concentration, often require a strict physiological conditions or engineering of proteins in some cases.⁸⁶

3.4 Bioconjugation of gold nanoparticles

Bioconjugation is an extension of previously described approaches of chemical and physical conjugation (fig. 13).²⁰ It's an effective approach to introduce extra functionality into nanoparticles by using various functional molecules, including small molecules like lipids, vitamins, sugars, peptides, and larger ones such as protein, polymers, enzymes, RNA and DNA.^{7,83} Biological processes are often based on highly specific interactions between biomolecules. These interactions include receptor and target interaction, antibody-antigen interaction, enzyme and substrate interaction, and complementary base pair of nucleic acid.⁷ They can react directly and rigidly to specific sites of different kinds of nanoparticles. Taking advantages of those bioconjugate interactions of nanomaterials formulation is essential for future applications.²⁰ The large surface area of nanoparticles enables to conjugate them with various sensors, contrast agents, targeting molecules, drugs and genes.⁸³ For example, nanoparticles that are bioconjugated with DNA have been used to specifically recognize target genetic materials.⁸⁶ Their tunable optical properties make them ideal for the selective and

sensitive detection of analytes⁸⁷. Additionally, non-toxic nature, good water solubility, high biocompatibility and well-defined surface chemistry make them promising bioimaging materials. Even though the coupling strategies are highly efficient and specific, it still remains challenging to control them fully.⁸³ The main challenge is to control the orientation of the biomolecules attached to NPs.

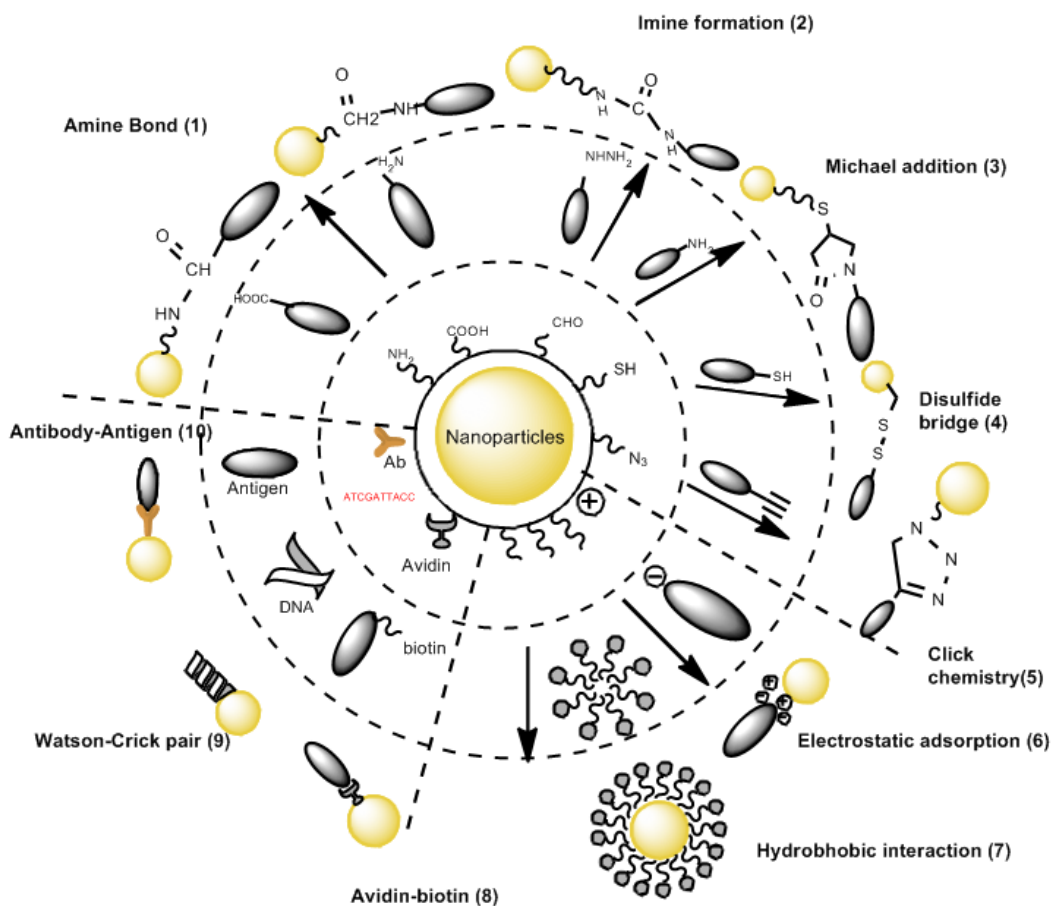


Figure 13. Examples of nanoparticle bioconjugation protocols.¹⁰⁴

The use of cross-linking agents is a common conjugation strategy. Crosslinking reagents are molecules that contain two or more functional groups which control the binding orientation, therefore, are capable of attaching to specific functional groups on another molecule. They can have different chemical reactivity and properties that affect their behavior depending on the application. Common linker chemistry is based on the reactions between sulfhydryl-containing biomolecules and amine-modified nanoparticles. Commercially available N-hydroxysuccinimide (NHS) ester and 3-sulfo-NHS ester derivatives of organic dyes, biotin can be conjugated to primary or secondary amine functionalized nanoparticles through the amide bond. The reaction mechanism can form covalent complexes between ligands and nanoparticles. Nanoparticles

functionalized with carbonyl group can be covalently conjugated to amine group through amide linkage.⁸⁶ Carbodiimide chemistry employs mild reaction condition and is effective for the attachment of molecules bearing single amine group. Moreover, it gives versatility to bioconjugation for a wide variety of protein and enzyme molecules.¹¹⁰ However, it remains difficult to control the binding orientation, leading to loss of functionality of the targeting ligands.⁸⁶

