Design of organic ligands for controlled assembly and triggered aggregation of gold nanoparticles for chemical detection

Wu, Shaojue

2015

Wu, S. (2015). Design of organic ligands for controlled assembly and triggered aggregation of gold nanoparticles for chemical detection. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/65637>

<https://doi.org/10.32657/10356/65637>

**NANYANG
TECHNOLOGICAL
UNIVERSITY**

**DESIGN OF ORGANIC LIGANDS FOR CONTROLLED
ASSEMBLY AND TRIGGERED AGGREGATION OF GOLD
NANOPARTICLES FOR CHEMICAL DETECTIONS**

SHAOJUE WU

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

2015

**Design of Organic Ligands for Controlled Assembly and
Triggered Aggregation of Gold Nanoparticles for Chemical
Detection**

SHAOJUE WU

School of Physical and Mathematical Sciences

A thesis submitted to the Nanyang Technological University
in partial fulfillment of the requirement for the degree of
Doctor of Philosophy

2015

ACKNOWLEDGMENTS

I would like to express my great gratitude to my supervisor, Associate Professor Yanli Zhao for his valuable guidance and support. He gave me a lot of help and suggestions during my graduate study. His enthusiasm in scientific research has great effect on my study.

I am thankful to my lab colleagues: Mr. Chung Yen Ang, Miss Si Yu Tan, Miss Nguyen Kim Truc, Dr. Zhong Luo, Dr. Huangcheng Zhang, Dr. Peizhou Li, for their kind help.

In the end, I am also grateful for my family for their endless support in the past 4 years. Without their support, I couldn't take the challenges that I encountered during my PhD research.

TABLE OF CONTENTS

Acknowledgements.....	I
Table of contents.....	II
Abstract.....	V
Chapter 1: Introduction	
1.1 Abstract.....	1
1.2 Self-assembly of gold nanoparticles to plasmonic clusters.....	2
1.3 One-dimensional self-assembly of gold nanoparticles to plasmonic chains or helixes.....	11
1.4 Self-assembly of gold nanoparticles to plasmonic micelles and vesicles.....	14
1.5 Self-assembly of nanoparticles to colloidal supraparticles.....	18
1.6 Application of gold nanoparticle aggregation in colorimetric detection.....	29
1.7 References.....	37
Chapter 2: An Imine-Based Approach to Prepare Amine-Functionalized Janus Gold Nanoparticles.	
2.1 Introduction.....	48
2.2 Results and discussion.....	60
2.3 Conclusion.....	71
2.4 Experimental section.....	72

2.4.1 Materials and instruments.....	72
2.4.2 Synthesis of organic compounds and nanoparticles.....	73
2.5 References.....	80
 Chapter 3: Byproduct-Induced <i>In-Situ</i> Formation of Monodisperse Gold Colloidal Supraparticles.	
3.1 Introduction.....	83
3.2 Results and discussion.....	86
3.3 Conclusion.....	99
3.4 Experimental section.....	100
3.4.1 Materials and instruments.....	100
3.4.2 Synthetic procedures of thiol ligands.....	101
3.4.3 Formation procedure of gold supraparticles.....	110
3.5 References.....	111
 Chapter 4: Oxidation-Triggered Hydrophilicity Change of Gold Nanoparticles for Naked Eye Detection of Hydrogen Peroxide.	
4.1 Introduction.....	113
4.2 Results and discussion.....	117
4.3 Conclusions.....	128
4.4 Experimental section.....	129
4.4.1 Materials and instruments.....	129
4.4.2 Organic synthesis.....	130
4.4.3 Preparation of H ₂ O ₂ -responsive AuNPs.....	135

4.5 References.....	136
List of Publications.....	138

ABSTRACT

The plasmonic properties of gold nanoparticles (AuNPs) hold great potential in various applications. Their plasmonic properties are closely related to their shapes and aggregation states. A way to obtain nanostructures with altered plasmonic properties is by assembling AuNPs into higher-ordered structures. In this thesis, firstly, we developed a method to prepare AuNPs asymmetrically modified by two ligands (Janus AuNPs). A small patch of AuNP surface was selectively modified with amino groups while the rest of surface was passivated by polyethylene glycol. Such asymmetrically modified AuNPs could be used to fabricate plasmonic nanoclusters with altered plasmonic properties. Secondly, we discovered a phenomenon that AuNPs could spontaneously aggregate to form spherical colloidal superparticles when thiol molecules with a middle tetraethylene glycol segment and a hydrocarbon terminal (HS-TEG-hydrocarbon) were used as ligands in the synthesis. We found that a byproduct generated from the chemical reaction also played a key role in the formation of AuNPs. Finally, we designed a novel ligand to modified AuNPs and made them responsive to hydrogen peroxide. The ligand contains a phenylboronate moiety that can react with hydrogen peroxide and subsequently cause fragmentation of the molecule, leading to aggregation of AuNPs accompanied with a color change from red to blue. This AuNPs-based platform can be used for colorimetric detection of hydrogen peroxide and disease biomarkers after coupling to ELISA technique.

Synthesis of Organic Ligands for Controlled Assembly and Triggered

Aggregation of Gold Nanoparticles

Chapter 1

Introduction

1.1 Abstract

Gold nanoparticles are among the most extensively studied nanomaterials¹. Their optical and electronic properties endow them with great potential in several practical applications, such as chemical catalysis², sensing³, therapeutic agent delivery^{4,5}, diagnostics⁶, bio-imaging and photodynamic therapy^{7,8}. With single nanoparticles, their optical and electronic properties are tunable by changing their sizes, shapes and compositions (e.g. alloy nanoparticles). Their optical and electronic properties are also strongly dictated by their aggregation state; when two gold nanoparticles are brought into proximity, the oscillations of their valence electrons couple to each other, which results in altered optical and electronic behavior⁹. This coupling effect occurs through a dielectric medium and is exploited to make nanoparticle assemblies with new capabilities. The key point in making nanoparticle assemblies lies in developing methods to control the movement of nanoparticles in solution; this is the so called bottom-up method for nanoparticle fabrication. Though assembling of pristine gold nanoparticles through delicate control of physical properties (e.g. ionic strength) of solvents or through use of multiple-phase solvent systems (e.g. emulsions) is possible, assembling of gold nanoparticles through surface

modification with small organic ligands, polymers or biological macromolecules is the major way to control the aggregation behavior of gold nanoparticles in solution. Organic molecules containing thiol, phosphine, and NHC carbene¹⁰ can readily bond with surface gold atoms of gold nanoparticles to form a self-assembled monolayer around gold nanoparticles, which provides great convenience in the design and synthesis of organic ligands to modify and functionalize gold nanoparticles.

As nanoscale building blocks, gold nanoparticles can be assembled into various assemblies, such as clusters, chains, micelles, vesicles and supraparticles, superlattices, for different applications. Through the interactions of surface ligands, these modified gold nanoparticles can undergo self-assembly process under pre-designed pathways. The forces that can be exploited to assemble gold nanoparticles include hydrophobic force, electrostatic attraction, hydrogen bonding and covalent bonding.

1.2 Self-assembly of gold nanoparticles into plasmonic clusters.

Plasmonic clusters are noble metal nanoparticle ensembles containing discrete numbers of nanoparticles. Due to the inter-particle plasmon coupling effect, the collective plasmonic property of plasmonic clusters is largely altered from that of the mathematical addition of the single nanoparticles, a phenomenon that is analogous to that of atom-molecule relationship¹¹⁻¹³. The plasmonic shift could be exploited to construct plasmon ruler to probe the activity of enzymes^{14,15}. The plasmon coupling also creates a particularly dense

electromagnetic field at the junction of two closely positioned nanoparticles. This so called “hot spot” is critically important for surface-enhanced Raman spectroscopy application¹⁶.

To date, there have been several methods to fabricate plasmonic clusters with the use of gold nanoparticles. One of the earliest and most powerful methods to construct plasmonic clusters is by using biological macromolecules such as oligonucleotides^{17,18}. Single-stranded oligonucleotides were conjugated to gold nanoparticles, and upon addition of complementary single-stranded DNA chains as template, these gold nanoparticles were brought together via base pairing interaction (Figure 1).

Figure 1. DNA-directed assembly of gold nanoparticles into plasmonic dimers and trimers. Reproduced with permission from ref. 18, copyright 1999, Wiley interscience.

Gold nanoparticle clusters with more complex structures could also be assembled by using DNA nanostructures as templates. By delicate sequencing and length design, pyramidal nanostructures of DNA could be made, and gold nanoparticles could be specifically placed at the corner positions to form

nanoclusters of tetrahedral geometry. By using four different sizes of gold nanoparticles or four nanoparticles of different materials, gold nanoparticle pyramids with chiroptical activity could be obtained (Figure 2a)^{19,20}. More complex structures were also created with use of DNA polyhedral frameworks as templates. Gold nanoparticles modified with single-stranded DNA could be either encapsulated inside or placed outside DNA nanocages to form nanoparticle assemblies with various molecule-like structures (Figure 2b)²¹. Similarly, by using DNA rings as templates, single-stranded DNA conjugated gold nanoparticles could be precisely arranged to form nanoparticles rings with or without broken symmetries (Figure 2c)²².