Nanoparticles can be conjugated to antibodies by using variety of methods.¹⁶ One of the most common biological interactions is to use biotinylated gold nanoparticles and attaching them to avidin/streptavidin through strong coupling between biotin and avidin. The specific and strong bonding between biotin-avidin has allowed this approach to be used in many other application.⁸³ Similarly, fluorescent dyes, drugs, amino groups in antibodies, proteins, peptides or DNAs can be biotinylated and further attached in one of the free biotin binding pockets in the streptavidin-functionalized gold nanoparticle.⁷ Recently, the coupling of protein to gold nanoparticles was studied by using strong binding between biotin and streptavidin in comparison to carbodiimide chemistry.¹¹⁰ First, the gold particles and catalase were biotinylated and then coupled together by using a streptavidin crosslinker.¹¹⁰ The biotin-streptavidin binding required two-step synthesis procedure and the enzyme bound particle turned out to be stable to be used for further studies.

The selectivity and sensitivity response of gold nanoparticles to the biological environment plays a crucial role for the biomedical applications. Therefore, conjugation strategies can be used to connect of biologically active molecules, such as oligonucleotides, DNA hairpins, peptides, antibodies, proteins, fluorescent proteins and organic dyes, and many other biological specimens to the surface of gold nanoparticles for construction of biosensors.⁷ Previously mentioned, site-specific conjugation of maleimide functionalized $\text{Au}_{102}(\text{pMBA})_{44}$ cluster to enteroviruses was recently published by Marjomäki *et al.*¹⁵ This covalent bioconjugation of gold particle to viral surface is of great importance tool for tracking of biological systems, for example, in understanding the pathogenesis of virus infection.

Surface plasmon resonance properties of gold nanomaterial are extensively applied in biological applications especially in a large variety of light-based techniques. Therefore, they can be conveniently adapted as powerful sensors of DNA hybridization processes,

pathogens, protein-protein interactions, antigens, and various toxic materials. For example, the blue shift in the SPR band is observed when the gold nanoparticles functionalized with oligonucleotides sense complementary DNA strands. Surface plasmon resonance properties are used when sensing of human chorionic gonadotropin (b-hCG). Antibody-functionalized gold nanoparticles are exploited in pregnancy tests exhibiting such a shift in the SPR band. Moreover, gold nanoparticles have been used to develop assays for cholera cells detection by using the SPR sensor technique. The information of biological interaction can be obtained by carefully monitoring SPR coupling characteristic. They have also received great attention because of detection of the DNA, aptamers or oligonucleotides with the use of functionalized AuNPs are straightforward and simple.¹⁰⁶

Additionally, the fluorescence properties of dye-functionalized oligonucleotides to complementary DNA strands have found various applications in biosensing. Nanoparticles functionalized with amine groups can be conjugated with fluorescence dyes.⁸⁶ Recently, Liu group reported the successful application of Au nanoclusters as a fluorescent sensor in the detection of cyanide with high selectivity and sensitivity.⁴ Furthermore, the fluorescent clusters can be applied to the detection of heavy ions in the environment. The enhancement in the light scattering intensity of nanoparticle-aggregates via protein-protein interaction is utilized in pharmacology to sense molecules such as adenosine, urinary, immunoglobulins and toxic substances including arsenic or pathogenic bacteria and viruses.

In addition to fluorescence properties, green fluorescent protein (GFP) functionalized nanoparticles are used cells as a biosensing method for the detection and labeling of cancer cells and microorganism. Wu *et al.* applied gold nanoparticles with near-infrared (NIR) emission in tumor fluorescence imaging in vivo.⁸⁶ The imaging signal could be well distinguished from the background and the studies confirmed no potential toxicity to the body. Moreover, a functionalized gold nanoparticle with the proper molecule such as oligonucleotide or dye is also useful for Raman- or SERS-based detection of pathogens or complementary DNA strands.

Gu *et al.* described efficient method for cancer cell imaging which was based on fluorescence dye-modified chitosan-coated magnetic nanoparticles.⁸⁶ Aldehyde functionalized nanoparticles were recently used to synthesize tumor-targeted

multifunctional viral nanoparticles using hydrazone ligation reaction.⁸⁶ Moreover, the ligation methods enable introduction of different peptides. The hydrazide functionalized nanoparticles can react directly with anticancer drugs containing carbonyl group.⁸⁶

4 CONCLUSION

A typical gold nanoparticle is a metallic particle surrounded by a dense, protecting ligand layer whose diameter is in nanoscale.³ Metal NPs have become important scientific tool, due to their special advantages, in many fields such as electronics, photochemical, biomedicine and chemistry. Their extremely small size, stability, high surface area, non-cytotoxicity and tuneable optical, chemical and physical properties have found wide spread applications in imaging, target drug delivery, therapeutics and diagnosis. During recent years, tremendous attention has been paid towards controlling the size and the surface chemistry of gold nanoparticles, mainly because all their unique properties are influenced by their diameter and protecting ligand layer.¹² One of the main advantages of gold nanoparticles is that these particles are biocompatible and can be conjugated with small biomolecules, such as enzymes, proteins, carboxylic acids amino acids and DNA, resulting in endless functionalities.

The particular interest in this thesis was in nanoclusters that can be categorized by size with diameter below 2 nm, and containing less than 200 atoms.³ These, monolayer protected gold nanoclusters are interesting because they possess unique chemical and physical properties which can be tuned by changing their size. Moreover, metal nanoclusters form a very important intermediate size regime between bulk materials and discrete atoms. Typically, they are composed of definite number of atoms and possess stable structure.³ The reduced size of metal structures has strong implications on their properties. The presence of quantum effects contribute for the appearance of new electronic, optical and chemical properties such as photoluminescence, magnetism and catalytic activity.³

Monolayer protected nanoclusters can be prepared by the chemical reduction of metal salt in the presence of the protecting ligand. The precise control and stability of the clusters are the most critical factors. The ligand which adsorb on the nanoparticles' surface prevents them from aggregation and increase the stability. Besides, the ligand

layer allows introducing of functionalities by various surface functionalization reactions.

Most of the particles can be synthesised in the organic solution. Therefore, surface modification reactions are of great importance because they can possibly provide new properties or functionality to the particles. In these cases, ligand exchange or ligand conjugation strategy can be applied. Introducing different molecules, compounds, and bridging groups to the nanoparticle's surface enable large number of possibilities with a variety of functionalities. Conjugation of nanoparticles with biomolecules allows them to interact nanoparticles specifically with biological systems. For example, nanoparticles can be site-specifically conjugated with biomolecules by molecular recognition.¹⁷ By choosing appropriate modification strategy molecules, surfaces, cells or biomolecules can be tuned, bringing new functionalities and unique properties.

For that reason, modified particles need to be characterized by using various analytical tools, depending on the nature of particles. Surface modification reactions often directly influence particle's physical and chemical properties, therefore transmission electron microscopy (TEM) can be used as preliminary tool to confirm particle size and samples' morphology. The optical properties of gold clusters can be studied using UV-vis spectroscopy. It is a valuable tool to study nanoparticles size, agglomeration state and concentration.

Generally, the properties of nanoparticles have proved to be promising in labelling and imaging, sensing and detection and as an active elements for optical sensitizing, delivery vehicles and heat mediation.¹⁷ Additionally, due to the well-defined structure of monolayer protected gold nanoclusters (MPC), functionalization surface can be examined with atomic precision. Several approaches have been used to modify MPC, such as dynamic linking of atomically precise $\text{Au}_{20}(\text{SR})_{16}$ clusters via weakly interacting diglyme molecules¹⁰⁷, and self-assembly of gold nanoclusters driven by interaction of surface ligands and proteins¹¹¹. Ligand exchange synthesis have been utilized to develop a large scale, facile synthesis of $\text{Au}_{38}(\text{SC}_{12}\text{H}_{25})_{24}$ cluster.⁶¹ It involved glutathione capped nanoclusters as starting material, which further were exchanged with $\text{HSC}_{12}\text{H}_{25}$ under etching procedure. Shischiu *et al.* also used a phosphine stabilized Au_{11} cluster to obtain glutathione (GSH) protected Au_{25} nanoclusters under optimized conditions.⁴ Moreover, the conversion of

$\text{Au}_{38}(\text{SC}_2\text{H}_4\text{Ph})_{24}$ to $\text{Au}_{36}(\text{SPh-tBu})_{24}$ was the first example of size transformation caused by the thiol-to-thiol ligand exchange.³ Monolayer protected gold nanoparticles were also successfully adapted in conjugation method with biological material which enable to study pathogenesis of virus infection by tracking the virus in tissues and cells.¹⁵

The chemistry of surface monolayer protected nanoclusters is a versatile and rapidly developing field. However still not too many functionalization studies exist so far.¹⁷ Several major issues need to be worked out for future applications. The main challenge remains in developing a suitable strategy that enables chemical control in surface modification and bioconjugation strategies of gold nanoparticles. Fundamental understanding of the molecular mechanism of the growth of nanoclusters and the principle of size control would lead to investigation of their properties and also for crystallization trials.³ Structure determination and characterization methods of clusters may help to understand the relationship between the structure and clusters properties. Moreover, from the atomic packing structure the electronic properties of clusters can be evaluated. The evaluation of the cluster's behaviour would lead to a fundamental insight into their property-structure correlation, providing new principles for the design of functional nanoparticles.