Figure 2. DNA-templated assembly of more complicated gold nanoparticle nanoclusters. Reproduced with permission from (a) ref. 19, copyright 2009, American Chemical Society (b) ref. 21, copyright 2015, American Chemical Society (c) ref. 22, copyright 2015, American Chemical Society.

Besides the powerful DNA-based techniques, self-assembly of plasmonic clusters can also be realized by changing the solvent's properties like ionic

strength to induce aggregation of nanoparticles or by using small organic molecules or polymers as mediators in the process. The nanoparticles in solution have a tendency to aggregate to minimize the overall surface energy of the colloidal system. Gold nanoparticles produced by citrate reduction method have negative surface charges. The electrostatic repulsions between these nanoparticles keep the colloidal solution stable. Upon elevation of the ionic strength of the solution or decrease in the pH of the solution, the electrostatic repulsion of these nanoparticles could be reduced, which results in aggregation of nanoparticles. By finely controlling the extent of these changes, the extent of aggregation would be controlled and thus clusters of nanoparticles could be obtained. This is a simple way to obtain plasmonic clusters, but mixed oligomers of nanoparticles are usually obtained and further purification by methods such as differential centrifugation is needed (Figure 3a, b)^{23,24}. Other than this electrostatic repulsion elimination method, covalent bonding was also used to bring nanoparticles together to form clusters. For example, organic coupling reactions forming amide and azo linkages were used to connect nanoparticles. When the reaction was conducted within dilute condition, mixtures of dimers, trimers, tetramers etc. were obtained (Figure 3c)²⁵. Such covalent bonding based technique could also be done with the use of substrates like glass slides to obtain more complex structures like core-satellite structures with broken symmetry (Figure 3d)²⁶. Non-covalent forces, such as hydrophobic force, can also be employed to assemble nanoparticles into clusters. Gold

nanoparticles decorated with both hydrophilic and hydrophobic polymer brushes possess amphiphilicity. Upon addition of solvent incompatible with one brush, the stability of gold nanoparticles decrease and aggregation occurred. Through careful control of nanoparticle concentration, the extent of aggregation could be controlled and dimers of gold nanoparticles could be obtained (Figure 4a)²⁷. A similar strategy was employed to assemble gold nanoparticles into plasmonic clusters. The use of amphiphilic block copolymers in this case helps to limit the progress of aggregation and encapsulate the obtained clusters to make them stable in solution. These clusters are polyhedral with much richer internal configurations. The hydrophobic force provided from the long polystyrene ligand endows constituent nanoparticles with sufficient mobility within the aggregates and thus achieve the minimum energy state. This is a key factor for the aggregates to possess regular internal configuration (Figure 4b)²⁸.

Figure 3. Fabrication of gold nanoparticle clusters based on control on the electrostatic repulsion (a, b) and use of covalent bonding (c, d). Reproduced with permission from (a) ref. 23, copyright 2008, American Chemical Society, (b) ref. 24, copyright 2009, American Chemical Society, (c) ref. 25, copyright 2010, American Chemical Society (d) ref. 26, copyright 2012, American Chemical Society.

Figure 4. Fabrication of gold nanoparticle dimers and polyhedrons based on control of hydrophobic forces. Reproduced with permission from (a) ref. 27, copyright 2011, American Chemical Society, and (b) ref. 28, copyright 2013, American Chemical Society.

Non-spherical nanoparticles, such as nanorods, could also be assembled into plasmonic clusters. The anisotropic geometry of gold nanorods leads to assembly with either end-to-end or side-by-side configuration. Gold nanorods are synthesized with their surfaces capped with two layers of cetyltrimethylammonium bromide (CTAB), a structure similar to lipid bilayers. The curvature at the ends of gold nanorods makes the CTAB layers less dense

and more easily replaced. Thus it is possible to specifically modify the ends of gold nanorods while leaving the sides of rods unchanged. This feature of surface ligand distribution makes it easy to fabricate end-to-end assemblies of gold nanorods (Figure 5a)²⁹. Combined with steric hindrance, gold nanodumbbells with similar surface chemistry could be assembled into crosslike dimers (Figure 5b)³⁰.

Figure 5. Self-assembly of gold nanoparticle dimers with end-to-end and crosslike configurations. Reproduced with permission from (a) ref. 29, copyright 2014, American Chemical Society and (b) ref. 30, copyright 2012, American Chemical Society.

These plasmonic clusters have diverse applications. The interaction of plasmonic clusters with light generates a particularly strong electromagnetic field at the gap between two closely apposed nanocrystals³¹. This gap thus serves as nanoantenna to focus intense electromagnetic field and tremendous enhancements in fluorescence^{32,33}, IR absorption³⁴ and Raman scattering³⁵

could be achieved. With control of the dimension of the gaps, the enhancement will be high enough for single molecule detection. A way to reduce the dimension of gaps between two nanoparticles is by growing silver layers around gold nanoparticles of dimers. Initially, the distance between the two gold nanoparticles is long due to the length of DNA linkages. Then positively charged silver ions are absorbed by the negatively charged DNA layers and reduced by sodium ascorbate to form silver layers around gold cores. Under careful control of the quantity of silver ions, the thickness and thus dimensions of the gaps could be controlled. The dye molecules located inside the gaps are thus experiencing a strong electromagnetic field and can be detected with single molecule sensitivity in the SERS (Figure 6a)³⁶. Crevice areas on junctions between two connected nanoparticles are structures similar to gaps between two closely apposed nanoparticles. The thickness of the crevice areas thus serves as a factor affecting the strength of electromagnetic fields; the thinner the crevice, the stronger the field. The thickness can be tuned via controlling the size of one nanoparticle (Figure 6b)³⁷. A structure similar to crevice could also be synthesized via depositing a metal layer on nanoparticle cores to form core-shell structures with nanobridged hollow gaps (Figure 6c)³⁸. The above three structures are all fabricated with DNA conjugated nanoparticles, demonstrating the advantage and versatility of DNA based techniques. Another DNA based technique utilizes the DNA origami technology to form a three-layered DNA structure that bridges two gold nanoparticles together. The

DNA origami bridge thus serves as a gap with strong electromagnetic fields to accommodate analytes such as dye molecules for SERS analysis³⁹. The same with the case of spherical nanoparticles, hot-spots can also be fabricated with anisotropic nanorods as building blocks^{29,40}. Besides the extensive explored application in optical spectroscopic analysis, other applications like plasmon rulers^{14,15,41} and drug delivery⁴² were also studied for nanoclusters of gold nanoparticles.

Figure 6. Formation of SERS hot-spots in dimer or core-shell nanostructures. Reproduced with permission from (a) ref. 36, copyright 2010, NPG, (b) ref. 37, copyright 2014, American Chemical Society, (c), ref. 38, copyright 2011, NPG.

1.3 One-dimensional self-assembly of gold nanoparticles to plasmonic chains or helices.

Plasmonic chains of gold nanoparticles are one dimensional assembly of gold nanoparticles. They possess altered optical properties from that of clusters.

Today, many approaches have been developed for the fabrication of nanoparticle chains. They include salt/pH-induced reduction of electrostatic repulsion of charged gold nanoparticles⁴³⁻⁴⁵, small organic ligands/polymers bridged nanoparticle chains^{46,47}, polymer-templated assembly of nanoparticle chains⁴⁸, solid template assisted fabrication of nanoparticle chains⁴⁹, and DNA templated assembly of nanoparticles chains⁵⁰⁻⁵². Chains fabricated by the salt/pH induced assembly process are non-covalently linked and less stable, compared with covalently linked chains. While most methods produce linear chains, DNA-based methods provide much richer and more complex structures. For example, single-stranded DNA conjugated gold nanoparticles can be incorporated into planar DNA tile array. The subsequent self-assembling of DNA tiles into tube conformations leads to spiral three-dimensional arrangement of these gold nanoparticles (Figure 7a)⁵⁰. DNA origami 24-helix bundles carrying nine helically arranged attachment sites were used to assemble single-stranded DNA conjugated gold nanoparticles into helix structures with tunable handedness (Figure 7b)⁵¹.

Figure 7. Formation of gold nanoparticle helix through DNA-mediated assembly. Reproduced with permission from (a) ref. 50, copyright 2009, AAAS, and (b) ref. 51, copyright 2012, NPG.