5 REFERENCES

1. Sardar R., Funston A.M., Mulvaney P., Murray R.W., Gold Nanoparticles : Past, Present, and Future, *Langmuir*, **2009**, 25, 13840-13851.
2. Daniel M.C., Astruc D., Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications Toward Biology, Catalysis, and Nanotechnology, *Chem. Rev.*, **2004**, 104, 293-346.
3. Jin R., Atomically precise metal nanoclusters: stable sizes and optical properties, *Nanoscale*, **2015**, 7, 1549-1565.
4. Lu Y., Chen W., Sub-nanometre sized metal clusters: from synthetic challenges to the unique property discoveries, *Chem. Soc. Rev.*, **2012**, 134, 3594-3623.
5. Jin R., Quantum sized , thiolate-protected gold nanoclusters, *Nanoscale*, **2010**, 2, 343-362.
6. X. Le Guevel, O. Tagit, C.E. Rodríguez, V. Trouillet, M.P. Leal, and N. Hildebrandt, Ligand effect on the size, valence state and red/near infrared photoluminescence of bidentate thiol gold nanoclusters, *Nanoscale*, **2014**, 6, 8091-8099.
7. Biju V., Chemical modifications and bioconjugate reactions of nanomaterials for sensing, imaging, drug delivery and therapy, *Chem. Soc. Rev.*, **2014**, 43, 744-764.
8. Philip R., Chantharasupawong P., Qian H., Jin R., Thomas J., Evolution of nonlinear optical properties: From gold atomic clusters to plasmonic nanocrystals, *Nano Lett.*, **2012**, 12, 4661-4667.
9. Niihori Y., Kikuchi Y., Kato A., Matsuzaki M., Negishi Y, Understanding Ligand-Exchange Reactions on Thiolate-Protected Gold Clusters by Probing Isomer Distributions Using Reversed-Phase High-Performance Liquid Chromatography, *J. Am. Chem. Soc.*, **2015**, 9, 9347-9356.
10. Jin R., Qian H, Controlling Nanoparticles with Atomic Precision: The Case of Au₁₄₄(SCH₂CH₂Ph)₆₀, *Nano Lett.*, **2009**, 9, 4083-4087.
11. Walter M., Akola J., Lopez-Acevedo O., Jadzinsky P.D., Calero G., Whetten R.L., Ackerson C.J., Grönbeck H., Häkkinen H, A unified view of ligand-protected gold clusters as superatom complexes., *PNAS*, **2008**, 105, 9157-9162.
12. Jin R., Qian H., Wu Z., Zhu Y., Zhu M., Mohanty A., Garg N., Size Focusing: A Methodology for Synthesizing Atomically Precise Gold Nanoclusters, *J. Phys. Chem. Lett.*, **2010**, 1, 2903-2910.
13. Caragheorgheopol A., Chechik V., Mechanistic aspects of ligand exchange in Au

- nanoparticles, *Phys.Chem.Chem.Phys.*, **2008**, *10*, 5029-5041.
14. Heinecke C.L., Thomas W. Ni., Malola S., Mäkinen V., Wong O.A., Häkkinen H. Ackerson C.J., Structural and Theoretical Basis for Ligand Exchange on Thiolate Monolayer Protected Gold Nanoclusters, *J. Am. Chem. Soc.*, **2012**, *134*, 13316-13322.
 15. Marjomäki V., Lahtinen T., Martikainen M., Koivisto J., Malola S., Salorinne K, Site-specific targeting of enterovirus capsid by functionalized monodisperse gold nanoclusters, *PNAS*, **2013**, *111*, 1277-1281.
 16. Morachis M., Mahmoud E.A., Almutairi A., Physical and Chemical Strategies for Therapeutic Delivery by Using Polymeric Nanoparticles, *Pharmacol. Rev.*, **2012**, *64*, 505-519.
 17. Rashid R., Murtaza G., Zahra A., Gold Nanoparticles: Synthesis and Applications in Drug, *Trop. J. Pharm. Res.*, **2014**, *13*, 1169-1177.
 18. Brust M., Walker M., Bethell D., Schiffrin D.J., Whyman R., Synthesis of Thiol-derivatised Gold Nanoparticles in a Two-phase Liquid-Liquid System, *J. Chem. Soc., Chem. Commun.*, **1994**, 801-802.
 19. Templeton A.C., Wuelfing W.P., Murray R.W., Monolayer-Protected Cluster Molecules Characterization of MPCs, *Acc. Chem. Res.*, **2000**, *33*, 27-36.
 20. Neouze M.A., Schubert U., Surface Modification and Functionalization of Metal and Metal Oxide Nanoparticles by Organic Ligands, *Chem. Review.*, **2008**, *139*, 183-195.
 21. Pattekari P., Zheng Z., Zhang X., Levchenko T., Torchilin V., Lvov Y., Top-down and bottom-up approaches in production of aqueous nanocolloids of low solubility drug paclitaxel, *Phys. Chem. Chem. Phys.*, **2011**, *13*, 9014-9019.
 22. Shimomura M., Sawadaishi T, Bottom-up strategy of materials fabrication: a new trend in nanotechnology of soft materials, *elsevier.*, **2001**, *6*, 11-16.
 23. Sardar R., Shumaker-Parry, J.S., Spectroscopic and Microscopic Investigation of Gold Nanoparticle Formation: Ligand and Temperature Effects on Rate and Particle Size, *J. Am. Chem. Soc.*, **2011**, *133*, 8179-8190.
 24. Jadzinsky P. D. *et al.*, Structure of a Thiol, *Science*, **2007**, *318*, 430-433.
 25. T. G. Schaaff *et al.*, Ascensio J, Isolation of Smaller Nanocrystal Au Molecules: Robust Quantum Effects in Optical Spectra, *J. Am. Chem. Soc.*, **1997**, *101*, 7885-7891.
 26. Goulet P.J.G., Lennox, R.B., New insights into Brust-Schiffrin metal nanoparticle synthesis, *J. Am. Chem. Soc.*, **2010**, *132*, 9582-9584.