The linear assembly of anisotropic gold nanorods is much easier than that of spherical gold nanoparticles with isotropic distribution of surface ligands, since gold nanorods have intrinsic anisotropic distribution of surface CTAB ligands due to the difference of curvature of the end and side areas. The specific functionalization at the end areas of gold nanorods provides a convenient way for asymmetric modification, which greatly facilitates the linear assembly of gold nanorods into plasmonic polymers. Gold nanorods are firstly end-functionalized with polystyrene in DMF and then addition of water, which is poor solvent for polystyrene, drives the linear self-assembly of gold nanorods. Notably, the self-assembly process is quite similar to that of reaction-controlled step-growth polymerization (Figure 8a)⁵³. When nanorods of different dimensions or compositions were mixed for self-assembly, copolymers of nanorods formed⁵⁴. Another powerful technique based on DNA origami technology was employed to construct nanorod helix. DNA origami templates with 'X' pattern of DNA capturing strands were mixed with gold nanorods conjugated with DNA strands with complementary sequences, which lead to positioning of these gold nanorods on the two sides of DNA origami with specific orientation (Figure 8b)⁵².

Figure 8. Linear (a) and helical (b) assembly of gold nanorods. Reproduced with permission from (a) ref. 53, copyright 2010, AAAS and (b) ref. 52, copyright 2015, American Chemical Society.

1.4 Self-assembly of gold nanoparticles to plasmonic micelles and vesicles.

Plasmonic micelles or vesicles are hollow spherical nanoparticle assemblies. Micelles or vesicles in aqueous solution are usually products of self-assembly of amphiphilic molecules such as lipids and block copolymers. One way to impart amphiphilicity to nanoparticles is by coating two distinct polymer brushes (one hydrophilic, the other hydrophobic) onto surface of gold nanoparticles. These gold nanoparticles exhibit amphiphilicity similar to block copolymers and could self-assemble in water into vesicular structures (Figure 9a)⁵⁵. Another way to impart amphiphilicity to gold nanoparticles is similar to the above method, but with the use of linear block-copolymer with a hydrophobic block and hydrophilic block. After rehydration, these amphiphilic block copolymer coated nanoparticles self-assemble into vesicular or tubular structures, a behavior resembling amphiphilic polymers (Figure 9b)⁵⁶. A third way to impart amphiphilicity to gold nanoparticles is by fabricating a

polymer-nanoparticle-polymer hybrid tri-block copolymer. A tri-block copolymer consisting of poly (ethylene oxide) and polystyrene outer blocks and a middle block that can bind to gold nanoparticles was mixed with gold nanoparticles. When the size of gold nanoparticles is comparable to the hydrodynamic dimension of the middle block, only one nanoparticle would be wrapped by the middle block and subsequently, a polymer-nanoparticle-polymer hybrid material was obtained. Since, the outer two blocks are hydrophilic and hydrophobic respectively, this hybrid compound exhibits amphiphilicity and can self-assemble into micelles, rods and vesicles (Figure 9c)⁵⁷. The above examples are nanoparticle vesicular assemblies with regular spherical morphology. Vesicular assemblies with much richer structures such as Janus vesicles form when amphiphilic nanoparticles are co-assembling with amphiphilic block copolymers. The phase separation was simulated and entropic attraction of between nanoparticles in the membranes was reported to be the dominant factor governing this process⁵⁸.

Figure 9. Self-assembly of gold nanoparticles into vesicular structure directed by amphiphilic block copolymers. Reproduced with permission from (a) ref. 55, copyright 2011, American Chemical Society, (b) ref. 56, copyright 2012, American Chemical Society and (c) ref. 57, copyright 2012, American Chemical Society.

Apart from block copolymers, small organic ligands and polypeptides can also be used to direct assembly of gold nanoparticles into vesicles. For example, a semi-fluorinated ligand was used to passivate gold nanoparticles and direct them to self-assemble into vesicles in THF (Figure 10a)⁵⁹. The bundling of the outer oligo ethylene glycol (OEG) and fluorinated OEG segments in the ligands serves as the main driving force for the self-assembling process. It was proposed by the authors that the incompatibility of these two segments in THF provided the source of amphiphilicity underlying the vesicular self-assembly process. Peptides can also serve as mediators for vesicular assembly of gold nanoparticles. It was reported that peptide, C6-AA-PEP_{Au}, (PEP_{Au} = AYSSGAPPMPPF), could direct assembly of gold nanoparticles into vesicles

(Figure 10b)⁶⁰. It was suggested by the authors that hollow self-assembled C₆-AA-PEP_{Au} structures are the entities that direct the vesicular assembly of gold nanoparticles.

Figure 10. Self-assembly of gold nanoparticles directed by oligo ethylene glycol ligands (a) and peptides (b). Reproduced with permission from (a) ref. 59, copyright 2012, American Chemical Society and (b) ref. 60, copyright 2010, American Chemical Society.

The main application of these plasmonic micelles or vesicles is used as delivery platform of therapeutic agents for treatment of diseases such as cancers. The hybrid nature of plasmonic vesicles endows them with functions derived from both organic and inorganic components. The organic components can be integrated with chemical entities that are cleavable by enzymes or thiols in cells, thus these plasmonic vesicles can be disrupted by these bio-species for release of therapeutics, which is similar to the case of liposomes or polymer-based micelles/vesicles. At the same time, the added plasmonic property derived from incorporation of gold nanoparticles allows the use of photothermal effect

derived from gold nanoparticles for triggering the disruption of these plasmonic vesicles and subsequent drug release or directly generating heat to cause apoptosis of cancer cells (Figure 11)⁶¹⁻⁶⁴. The near-field enhancement of electromagnetic field of gold nanoparticles incorporated in the vesicles also provides a tool for imaging using surface enhanced Raman spectroscopy^{64,65}.

Figure 11. Application of plasmonic vesicles for delivery and controlled release of therapeutics, SERS imaging, photothermal therapy. Reproduced with permission from ref. 64, copyright 2013, American Chemical Society.

1.5 Self-assembly of nanoparticles to colloidal supraparticles

Supraparticles are discrete three-dimensional assemblies of nanoparticles. Different from micelles/vesicles mentioned above that are hollow structures, supraparticles are solid structures with three-dimensionally continuous distribution of nanoparticles. The arrangement of constituent nanoparticles can

be long-range ordered (crystalline) or disordered (amorphous), depending on the conditions in which they are fabricated. Methods used to form supraparticles can be divided into two categories, templated and non-templated methods. Templated methods rely on molecules (surfactants or macromolecules) to spatially direct and constrain the aggregation of nanoparticles. Non-templated methods do not rely on such molecules. Instead, aggregation of nanoparticles spontaneously takes place and the morphology of aggregates follows balance of forces involved in the aggregation and minimum overall energy. In templated methods, the forces used to fabricate supraparticles include hydrophobic (solvophobic) force⁶⁶⁻⁷⁰, hydrogen bonding⁷¹, host-guest complexation⁷² and covalent bonding⁷³. While supraparticles prepared by hydrogen bonding, host-guest complexation and covalent bonding are disordered in internal arrangement of constituent nanoparticles, supraparticles fabricated by using hydrophobic (solvophobic) force usually possess long-range ordered internal arrangement of nanoparticles. The difference should arise from the different degree of mobility of nanoparticles within supraparticles to achieve positions with lowest energy state. In non-templated methods, supraparticles form *in-situ* in the synthetic solution, without addition of external additives. Surface tension induced nanocrystallite aggregation mechanism was proposed to explain the phenomenon⁷⁴.

A versatile method to fabricate supraparticles is based on oil-in-water emulsion. Hydrophobic nanoparticles were dispersed in oil phase and

emulsification by surfactants leads to oil-in-water emulsions where nanoparticles are contained within oil droplets. Subsequent evaporation of the cyclohexane solvent in these oil droplets drives the assembly of nanoparticles within the oil droplets. Since these hydrophobic nanoparticles are passivated with alkyl ligands, the ligand layers of adjacent nanoparticles can interdigitate into each other and the alkane chains of surfactants can interdigitate into the ligand layers of outermost nanoparticles. Supraparticles fabricated with this method can be well dispersed in water. Besides spherical nanoparticles, nanoparticles of other shapes such as nanoplates and nanorods can also be applied in this method to fabricate supraparticles (Figure 12)⁶⁷. In principle, nanoparticles with similar surface chemistry can all be fabricated into supraparticles, regardless of their compositions. Importantly, the internal arrangement of constituent nanoparticles is long-range ordered. This supracrystalline feature comes from the good mobility of nanoparticles within supraparticles, since they are brought together by hydrophobic force which allows them to reconfigure to maximize the interaction between nanoparticles and between nanoparticles and the liquid-liquid interface⁶⁹.

Figure 12. Fabrication of supraparticles by emulsification-evaporation method. Reproduced with permission from ref. 67, copyright 2007, Wiley interscience.

Another method to fabricate supraparticles is through controlled induction of solvophobic interaction⁶⁸, in which decomposition of nanoparticle micelles induces aggregation of nanoparticles to form supraparticles. Hydrophobic van der Waals interaction between hydrocarbon chains of ligands on nanoparticles and hydrocarbon chains of surfactants drives the formation of nanoparticle micelles. Transferring of the nanoparticle micelles from water to ethylene glycol weakens the interaction and leads to loss of surfactants from the nanoparticle micelles, which induces solvophobic interaction between nanoparticles and ethylene glycol and eventually aggregation of these

nanoparticles to form supraparticles (Figure 13a)⁶⁸. The arrangement of constituent nanoparticles also exhibit ordering due to the good mobility of these nanoparticles within supraparticles. When the same fabrication method was applied to fabricate nanorods and nanocubes, supraparticles with more complex internal structures could be obtained. In the case of nanocubes, the shape of obtained nanoparticles can be either spherical or cubic, which can be tuned by controlling the interaction between constituent nanocubes (Figure 13b)⁷⁵. This report demonstrated the importance of wettability of nanoparticle surfaces by solvent molecules to assembly of supraparticles. When anisotropic nanorods were used as building blocks, supraparticles with multiple supracrystalline domains or single-domain needle-like supraparticles with parallel alignment of constituent nanorods could be obtained (Figure 13c)⁷⁶. Compared with spherical nanoparticles, the anisotropic interactions between nanorods give rise to supraparticles with much more complex structures.

Apart from hydrophobic/solvophobic interactions based methods, Supraparticles can also be fabricated based on organic molecules as mediator. Polymer chains functionalized with diaminotriazine can mediate the formation of spherical aggregates of gold nanoparticles stabilized with thymine terminated alkanethiol ligands, through the diaminotriazine-thymine

Figure 13. Fabrication of supraparticles through the controlled induction of solvophobic interactions. Spherical nanoparticles (a), nanocubes (b) and nanorods (c) are used as building blocks. Reproduced with permission from (a) ref. 68, copyright 2007, American Chemical Society, (b) ref. 75, copyright 2012, American Chemical Society and (c) ref. 76, copyright 2012, AAAS.

recognition which is hydrogen bonding in nature. This early work was named polymer mediated ‘bricks and mortar’ strategy (Figure 14a)⁷¹. The same

strategy was applied to assemble gold nanoparticles through beta-cyclodextrin-adamantane host-guest complexation, which is hydrophobic force in nature (Figure 14b)⁷². Small organic ligand, such as tetradentate thioether, was employed to mediate spherical assembly of gold nanoparticles (Figure 14c)⁷³. A shared feature of supraparticles fabricated by methods mention above is lack of supracrystalline arrangement of constituent nanoparticles. The reason could be the poor mobility of nanoparticles within supraparticles after multiple binding or complexing with the molecular mediators.

Figure 14. Fabrication of supraparticles by polymer/multi-dentate organic molecules mediated assembly of nanoparticles. Reproduced with permission from (a) ref. 71, copyright 2000, NPG, (b) ref. 72, copyright 2010, Wiley interscience and (c) ref. 73, copyright 2002, American Chemical Society.

Spontaneous aggregation of nanoparticles in synthetic solutions without addition of mediators is another pathway for the formation of supraparticles.

The early works by Matijević and Privman are pioneering studies on this subject^{74,77}. In their works, high-temperature hydrolysis reaction leads to the formation of monodisperse metal oxide sols. The supraparticle formation is a two-stage process. In the first stage, nanoparticles form from the supersaturation solution; in the second stage, these nanoparticles aggregate into larger and usually spherical aggregates that are now termed as supraparticles. Surface tension induced nanocrystallite aggregation was proposed as mechanism to describe the formation process. This high-temperature hydrolysis reaction technique was recently applied to synthesize metal oxide and chalcogenide supraparticles^{4,78,79} in high boiling organic solvents such as 1-octadecene and diethylene glycol (Figure 15a, b). High resolution transmission electron spectroscopy reveals that within these supraparticles, adjacent nanoparticles crystallographically align with each other, which indicates the presence of an oriented attachment⁸⁰ process during the aggregation of precursor nanoparticles. Different from supraparticles prepared by hydrophobic interaction or organic mediator based methods, where constituent nanoparticles remain separated from each other by organic ligand layers, constituent nanoparticles in the supraparticles prepared via high temperature hydrolysis reaction are crystallographically interconnected and the arrangement of them is usually random.

In 2011, a new method was developed to form supraparticles via spontaneous self-assembly of nanoparticles⁸¹. The process was explained based

on the balance between electrostatic repulsion and interparticle van de Waals attraction. In their system, constituent nanoparticles retain their individuality; crystallographical merge does not occur in this case, which is different from that of the case of high temperature hydrolysis reaction mentioned above. Interestingly, the precursor nanoparticles are polydisperse in size distribution but the obtained supraparticles are monodisperse in size distribution, and the internal distribution of constituent nanoparticles are hierarchically complex with a loosely packed core and densely packed shell of nanoparticles. Core-shell supraparticles with different materials and shapes at the core and shell zones can also be assembled in this system, demonstrating the versatility of this method (Figure 15c).

Figure 15. Formation of metal oxide supraparticles via high temperature hydrolysis reaction in high boiling organic solvents (a, b) and metal chalcogenide supraparticles via balance of electrostatic repulsion and interparticle van de Waals attraction (c). Reproduced with permission from (a) ref. 78, copyright 2006, Wiley interscience, (b) ref. 80, copyright 2008, Wiley interscience and (c) ref. 82, copyright 2011, NPG.

As aggregates of nanoparticles, supraparticles have potential applications derived from the physical properties of constituent nanoparticles and added benefits from the higher-order structure. Currently, reported applications include cancer therapy^{72,82}, catalysis⁸³, dye-sensitized solar cells⁸⁴ and lithium ion battery electrode^{85,86}. Photothermal/magnetothermal effects aroused from constituent gold nanoparticles/magnetite nanoparticles can be used to enhance the therapeutic effects for cancers when combined with the use of drugs. Compared with single nanoparticles, supraparticles can carry much more drugs and have better heating effect due to the collective effect of constituent nanoparticles. Moreover, the big size (ca. 100-200 nm) of supraparticles could lead to reduced clearance and improved retention within tumors^{87,88}, compared with small-size single nanoparticles. An appealing advantage of supraparticles in drug delivery application is that they can also be cleared from the body via renal clearance (a feature of small nanoparticles with size below 5nm⁸⁷) upon dissociation into small nanoparticles when they finish the role as a delivery form⁴². Using supraparticles as supported catalyst can also address some tough problems in noble metal catalysts. Aggregation of catalytically active particles on supports and detachment of these particles from supports are the two problems encountered in current supported noble metal catalysts. By trapping catalytical particles inside the matrix of supraparticles, aggregation and detachment of catalytic particles within/from the supports are prohibited due to the three-dimensional constraints on the movement of catalytic particles, while

the mesoporous structures of supraparticle matrix allows efficient contact of catalytic particles with reactants in solution phase (Figure 16a)⁸³. The mesoporous structures of supraparticles also benefit their application in lithium ion battery anode materials. Today, metal oxide nanoparticles based anode materials suffer from excessive generation of solid-electrolyte interphase (SEI) on the surface of nanoparticles and unsatisfactory cyclic stability. Fabrication of nanoparticles into the form of supraparticles can constrain the growth of SEI on the outer surface of supraparticles. As-fabricated supraparticles also exhibit excellent cyclic stability and rate capability (Figure 16b)⁸⁵. The same strategy can also be applied to silicon nanoparticles based anode materials. In this case, the supraparticles fabricated have a pomegranate structure with void space for each encapsulated silicon nanoparticles to expand and contract during cycling (Figure 16c)⁸⁶. The higher-order structure of supraparticles also can be advantageous for fabrication of dye-sensitized solar cell electrodes. The 200-300 nm size of supraparticles can facilitate multiple reflection of light within the internal structure of electron films made from supraparticles⁸⁹, while the mesoporous structure of supraparticles allows efficient absorption of dye molecules to each constituent nanoparticle. This hierarchical structure of electrode films fabricated from supraparticles thus possesses higher energy conversion efficiency than films fabricated directly from nanoparticles⁸⁴.

Figure 16. Application of supraparticles as catalyst support and lithium ion battery anodes. Reproduced with permission from (a) ref. 84, copyright 2011, Wiley interscience, (b) ref. 86, copyright 2013, American Chemical Society and (c) ref. 87, copyright 2014, NPG.

1.6 Application of gold nanoparticle aggregation in colorimetric detection.

Colorimetric detection is a technique with high convenience and low cost, and maintains supreme sensitivity⁹⁰⁻⁹². Gold nanoparticles are ideal signal generators since they exhibit distinctive color change between their dispersed and aggregated states. Metal ions such as Pb²⁺, Cu²⁺, Hg⁺, and small biological molecules such as cysteine, large biomacromolecules like enzyme and some disease related proteins are all among the target analytes⁹³⁻¹⁰⁰.