27. Barngrover B.M., Aikens C.M, The Golden Pathway to Thiolate-Stabilized Nanoparticles: Following the Formation of Gold(I) Thiolate from Gold(III) Chloride, *J. Am. Chem. Soc.*, **2012**, *134*, 12590-12595.
28. Qian H., Jin R, Ambient Synthesis of Au₁₄₄(SR)₆₀ Nanoclusters in Methanol, *J. Am. Chem. Soc.*, **2011**, *23*, 2209-2217.
29. Liu C., Yan C., Lin J., Yu C., Huang J., Li G., One-pot synthesis of Au₁₄₄(SCH₂Ph)₆₀ nanoclusters and their catalytic application, *J. Mater. Chem. A Mater. energy Sustain.*, **2015**, *3*, 20167-20173.
30. Negishi Y., Nobusada K., Tsukuda T., Glutathione-Protected Gold Clusters Revisited : Bridging the Gap between Gold(I)-Thiolate Complexes and Thiolate-Protected Gold Nanocrystals, *Am. Chem. Soc.*, **2005**, *127*, 5261-5270.
31. Shichibu Y., Negishi Y., Tsukuda T., Teranishi T., Large-Scale Synthesis of Thiolated Au₂₅ Clusters via Ligand Exchange Reactions of Phosphine-Stabilized Au₁₁ Clusters, *J. Am. Chem. Soc.*, **2005**, *127*, 13464-13465.
32. Wu, Z., Suhan, J., Jin R., *J. Mater.*, One-Pot synthesis of atomically monodisperse , thiol-functionalized Au₂₅ nanoclusters, *J. Mater. Chem.* **2009**, *19*, 622.
33. Zhu M., Lanni E., Garg N., Bier M.E., Jin R, Kinetically Controlled , High-Yield Synthesis of Au₂₅ Clusters, *J. Am. Chem. Soc.*, **2008**, *130*, 1138-1139.
34. Wu Z., Jin R, On the Ligand's Role in the Fluorescence of Gold Nanoclusters, *Nano Lett.*, **2010**, *10*, 2568-2573.
35. Wu Z., Gayathri C., Gil RR., Jin R, Probing the Structure and Charge State of Glutathione-Capped Au₂₅(SG)₁₈ Clusters by NMR and Mass Spectrometry, *J. Am. Chem. Soc.*, **2009**, *131*, 6535-6542.
36. Jadzinsky P.D., Calero G., Ackerson C.J., Bushnell D.A., Kornberg R.D, Structure of a thiol monolayer-protected gold nanoparticle at 1.1 Å resolution, *Science*, **2007**, *318*, 430-433.
37. Levi-kalisman Y., Jadzinsky P.D., Kalisman N., Tsunoyama H., Tsukuda T., Bushnell D.A., Kornberg R.D, Synthesis and Characterization of Au₁₀₂(p-MBA)₄₄ Nanoparticles, *J. Am. Chem. Soc.*, **2011**, *133*, 2976-2982.
38. Salorinne K., Lahtinen T., Malola S., Solvation Chemistry of Water-Soluble Thiol-Protected Gold Nanocluster Au₁₀₂ from DOSY NMR Spectroscopy and DFT Calculations, *Nanoscale*, **2014**, *6*, 1-6.
39. Nishigaki J., Tsunoyama R., Tsunoyama H., Ichikuni N., Yamazoe S., Negishi Y., Ito M., Matsuo T., Tamao K., Tsukuda T., A New Binding Motif of Sterically Demanding Thiolates on a Gold Clusters, *J. Am. Chem. Soc.*, **2012**, *134*, 14295-

14297.

40. Chen Y., Zeng C., Kau DR., Jin R., Tuning the Magic Size of Atomically Precise Gold Nanoclusters via Isomeric Methylbenzenethiols, *J. Am. Chem. Soc.*, **2015**, *137*, 3603-3609.
41. Azubel M., Koivisto J., Malola S., Bushnell D., Hura G.L., Koh A.L., Tsunoyama H., Tsukuda T., Kornberg RD, Electron microscopy of gold nanoparticles at atomic resolution, *Science*, **2014**, *345*, 909-912.
42. Zeng C., Chen Y., Das A., Jin R., Transformation Chemistry of Gold Nanoclusters: From one Stable Size to Another, *J. Am. Chem. Soc.*, **2015**, *137*, 2976-2986.
43. Wolfe R.L., Murray R.W., Analytical Evidence for the Monolayer-Protected Cluster Au₂₂₅ [(S(CH₂)₅CH₃)]₇₅, *Anal. Chem.*, **2006**, *78*, 13322-13328.
44. Hostetler M.J., Wingate J.E., Zhong C., Harris J.E., Vachet R.W., Clark M.R., Londono J.D., Green S.J., Stokes J.J., Wignall G.D., Glish G.L., Porter M.D., Evans N.D., Murray R.W, Alkanethiolate Gold Cluster Molecules with Core Diameters from 1.5 to 5.2 nm : Core and Monolayer Properties as a Function of Core Size, *Langmuir*, **1998**, *14*, 17-30.
45. Marbella L.E., Millstone J.E, NMR Techniques for Noble Metal Nanoparticles, *J. Am. Chem. Soc.*, **2015**, *137*, 2721-2739.
46. Qian H., Zhu M., Wu Z., Jin R., Quantum sized gold nanoclusters with atomic precision, *Acc. Chem. Res.*, **2012**, *45*, 1470-1479.
47. Zeng C., Chen Y., Li G., Jin R., Synthesis of a Au₄₄(SR)₂₈ nanocluster: structure prediction and evolution from Au₂₈(SR)₂₀, Au₃₆(SR)₂₄ to Au₄₄(SR)₂₈, *Chem. Commun.*, **2014**, *50*, 55-57.
48. Akola J., Walter M., Whetten R.L., Häkkinen H., Grönbeck H., On the Structure of Thiolate-Protected Au₂₅, *J. Am. Chem. Soc.*, **2008**, *130*, 3756-3757.
49. Zhu M., Aikens C.M., Hollander F.J., Schatz G.C., Jin R., Correlating the crystal structure of a thiol-protected Au₂₅ cluster and optical properties, *J. Am. Chem. Soc.*, **2008**, *130*, 5883-5885.
50. Lopez-Acevedo O., Akola J., Whetten R.L., Grönbeck H., Häkkinen H., Structure and bonding in the ubiquitous icosahedral metallic gold cluster Au₁₄₄(SR)₆₀, *J. Phys. Chem. C*, **2009**, *113*, 5035-5037.
51. Heaven M.W., Dass A., White P.S., Holt K.M., Murray R.W, Crystal structure of the gold nanoparticle [N(C₈H₁₇)₄][Au₂₅(SCH₂CH₂Ph)₁₈], *J. Am. Chem. Soc.*, **2008**, *130*, 3754-3755.