Among many toxic heavy metal ions, Pb²⁺ has received much attention^{101,102}, due to its adverse effects to human health¹⁰³. A DNzyme directed assembly of gold nanoparticles method was developed for specific

detection of Pb^{2+} . The DNAzyme consists of an enzyme strand and a substrate strand. In the presence of Pb^{2+} , the enzyme strand will catalyze the cleavage of the substrate strand. A Pb^{2+} detection system was designed based on this cleavage process. A substrate DNA strand was designed with a middle portion with sequence that can recognize the DNAzyme strand. On the two ends of the middle portion are two strands with sequence complementary to the single-stranded DNA attached to the surface of AuNPs. The detection system is based on these three components: DNAzyme strand, the substrate strand and single-stranded DNA conjugated AuNPs. Mixing these three components results in aggregation of gold nanoparticles and a blue-colored nanoparticle solution. The DNAzyme strand has hybridized with the middle recognition portion at this step. Then, with the presence of Pb^{2+} , the DNAzyme strands catalyze the hydrolytic cleavage of the recognition portion, which leads to dissociation of gold nanoparticle aggregates and a red-colored solution. The color of the solution is thus depending on the concentration of Pb^{2+} (Figure 17a)^{93,94}. A method was developed to detect Cu^{2+} with high specificity, based on Cu(I) catalyzed 1, 3-dipolar cycloaddition of azide and alkyne. The design is simple. Gold nanoparticles are capped with azide and alkyne, respectively. In the presence of Cu^{2+} , which is reduced to Cu^+ by sodium ascorbate, the azide and alkyne groups on gold nanoparticles undergo cycloaddition reaction, which brings gold nanoparticles together to form aggregates. Thus, in the presence of Cu^{2+} ions, the color of solution changes from red to blue. This method can

detect Cu^{2+} in concentrations as low as 50 μM after overnight reaction. The chemical specificity of the click reaction endows the high selectivity of this detection platform (Figure 17b)⁹⁵. Hg^{2+} ions are among the most dangerous heavy metal ions and detection of them in aqueous solutions like drinking water and discharged waste water is very important for human health. A method to detect Hg^{2+} ions is based on the thymidine- Hg^{2+} -thymidine coordination chemistry. Gold nanoparticles conjugated with complementary DNA strands undergo aggregation similarly to gold nanoparticles conjugated with perfect complementary DNA strands. These aggregates also have narrow melting temperature range. However, in the presence of Hg^{2+} ions, the melting temperature range broadens. The extent of broadening of the melting temperature range is related to the concentration of Hg^{2+} ions. Based on the measurement of the change of the melting temperature range, the concentration of Hg^{2+} ions can be determined (Figure 17c)⁹⁷. The formed aggregates can be dissociated via abstraction of Hg^{2+} ions of the thymidine- Hg^{2+} -thymidine coordination complex by cysteine, based on which a method was developed to detect cysteine⁹⁸. Another method for Hg^{2+} detection was developed based on the stronger bonding ability of Hg^{2+} ions towards thiolates than gold. Hg^{2+} ions abstract surface thiol ligands of gold nanoparticles, which destabilizes gold nanoparticles and results in their aggregation⁹⁶.

Detection of DNA through aggregation of gold nanoparticles was based on the early work of Mirkin¹⁰⁴. In their work, introduction of target DNA single strands into solution of gold nanoparticles tethered with DNA single strands with the complementary sequence leads to the formation of an extended polymeric gold nanoparticles/polynucleotide aggregate, accompanied with a color change from red to purple due to the red-shift of surface plasmon resonance of gold nanoparticles. These aggregates possess a sharp melting transition that enables the differentiation of a variety of imperfect targets. The differentiation can reach one base level¹⁰⁵. A method based on high-fidelity Tth DNA ligase (DNA ligase from *Thermus thermophilus*¹⁰⁶) was developed to

detect point mutation¹⁰⁷. Both allele-specific discriminating strands and common strands were tethered to gold nanoparticles. Introduction of target strands made both gold nanoparticle samples aggregate via base pairing. The perfect base match between allele-specific strands and target strands allowed the two strands to be covalently ligated by high-fidelity Tth DNA ligase, while the mismatch between common strands and target strands did not. After both samples were heated to denature the DNA strands, the covalently ligated aggregates in the sample with allele-specific strands did not dissociate, while the sample with common strands dissociated. This method allows detection of point mutation with color change observable by naked eyes and does not require accurate temperature control¹⁰⁷.

It was also found that single-stranded DNA could be absorbed onto the surface of gold nanoparticles via electrostatic interaction between exposed bases and gold nanoparticles. In contrast, double stranded DNA couldn't absorb to gold nanoparticles as single-stranded DNA did^{108,109}. The rigid duplex structure of double stranded DNA was supposed to limit the uncoiling of DNA strands so that exposure of bases couldn't take place. Importantly, it was found that gold nanoparticles attached by single-stranded DNA could resist aggregation when exposed to high concentration of salts. This phenomenon was then employed for sensitive detection of specific DNA sequence. The target DNA strands hybridize with the probe single-stranded DNA sequence to form rigid duplex, which deprives the probe single-stranded DNA of the ability to

stabilize gold nanoparticles in solution of high-concentration salts. This detection method also possesses single mismatch sensitivity and is advantageous in the way that no chemical modifications on probe DNA, target DNA and gold nanoparticles are needed.

The stabilizing effect of DNA molecules on gold nanoparticles attached against salt-induced aggregation was suggested to relate to the conformation of DNA molecules on nanoparticle surface. It was studied that single-stranded DNA tethered to gold nanoparticles via Au-S bonds stabilizes gold nanoparticles more effectively than double-stranded DNA¹¹⁰. The exact mechanism is not clear yet, but it was supposed that entropic loss associated with the formation of rigid duplex upon hybridization with complementary strands might be the reason. It was also found that folding of DNA aptamers on the surface of gold nanoparticles affects the stability of gold nanoparticles in salt-induced aggregation¹¹¹. Gold nanoparticles attached with folded aptamers are more stable than the ones that attached with unfolded aptamers. It was found that folded aptamers were more extended on the surface of gold nanoparticles than unfolded DNA, as unfolded DNA collapsed on the surface of gold nanoparticles. This phenomenon was utilized to detect the target molecules that can be recognized by aptamers and cause folding of aptamers, through measurement of the enhanced stability of aptamers conjugated gold nanoparticles in salt-induced aggregation.

Apart from polynucleotides, proteins such as lectin, kinase and

cancer-related proteins are also detected with the use of gold nanoparticle aggregation as signal generation method. Lectin, for example, can cause aggregation of gold nanoparticles modified with β -D-galactose residues¹¹². The detection sensitivity of this simple colorimetric assay (lectin concentration about 1 μ g/mL, or 1 ppm) was comparable with that of ELISA. Kinase is also an important enzymes associated with phosphorylation of proteins. Identification of kinase, their substrates and potential inhibitors is very important for both fundamental research and practical application like drug discovery. A gold nanoparticle based colorimetric assay for probing kinase activity in the presence of inhibitors was developed¹¹³. Gold nanoparticles were conjugated with a peptide sequence that is substrate to a specific kinase. Then, biotinylation of these gold nanoparticles by kinase and γ -biotin-ATP could be controlled by the existence of inhibitors to the kinase. In the presence of inhibitors, gold nanoparticles were not biotinylated, thus did not aggregate after mixing with gold nanoparticles conjugated with avidin. In contrast, in the absence of inhibitors, gold nanoparticles were successfully biotinylated and underwent aggregation after mixing with gold nanoparticles conjugated with avidin. This difference in color change thus can be used to probe the activity of kinase in the presence of a certain kind of inhibitors. Single-stranded DNA binding protein is a key protein involved in various DNA activities such as DNA replication and repair. It can bind with single stranded DNA with high affinity. This protein-DNA complex can provide stronger stabilization effect on

gold nanoparticles that the protein or single-stranded DNA alone. Therefore, the aggregate state of gold nanoparticles in salt-induced aggregation test can reflect the quantity of the protein-DNA complex, which in turn provides information on the quantity of target single-stranded DNA that do not hybridize with the probe single-stranded DNA and so the information of the sequence of target DNA. This method was reported to be able to discriminate single base mismatch between target DNA and probe DNA¹¹⁴. β -Lactamases are a family of bacterial enzymes involved in bacterial resistance to β -lactam antimicrobial reagents. Screening of their inhibitors are thus of great importance clinically. β -Lactamases can efficiently catalyze the hydrolysis of β -lactam ring in penicillins and cephalosporins to generate thiol groups from the 3' position. This process was employed to construct a substrate that can generate di-thiols upon β -lactamase treatment. The generated di-thiol molecules could induce aggregation of gold nanoparticles, a process accompanied with a red-to-blue color change. Therefore, the enzyme activity could be probed by the color change. In the presence of potential inhibitors, the enzyme activity decreased, so the extent of red-to-blue color change decreased. This method could thus be used for inhibitor screening¹¹⁵.

The color change from aggregation of gold nanoparticles can also be used in enzyme-linked immunosorbent assay (ELISA) technique as signal generation mechanism. For example, an ELISA platform was recently developed based on acetylcholinesterase-catalyzed hydrolysis of acetylthiocholine, an analogue of

acetylcholine, to detection pathogens. The released thiocholine molecules can cause aggregation of gold nanoparticles, which indicates the quantity of acetylcholinesterase and thus pathogens in the test sample (Figure 18a)¹¹⁶. In this work, magnetic beads were used to load a large quantity of acetylcholinesterase and detection antibody, which greatly enhanced the sensitivity of the assay. A second example used different growth and aggregation behavior of gold nanoparticles in different concentrations of hydrogen peroxide solutions. The narrow hydrogen peroxide concentration range where the different growth and aggregation behavior take place was very narrow (only 20 μ M), which enabled the use of catalase to tune the concentration of hydrogen peroxide in the ELISA technique. The narrow concentration range mentioned above, together with the high catalytic activity of catalase, makes the sensitivity of the assay extremely low (Figure 18b)⁹⁹.

Figure 18. Detection of pathogens and cancer related proteins using ELISA with aggregation of gold nanoparticles as signal transducer. Reproduced with permission from (a) ref. 105, copyright 2013, Wiley interscience and (b) ref. 106, copyright 2012, NPG.

1.7 References

- (1) Daniel, M.-C.; Astruc, D. *Chem. Rev.* **2004**, *104*, 293.

- (2) Thompson, D. T. *Nano Today* **2007**, 2, 40.
- (3) Anker, J. N.; Hall, W. P.; Lyandres, O.; Shah, N. C.; Zhao, J.; Van Duyn, R. P. *Nat. Mater.* **2008**, 7, 442.
- (4) Gu, F. X.; Karnik, R.; Wang, A. Z.; Alexis, F.; Levy-Nissenbaum, E.; Hong, S.; Langer, R. S.; Farokhzad, O. C. *Nano Today* **2007**, 2, 14.
- (5) Dreaden, E. C.; Alkilany, A. M.; Huang, X.; Murphy, C. J.; El-Sayed, M. A. *Chem. Soc. Rev.* **2012**, 41, 2740.
- (6) Peng, G.; Tisch, U.; Adams, O.; Hakim, M.; Shehada, N.; Broza, Y. Y.; Billan, S.; Abdah-Bortnyak, R.; Kuten, A.; Haick, H. *Nat. Nanotechnol.* **2009**, 4, 669.
- (7) Huang, X.; El-Sayed, I. H.; Qian, W.; El-Sayed, M. A. *J. Am. Chem. Soc.* **2006**, 128, 2115.
- (8) Zhang, Y.; Qian, J.; Wang, D.; Wang, Y.; He, S. *Angew. Chem., Int. Ed.* **2013**, 52, 1148.
- (9) Choi, C. L.; Alivisatos, A. P. *Annu. Rev. Phys. Chem.* **2010**, 61, 369.
- (10) Crudden, C. M.; Horton, J. H.; Ebralidze, I. I.; Zenkina, O. V.; McLean, A. B.; Drevniok, B.; She, Z.; Kraatz, H.-B.; Mosey, N. J.; Seki, T.; Keske, E. C.; Leake, J. D.; Rousina-Webb, A.; Wu, G. *Nat. Chem.* **2014**, 6, 409.
- (11) Prodan, E.; Radloff, C.; Halas, N. J.; Nordlander, P. *Science* **2003**, 302, 419.
- (12) Halas, N. J.; Lal, S.; Chang, W.-S.; Link, S.; Nordlander, P. *Chem. Rev.* **2011**, 111, 3913.

- (13) Wang, H.; Brandl, D. W.; Nordlander, P.; Halas, N. J. *Acc. Chem. Res.* **2007**, *40*, 53.
- (14) Sonnichsen, C.; Reinhard, B. M.; Liphardt, J.; Alivisatos, A. P. *Nat. Biotechnol.* **2005**, *23*, 741.
- (15) Reinhard, B. M.; Sheikholeslami, S.; Mastroianni, A.; Alivisatos, A. P.; Liphardt, J. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 2667.
- (16) Fang, Y.; Seong, N.-H.; Dlott, D. D. *Science* **2008**, *321*, 388.
- (17) Alivisatos, A. P.; Johnsson, K. P.; Peng, X.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P.; Schultz, P. G. *Nature* **1996**, *382*, 609.
- (18) Loweth, C. J.; Caldwell, W. B.; Peng, X.; Alivisatos, A. P.; Schultz, P. G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1808.
- (19) Mastroianni, A. J.; Claridge, S. A.; Alivisatos, A. P. *J. Am. Chem. Soc.* **2009**, *131*, 8455.
- (20) Yan, W.; Xu, L.; Xu, C.; Ma, W.; Kuang, H.; Wang, L.; Kotov, N. A. *J. Am. Chem. Soc.* **2012**, *134*, 15114.
- (21) Li, Y.; Liu, Z.; Yu, G.; Jiang, W.; Mao, C. *J. Am. Chem. Soc.* **2015**, *137*, 4320.
- (22) Roller, E.-M.; Khorashad, L. K.; Fedoruk, M.; Schreiber, R.; Govorov, A. O.; Liedl, T. *Nano Lett.* **2015**, *15*, 1368.
- (23) Wang, X.; Li, G.; Chen, T.; Yang, M.; Zhang, Z.; Wu, T.; Chen, H. *Nano Lett.* **2008**, *8*, 2643.
- (24) Chen, G.; Wang, Y.; Tan, L. H.; Yang, M.; Tan, L. S.; Chen, Y.; Chen,

H. *J. Am. Chem. Soc.* **2009**, *131*, 4218.

(25) Maneeprakorn, W.; Malik, M. A.; O'Brien, P. *J. Am. Chem. Soc.* **2010**, *132*, 1780.

(26) Yoon, J. H.; Lim, J.; Yoon, S. *ACS Nano* **2012**, *6*, 7199.

(27) Cheng, L.; Song, J.; Yin, J.; Duan, H. *J. Phys. Chem. Lett.* **2011**, *2*, 2258.

(28) Urban, A. S.; Shen, X.; Wang, Y.; Large, N.; Wang, H.; Knight, M. W.; Nordlander, P.; Chen, H.; Halas, N. J. *Nano Lett.* **2013**, *13*, 4399.

(29) Stewart, A. F.; Lee, A.; Ahmed, A.; Ip, S.; Kumacheva, E.; Walker, G. C. *ACS Nano* **2014**, *8*, 5462.

(30) Grzelczak, M.; Sánchez-Iglesias, A.; Mezerji, H. H.; Bals, S.; Pérez-Juste, J.; Liz-Marzán, L. M. *Nano Lett.* **2012**, *12*, 4380.

(31) Wustholz, K. L.; Henry, A.-I.; McMahon, J. M.; Freeman, R. G.; Valley, N.; Piotti, M. E.; Natan, M. J.; Schatz, G. C.; Duyne, R. P. V. *J. Am. Chem. Soc.* **2010**, *132*, 10903.

(32) Bek, A.; Jansen, R.; Ringler, M.; Mayilo, S.; Klar, T. A.; Feldmann, J. *Nano Lett.* **2008**, *8*, 485.

(33) Kinkhabwala, A.; Yu, Z.; Fan, S.; Avlasevich, Y.; Mullen, K.; Moerner, W. E. *Nat. Photonics* **2009**, *3*, 654.

(34) Brown, L. V.; Zhao, K.; King, N.; Sobhani, H.; Nordlander, P.; Halas, N. J. *J. Am. Chem. Soc.* **2013**, *135*, 3688.

(35) McMahon, J. M.; Li, S.; Ausman, L. K.; Schatz, G. C. *J. Phys. Chem.*

C **2012**, *116*, 1627.

(36) Lim, D.-K.; Jeon, K.-S.; Kim, H. M.; Nam, J.-M.; Suh, Y. D. *Nat. Mater.* **2010**, *9*, 60.

(37) Lee, J.-H.; You, M.-H.; Kim, G.-H.; Nam, J.-M. *Nano Lett.* **2014**, *14*, 6217.

(38) Lim, D.-K.; Jeon, K.-S.; Hwang, J.-H.; Kim, H.; Kwon, S.; Suh, Y. D.; Nam, J.-M. *Nat. Nanotechnol.* **2011**, *6*, 452.

(39) Kühler, P.; Roller, E.-M.; Schreiber, R.; Liedl, T.; Lohmüller, T.; Feldmann, J. *Nano Lett.* **2014**, *14*, 2914.

(40) Osberg, K. D.; Rycenga, M.; Harris, N.; Schmucker, A. L.; Langille, M. R.; Schatz, G. C.; Mirkin, C. A. *Nano Lett.* **2012**, *12*, 3828.

(41) Liu, N.; Hentschel, M.; Weiss, T.; Alivisatos, A. P.; Giessen, H. *Science* **2011**, *332*, 1407.

(42) Chou, L. Y. T.; Zagorovsky, K.; Chan, W. C. W. *Nat. Nanotechnol.* **2014**, *9*, 148.