52. Zhang P., X-ray Spectroscopy of Gold–Thiolate Nanoclusters, *J. Phys. Chem. Soc.*, **2014**, *118*, 25291-25299.
53. Neukermans S., Janssens E., Silverans RE., Lievens P., Magic numbers for shells of electrons and shells of atoms in binary clusters, **2006**, 1-43.
54. Zhong J., Tang X., Tang J., Su J., Pei Y., Density Functional Theory Studies on Structure, Ligand Exchange, and Optical Properties of Ligand-Protected Gold Nanoclusters: Thiolate versus Selenolate, *J. Am. Chem. Soc.*, **2015**, *119*, 9205-9214.
55. H.Häkkinen, M.Moseler, O.Kostko, N. Morgner, M.Astruc Hoffmann, and B.V. Issendorff, Symmetry and Electronic Structure of Noble Metal Nanoparticles and the Role of Relativity, *Phys. Rev. Lett.*, **2004**, 93.
56. Qian H., Eckenhoff WT., Zhu Y., Pintauer T., Jin R., Total structure determination of thiolate-protected Au₃₈ nanoparticles, *J. Am. Chem. Soc.*, **2010**, *132*, 8280-8281.
57. Lopez-Acevedo O., Tsunoyama H., Tsukuda T., Hakkinen H., Aikens C. M., Chirality and Electronic Structure of the Thiolate-Protected Au₃₈ Nanocluster, *J. Am. Chem. Soc.*, **2010**, *132*, 8210-8218.
58. Jiang D., Luo W., Tiago M.L., Dai S., In Search of a Structural Model for a Thiolate-protected Au₃₈ Cluster, *J. Phys. Chem. Soc.*, **2008**, *112*, 13905-13910.
59. Pei Y., Gao Y., Zeng X.C., Structural Prediction of Thiolate-Protected Au₃₈: A Face-Fused Bi-icosahedral, *J. Am. Chem. Soc.*, **2008**, *130*, 7830-7832.
60. Qian H., Zhu M., Andersen UN., Jin R., Facile, Large-Scale Synthesis of Dodecanethiol-Stabilized Au₃₈ Clusters, *J. Phys. Chem.*, **2009**, *113*, 4281-4284.
61. Qian H., Zhu Y., Jin R., Size-Focusing Synthesis, Optical and Electrochemical Properties of Monodisperse Au₃₈(SC₂H₄Ph)₂₄ Nanoclusters, *J. Am. Chem. Soc.*, **2009**, *3*, 3795-3803.
62. Zhu M., Qian H., Jin R., Thiolate-Protected Au₂₀ Clusters with a Large Energy Gap of 2.1 eV, *J. Am. Chem. Soc.*, **2009**, *131*, 7220-7221.
63. Zeng C., Liu C., Chen Y., Rosi N.L., Jin R., Gold –Thiolate Ring as a Protecting Motif in the Au₂₀(SR)₁₆ Nanocluster and Implications, *J. Am. Chem. Soc.*, **2014**, *136*, 11922-11925.
64. Li G., Jin R., Atomically Precise Gold Nanoclusters as New Model Catalysts, *Acc. Chem. Res.*, **2013**, *46*, 1749-1758.
65. Reimers J.R., Wang Y., Cankurtaran B.O., Ford M.J., Chemical Analysis of the Superatom Model for Sulfur-Stabilized Gold Nanoparticles, *J. Am. Chem. Soc.*,