(43) Zhang, H.; Wang, D. *Angew. Chem., Int. Ed.* **2008**, *120*, 4048.

(44) Xia, H.; Su, G.; Wang, D. *Angew. Chem., Int. Ed.* **2013**, *52*, 3726.

(45) Kang, Y.; Erickson, K. J.; Taton, T. A. *J. Am. Chem. Soc.* **2005**, *127*, 13800.

(46) DeVries, G. A.; Brunnbauer, M.; Hu, Y.; Jackson, A. M.; Long, B.; Neltner, B. T.; Uzun, O.; Wunsch, B. H.; Stellacci, F. *Science* **2007**, *315*, 358.

(47) Hüsken, N.; Taylor, R. W.; Zigah, D.; Taveau, J.-C.; Lambert, O.;

- Scherman, O. A.; Baumberg, J. J.; Kuhn, A. *Nano Lett.* **2013**, *13*, 6016.
- (48) Sardar, R.; Shumaker-Parry, J. S. *Nano Lett.* **2008**, *8*, 731.
- (49) Shen, X.; Chen, L.; Li, D.; Zhu, L.; Wang, H.; Liu, C.; Wang, Y.; Xiong, Q.; Chen, H. *ACS Nano* **2011**, *5*, 8426.
- (50) Sharma, J.; Chhabra, R.; Cheng, A.; Brownell, J.; Liu, Y.; Yan, H. *Science* **2009**, *323*, 112.
- (51) Kuzyk, A.; Schreiber, R.; Fan, Z.; Pardatscher, G.; Roller, E.-M.; Hoge, A.; Simmel, F. C.; Govorov, A. O.; Liedl, T. *Nature* **2012**, *483*, 311.
- (52) Lan, X.; Lu, X.; Shen, C.; Ke, Y.; Ni, W.; Wang, Q. *J. Am. Chem. Soc.* **2015**, *137*, 457.
- (53) Liu, K.; Nie, Z.; Zhao, N.; Li, W.; Rubinstein, M.; Kumacheva, E. *Science* **2010**, *329*, 197.
- (54) Liu, K.; Lukach, A.; Sugikawa, K.; Chung, S.; Vickery, J.; Therien-Aubin, H.; Yang, B.; Rubinstein, M.; Kumacheva, E. *Angew. Chem., Int. Ed.* **2014**, *126*, 2686.
- (55) Song, J.; Cheng, L.; Liu, A.; Yin, J.; Kuang, M.; Duan, H. *J. Am. Chem. Soc.* **2011**, *133*, 10760.
- (56) He, J.; Liu, Y.; Babu, T.; Wei, Z.; Nie, Z. *J. Am. Chem. Soc.* **2012**, *134*, 11342.
- (57) Hu, J.; Wu, T.; Zhang, G.; Liu, S. *J. Am. Chem. Soc.* **2012**, *134*, 7624.
- (58) Liu, Y.; Li, Y.; He, J.; Duelge, K. J.; Lu, Z.; Nie, Z. *J. Am. Chem. Soc.* **2014**, *136*, 2602.

- (59) Niikura, K.; Iyo, N.; Higuchi, T.; Nishio, T.; Jinnai, H.; Fujitani, N.; Ijro, K. *J. Am. Chem. Soc.* **2012**, *134*, 7632.
- (60) Song, C.; Zhao, G.; Zhang, P.; Rosi, N. L. *J. Am. Chem. Soc.* **2010**, *132*, 14033.
- (61) He, J.; Huang, X.; Li, Y.-C.; Liu, Y.; Babu, T.; Aronova, M. A.; Wang, S.; Lu, Z.; Chen, X.; Nie, Z. *J. Am. Chem. Soc.* **2013**, *135*, 7974.
- (62) Lin, J.; Wang, S.; Huang, P.; Wang, Z.; Chen, S.; Niu, G.; Li, W.; He, J.; Cui, D.; Lu, G.; Chen, X.; Nie, Z. *ACS Nano* **2013**, *7*, 5320.
- (63) Niikura, K.; Iyo, N.; Matsuo, Y.; Mitomo, H.; Ijro, K. *ACS Appl. Mater. Interfaces* **2013**, *5*, 3900.
- (64) Song, J.; Pu, L.; Zhou, J.; Duan, B.; Duan, H. *ACS Nano* **2013**, *7*, 9947.
- (65) Song, J.; Zhou, J.; Duan, H. *J. Am. Chem. Soc.* **2012**, *134*, 13458.
- (66) Xu, H.; Cui, L.; Tong, N.; Gu, H. *J. Am. Chem. Soc.* **2006**, *128*, 15582.
- (67) Bai, F.; Wang, D.; Huo, Z.; Chen, W.; Liu, L.; Liang, X.; Chen, C.; Wang, X.; Peng, Q.; Li, Y. *Angew. Chem., Int. Ed.* **2007**, *46*, 6650.
- (68) Zhuang, J.; Wu, H.; Yang, Y.; Cao, Y. C. *J. Am. Chem. Soc.* **2007**, *129*, 14166.
- (69) Lacava, J.; Born, P.; Kraus, T. *Nano Lett.* **2012**, *12*, 3279.
- (70) S á nchez-Iglesias, A.; Grzelczak, M.; Altantzis, T.; Goris, B.; P é rez-Juste, J.; Bals, S.; Van Tendeloo, G.; Donaldson, S. H.; Chmelka, B. F.;

Israelachvili, J. N.; Liz-Marzán, L. M. *ACS Nano* **2012**, *6*, 11059.

(71) Boal, A. K.; Ilhan, F.; DeRouchey, J. E.; Thurn-Albrecht, T.; Russell, T. P.; Rotello, V. M. *Nature* **2000**, *404*, 746.

(72) Wang, S.; Chen, K.-J.; Wu, T.-H.; Wang, H.; Lin, W.-Y.; Ohashi, M.; Chiou, P.-Y.; Tseng, H.-R. *Angew. Chem., Int. Ed.* **2010**, *49*, 3777.

(73) Maye, M. M.; Chun, S. C.; Han, L.; Rabinovich, D.; Zhong, C.-J. *J. Am. Chem. Soc.* **2002**, *124*, 4958.

(74) Privman, V.; Goia, D. V.; Park, J.; Matijević, E. *J. Colloid Interface Sci.* **1999**, *213*, 36.

(75) Wang, T.; Wang, X.; LaMontagne, D.; Wang, Z.; Wang, Z.; Cao, Y. C. *J. Am. Chem. Soc.* **2012**, *134*, 18225.

(76) Wang, T.; Zhuang, J.; Lynch, J.; Chen, O.; Wang, Z.; Wang, X.; LaMontagne, D.; Wu, H.; Wang, Z.; Cao, Y. C. *Science* **2012**, *338*, 358.

(77) Matijevic, E. *Acc. Chem. Res.* **1981**, *14*, 22.

(78) Narayanaswamy, A.; Xu, H.; Pradhan, N.; Peng, X. *Angew. Chem., Int. Ed.* **2006**, *45*, 5361.

(79) Hu, X.; Gong, J.; Zhang, L.; Yu, J. C. *Adv. Mater.* **2008**, *20*, 4845.

(80) Penn, R. L.; Banfield, J. F. *Science* **1998**, *281*, 969.

(81) Xia, Y.; Nguyen, T. D.; Yang, M.; Lee, B.; Santos, A.; Podsiadlo, P.; Tang, Z.; Glotzer, S. C.; Kotov, N. A. *Nat. Nanotechnol.* **2011**, *6*, 580.

(82) Lee, J.-H.; Chen, K.-J.; Noh, S.-H.; Garcia, M. A.; Wang, H.; Lin, W.-Y.; Jeong, H.; Kong, B. J.; Stout, D. B.; Cheon, J.; Tseng, H.-R. *Angew.*

Chem., Int. Ed. **2013**, *52*, 4384.

(83) Chen, C.; Nan, C.; Wang, D.; Su, Q.; Duan, H.; Liu, X.; Zhang, L.; Chu, D.; Song, W.; Peng, Q.; Li, Y. *Angew. Chem., Int. Ed.* **2011**, *50*, 3725.

(84) Chou, T. P.; Zhang, Q.; Fryxell, G. E.; Cao, G. Z. *Adv. Mater.* **2007**, *19*, 2588.

(85) Lee, S. H.; Yu, S.-H.; Lee, J. E.; Jin, A.; Lee, D. J.; Lee, N.; Jo, H.; Shin, K.; Ahn, T.-Y.; Kim, Y.-W.; Choe, H.; Sung, Y.-E.; Hyeon, T. *Nano Lett.* **2013**, *13*, 4249.

(86) Liu, N.; Lu, Z.; Zhao, J.; McDowell, M. T.; Lee, H.-W.; Zhao, W.; Cui, Y. *Nat. Nanotechnol.* **2014**, *9*, 187.