- 2010**, *132*, 8378-8384.
66. Negishi Y., Sakamoto C., Ohyama T., Tsukuda T., Synthesis and the Origin of the Stability of Thiolate-Protected Au₁₃₀ and Au₁₈₇ Clusters, *J. Phys. Chem. Soc.*, **2012**, *3*, 1624-1628.
 67. Ho-Wu R., Yau S.H., Goodson T., Linear and Nonlinear Optical Properties of Monolayer-Protected Gold Nanocluster Films, *J. Am. Chem. Soc.*, **2016**, *10*, 562-572.
 68. S. Mustalahti S., Myllyperkiö P., Malola S., Lahtinen T., Salorinne K., Koivisto J., Häkkinen H., Pettersson M., Molecule-like Photodynamics of Au₁₀₂(pMBA)₄₄ Nanocluster, *ACS Nano*, **2015**, *9*, 2328-2335.
 69. Malola S., Lehtovaara L., J. Enkovaara, Häkkinen H., Birth of the Localized Surface Plasmon Resonance in Monolayer-Protected Gold Nanoclusters, *J. Am. Chem. Soc.*, **2013**, *7*, 10263-10270.
 70. Weissker, H.C., Escobar, H. B., Thanthirige, V. D., Kwak, K., Lee D., Ramakrishna, G., Whetten, R., López-Lozano X., Information on Quantum States Pervades the Visible Spectrum of the Ubiquitous Au₁₄₄ Gold Nanocluster *Nat. Commun.* **2014**, *5*, 1– 8.
 71. E. Hulkko, O. Lopez-Acevedo, J. Koivisto, Y. Levi-Kalisman, R. D. Kornberg, M. Pettersson, H. Häkkinen, Electronic and Vibrational Signatures of the Au₁₀₂(p- MBA)₄₄ Cluster, *J. Am. Chem. Soc.*, **2011**, *133*, 3752-3755.
 72. Takale B.S., Bao M., Yamamoto Y., Gold nanoparticle (AuNPs) and gold nanopore (AuNPore) catalysts in organic synthesis, *Org. Biomol. Chem.*, **2014**, *12*, 2005-2027.
 73. Lopez-Serrano A., Sanz-Landaluze J., Munoz-Olivas R., Nanoparticles: a global vision. Characterization, separation, and quantification methods. Potential environmental and health impact, *R. Soc. Chem.*, **2014**, *6*, 38-56.
 74. Yu L., Andriola A, Quantitative gold nanoparticle analysis methods : A review, *Talanta.*, **2010**, *82*, 869-875.
 75. Farrag M., Tschurl M., Heiz U., Chiral Gold and Silver Nanoclusters: Preparation, Size Selection, and Chiroptical Properties, *J. Am. Chem. Soc.*, **2013**, *25*, 862-870.
 76. Beck R.D., Weis P., Bräuchle G., Rockenberger J., Beck R.D., Weis P., Tandem time-of-flight mass spectrometer for cluster–surface scattering experiments, *Rev. Sci. Instrum.*, **1995**, *66*.
 77. Dass A., Mass Spectrometric Identification of Au₆₈(SR)₃₄ Molecular Gold

- Nanoclusters with 34-Electron Shell Closing, *J. Am. Chem. Soc.*, **2009**, *131*, 11666-11667.
78. Kimura K., Sugimoto N., Sato S., Yao H., Negishi Y., Size Determination of Gold Clusters by Polyacrylamide Gel Electrophoresis in a Large Cluster Region, *J. Phys. Chem. Soc.*, **2009**, *113*, 14076-14082.
 79. Tvedte L.M., Ackerson C.J, Size-Focusing Synthesis of Gold Nanoclusters with p-Mercaptobenzoic Acid, *J. Phys. Chem. Soc.*, **2014**, *118*, 8124-8128.
 80. Chaki N.K., Negishi Y., Tsunoyama H., Shichibu Y., Tsukuda T., Magic-Numbered Aun Clusters Protected by Glutathione Monolayers (n=18,21,25,,28,32,39): Isolation and Spectroscopic Characterization, *J. Am. Chem. Soc.*, **2004**, *126*, 6518-6519.
 81. Smith A.M., Marbella L.E., Johnston K.A., Hartmann M.J., Crawford S.E., Kozycz L.M., Seferos D.S., Millstone J.E., Quantitative Analysis of Thiolated Ligand Exchange on Gold Nanoparticles Monitored by ¹H NMR Spectroscopy, *J. Am. Chem. Soc.*, **2015**, *87*, 2771-2778.
 82. Farrag M., Tschurl M., Dass A., Heiz U., Infra-red spectroscopy of size selected Au₂₅, Au₃₈ and Au₁₄₄ ligand protected gold clusters, *Phys. Chem. Chem. Phys.*, **2013**, *15*, 12539-12542.
 83. Sperling R.A., Parak, W.J., Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles, *R. Soc.*, **2010**, *368*, 1333-1383.
 84. Cheng H., Yang L., Jiang Y., Huang Y., Sun Z., Zhang J., Adsorption kinetic process of thiol ligands on gold nanocrystals, *Nanoscale.*, **2013**, *5*, 11795-11800.
 85. Ni T.W., Tofanelli M.A., Phillips B.D., Ackerson C.J, Structural Basis for Ligand Exchange on Au₂₅(SR)₁₈, *J. Am. Chem. Soc.*, **2014**, *53*, 6500-6502.
 86. Yu MK., Park J., Jon S., Targeting Strategies for Multifunctional Nanoparticles in Cancer Imaging and Therapy, *Theranostics.*, **2012**, *2*, 1-44.
 87. Auger A., Samuel J., Poncelet O., Raccurt O., A comparative study of non-covalent encapsulation methods for organic dyes into silica nanoparticles, *Nanoscale Res. Lett.*, **2011**, *6*, 1-12.
 88. Heddle J.G., Gold Nanoparticle-Biological Molecule Interactions and Catalysis, *Catalyst.*, **2013**, *3*, 683-708.
 89. Caragheorgheopol A., Chechik V., Mechanistic aspects of ligand exchange in Au nanoparticles, *Phys. Chem. Chem. Phys.*, **2008**, *10*, 5029-5041.
 90. Tracy J.B., Crowe M.C., Parker J.F., Hampe O., Fields-zinna C.A., Dass A.,

- Murray R.W., Hill C., Carolina N., Karlsruhe F, Electrospray Ionization Mass Spectrometry of Uniform and Mixed Monolayer Nanoparticles: Au₂₅[S(CH₂)₂Ph]₁₈ and Au₂₅[S(CH₂)₂Ph]_{18-x}(SR)_x, *J. Am. Chem. Soc.*, **2007**, *129*, 16209-16215.
91. Guo R., Song Y., Wang G., Murray R.W., Hill C., Carolina N., Does Core Size Matter in the Kinetics of Ligand Exchanges of Monolayer-Protected Au Clusters, *J. Am. Chem. Soc.*, **2005**, *127*, 2752-2757.
 92. Hostetler M.J., Templeton A.C., Murray R.W., Hill C., Carolina N., Dynamics of Place-Exchange Reactions on Monolayer-Protected Gold Cluster Molecules, **1999**, *15*, 3782-3789.
 93. Templeton A.C., Chen S., Gross S.M., Murray R.W., Hill C., Carolina N., Water-Soluble, Isolable Gold Clusters Protected by Tiopronin and Coenzyme A Monolayers, *Langmuir*, **1999**, *13*, 66-76.
 94. Perumal S., Hofmann A., Scholz N., Eckart R., Graf C., Kinetics Study of the Binding of Multivalent Ligands on Size-Selected Gold Nanoparticles, *Langmuir*, **2011**, *27*, 4456-4464.
 95. Knoppe S., Michalet S., Bu T., Stabilization of Thiolate-Protected Gold Clusters Against Thermal Inversion: Diastereomeric Au₃₈(SCH₂CH₂Ph)_{24-2x}(R-BINAS)_x, *J. Phys. Chem. Soc.*, **2013**, *117*, 15354-15361.
 96. Knoppe S., Dharmaratne A.C., Schreiner E., Dass A., Bu T., Ligand Exchange Reactions on Au₃₈ and Au₄₀ Clusters: A Combined Circular Dichroism and Mass Spectrometry Study, *J. Am. Chem. Soc.*, **2010**, *132*, 16783-16789.
 97. Wan W., Yeow J.T.W., The effects of gold nanoparticles with different sizes on polymerase chain reaction efficiency, *Nanotechnology*, **2009**, *20*, 1-5.
 98. Woehrle G.H., Brown L.O., Hutchison J.E, Thiol-Functionalized, 1.5-nm Gold Nanoparticles through Ligand Exchange Reactions: Scope and Mechanism of Ligand Exchange, *J. Am. Chem. Soc.*, **2005**, *127*, 2172-2183.
 99. Rayavarapu R.G., Petersen W., Ungureanu C., Post J.N., Leeuwen T.G. Van., Manohar S, Synthesis and Bioconjugation of Gold Nanoparticles as Potential Molecular Probes for Light-Based Imaging Techniques, *Int. J. Biomed. Imaging.*, **2007**, *2007*, 1-10.
 100. Leff D.V., Brandt L., Heath J.R., Synthesis and Characterization of Hydrophobic, Organically-Soluble Gold Nanocrystals Functionalized with Primary Amines, *Langmuir*, **1996**, *12*, 4723-4730.
 101. Woo K., Hong J., Surface Modification of Hydrophobic Iron Oxide

- Nanoparticles for Clinical Applications, *IEEE Trans. Magn.*, **2005**, *41*, 4137-4139.
102. Raines T., Kalia J., Advances in Bioconjugation, *Curr. Org. Chem.*, **2010**, *14*, 138-147.
 103. White M.A., Johnson J.A., Koberstein J.T., Turro N.J, Toward the Syntheses of Universal Ligands for Metal Oxide Surfaces : Controlling Surface Functionality through Click Chemistry, *J. Am. Chem. Soc.*, **2006**, *128*, 11356-11357.
 104. Chen L., *Surface Functionalization and Bioconjugation of Nanoparticles for Biomedical Applications*, 2014.
 105. Calzolari L., Franchini F., Gilliland D, Protein-Nanoparticle Interaction : Identification of the Ubiquitin - Gold Nanoparticle Interaction Site, *J. Am. Chem. Soc.*, **2010**, *10*, 3101-3105.
 106. Zeng S., Yong K., Roy I., A Review on Functionalized Gold Nanoparticles for Biosensing Applications, **2011**, 491-506.
 107. Compel, W. S., Wong, O. A., Chen, X., Yi, C., Geiss, R., Hakkinen, H., Knappenberger, K. L., Jr., Ackerson, C. J., Dynamic Diglyme-Mediated Self-Assembly of Gold Nanoclusters, *J. Am. Chem. Soc.*, **2015**, *9*(12), 11690-11698.
 108. DeVries, G. A. *et al*, Divalent Metal Nanoparticles, *Science*, **2007**, *315*, 358-362.
 109. Jiang S., Zhang Y, Upconversion Nanoparticle-Based FRET System for Study of siRNA in Live Cells, *Langmuir*, **2010**, *26*, 6689-6694.
 110. Hariharasudhan D. Chirra, Travis Sexton, Dipti Biswal, Louis B. Hersh, J. Zach Hilt., Catalase coupled gold nanoparticles: Comparison between carbodiimide and biotin-streptavidin methods, *Acta Biomater.*, **2011**, *7*, 2865-2872.
 111. Ackerson C.J., Jadzinsky P.D., Jensen G.J., Kornberg R.D., Rigid, Specific, and Discrete Gold Nanoparticle/Antibody Conjugates, *J. Am. Chem. Soc.*, **2006**, *128*, 2635-2640.
 112. Yi C., Tofanelli M.A., Ackerson C.J., Knappenberger K.L., Optical properties and electronic energy relaxation of metallic Au₁₄₄(SR)₆₀ nanoclusters, *J. Am. Chem. Soc.*, **2013**, *135*, 18222-18228.