(87) Soo Choi, H.; Liu, W.; Misra, P.; Tanaka, E.; Zimmer, J. P.; Itty Ipe, B.; Bawendi, M. G.; Frangioni, J. V. *Nat. Biotechnol.* **2007**, *25*, 1165.

(88) Zhou, C.; Long, M.; Qin, Y.; Sun, X.; Zheng, J. *Angew. Chem., Int. Ed.* **2011**, *50*, 3168.

(89) Wang, Z.-S.; Kawauchi, H.; Kashima, T.; Arakawa, H. *Coordin. Chem. Rev.* **2004**, *248*, 1381.

(90) Xia, F.; Zuo, X.; Yang, R.; Xiao, Y.; Kang, D.; Vallée-Bélisle, A.; Gong, X.; Yuen, J. D.; Hsu, B. B. Y.; Heeger, A. J.; Plaxco, K. W. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 10837.

(91) Zhou, Y.; Zhang, J. F.; Yoon, J. *Chem. Rev.* **2014**, *114*, 5511.

(92) Zhang, X.; Yin, J.; Yoon, J. *Chem. Rev.* **2014**, *114*, 4918.

(93) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2003**, *125*, 6642.

- (94) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2004**, *126*, 12298.
- (95) Zhou, Y.; Wang, S.; Zhang, K.; Jiang, X. *Angew. Chem., Int. Ed.* **2008**, *47*, 7454.
- (96) Liu, D.; Qu, W.; Chen, W.; Zhang, W.; Wang, Z.; Jiang, X. *Anal. Chem.* **2010**, *82*, 9606.
- (97) Lee, J.-S.; Han, M. S.; Mirkin, C. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 4093.
- (98) Lee, J.-S.; Ulmann, P. A.; Han, M. S.; Mirkin, C. A. *Nano Lett.* **2008**, *8*, 529.
- (99) de la Rica, R.; Stevens, M. M. *Nat. Nanotechnol.* **2012**, *7*, 821.
- (100) Liu, D.; Yang, J.; Wang, H.-F.; Wang, Z.; Huang, X.; Wang, Z.; Niu, G.; Hight Walker, A. R.; Chen, X. *Anal. Chem.* **2014**, *86*, 5800.
- (101) Godwin, H. A. *Curr. Opin. Chem. Biol.* **2001**, *5*, 223.
- (102) Lu, Y. *Chem-Eur. J.* **2002**, *8*, 4588.
- (103) Needleman, H. L. *Human Lead Exposure*; CRC Press: Boca Raton, FL, 1992.
- (104) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078.
- (105) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959.
- (106) Luo, J.; Bergstrom, D. E.; Barany, F. *Nucleic Acids Res.* **1996**, *24*, 3071.

- (107) Li, J.; Chu, X.; Liu, Y.; Jiang, J.-H.; He, Z.; Zhang, Z.; Shen, G.; Yu, R.-Q. *Nucleic Acids Res.* **2005**, *33*, e168.
- (108) Li, H.; Rothberg, L. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 14036.
- (109) Li; Rothberg, L. J. *J. Am. Chem. Soc.* **2004**, *126*, 10958.
- (110) Sato, K.; Hosokawa, K.; Maeda, M. *J. Am. Chem. Soc.* **2003**, *125*, 8102.
- (111) Zhao, W.; Chiuman, W.; Lam, J. C. F.; McManus, S. A.; Chen, W.; Cui, Y.; Pelton, R.; Brook, M. A.; Li, Y. *J. Am. Chem. Soc.* **2008**, *130*, 3610.
- (112) Otsuka, H.; Akiyama, Y.; Nagasaki, Y.; Kataoka, K. *J. Am. Chem. Soc.* **2001**, *123*, 8226.
- (113) Wang, Z.; Lévy, R.; Fernig, D. G.; Brust, M. *J. Am. Chem. Soc.* **2006**, *128*, 2214.
- (114) Tan, Y. N.; Lee, K. H.; Su, X. *Anal. Chem.* **2011**, *83*, 4251.
- (115) Liu, R.; Liew, R.; Zhou, J.; Xing, B. *Angew. Chem., Int. Ed.* **2007**, *46*, 8799.
- (116) Liu, D.; Wang, Z.; Jin, A.; Huang, X.; Sun, X.; Wang, F.; Yan, Q.; Ge, S.; Xia, N.; Niu, G.; Liu, G.; Hight Walker, A. R.; Chen, X. *Angew. Chem., Int. Ed.* **2013**, *52*, 14065.

Chapter 2

An Imine-Based Approach to Prepare Amine-Functionalized Janus Gold Nanoparticles

2.1 Introduction

Janus nanoparticles are nanoparticles with asymmetric distribution of compositions, either in the core materials or surface materials. There can be several kinds of asymmetry based on different shapes. The simplest one is spherical particle with different materials distributed on the two semispheres (Figure 1a). The relative sizes of the two faces can be tuned. For cylindrical and dish-shape objects, there can be two ways for asymmetric material distribution (Figure 1b-e). Dumbbells with different asymmetric characters can also be viewed as Janus particles (Figure 1f-k). Hollow structures with asymmetric material distribution on the two semispheres have also been assembled experimentally (Figure 1l)¹. The synthesis of Janus particles can be roughly divided into three methods: mask-functionalization, mixing and self-phase separation and self-assembly². The mask-functionalization method uses substrates to conceal one face of particle and protect it from chemical modifications that are applied to the other face of particle. The mixing and self-phase separation method takes advantage of the self-phase separation behavior of immiscible organic molecules on surfaces. Janus nanoparticles are created when their surface ligand monolayers phase-separate into two semi-spheres. However, there are relatively few examples that are reported to

successfully prepare Janus nanoparticles based on this method, since there are some requirements on the molecular structure of the two immiscible ligands. The self-assembly method also takes advantage of the self-phase separation behavior of immiscible molecules, but in this case, there is no existence of solid surface. This method is usually applied in cases with the used of polymers. In the aspect of materials, the core materials can be organic/polymeric soft materials or inorganic hard materials. In the aspect of dimensions, Janus particles can be in the range from a few nanometers to several hundreds of micrometers. In this section, we limit the discussion within spherical inorganic nanoparticles with asymmetric distribution of surface organic ligands.

Figure 1. Different kind of Janus particles. Reproduced with permission from ref. 1, copyright 2013, American Chemical Society.

Figure 2. Schematic representation of three methods used to prepare Janus nanoparticles. Reproduced with permission from ref. 2, copyright 2011, Elsevier.

The most widely employed method to prepare Janus gold nanoparticles (AuNPs) is based on the mask-functionalization method. Substrates used include glass slides (Silica nanoparticles, SiO₂NPs)³⁻⁶, resins⁷ or solid-state polymer crystals⁸. For the use of glass slides, their surfaces are silanized with a layer of amino groups, which imparts positive charge on the glass slides (Figure 3). AuNPs were then synthesized with the well known citrate reduction method, which provides AuNPs with negative surface charge. In aqueous solution, negatively charged AuNPs are absorbed onto the positively charged glass slides via electrostatic attraction. At this step, the surfaces of AuNPs that face to the glass slides are protected sterically from chemical modification in the subsequent step. It is crucial to prevent desorption of gold nanoparticle from glass slides during chemical modification. Thus, the pH of the aqueous solution used should ensure the protonation of amino groups on glass slides, and the concentration and length of ligands introduced to modify the surface of AuNPs facing to solution should be appropriate. Similarly, SiO₂NPs can also be used with this method as substrate. The large surface-to-volume ratio of SiO₂NPs makes them suitable for massive production of Janus particles. The advantage of this glass slide based masking technique lies in the convenience of preparation of the silanized substrate. Since citrate reduction method can provide monodisperse AuNPs with a large size range and AuNPs as prepared possess negative charge from the surface carboxylic acids, this glass slide based method is one of the most convenient methods so far to prepare Janus AuNPs.

Another potential advantage of using glass material as substrate is the capability to tune the ratio of the faces of AuNPs, by depositing additional layers of glass which can conceal the surface of AuNPs⁶. However, so far, there is no work reporting the use of this strategy, since it requires very high skilled operation to tune the thickness of glass layers with high precision. One potential shortcoming of this method lies in the inefficient release of AuNPs after the first chemical modification step. The easiness of release was reported to be associated with the size of AuNPs⁹. A way to release all AuNPs is by etching the glass substrate by sodium hydroxide solution or hydrogen fluoride solution⁴, but the possible adverse effect deriving from the harsh condition should be considered as the damage of modified ligand layers is likely. The compulsory use of water or other high-polarity solvents to maintain the electrostatic attraction between oppositely charged gold nanoparticle and glass substrates is also a shortcoming of this method, since this requirement excludes the use of hydrophobic ligands to modify AuNPs.

Figure 3. Preparation of Janus AuNPs by using glass slides as substrate. Reproduced with permission from ref. 3, copyright 2007, American Chemical Society.

Apart from glass slides/SiO₂NPs, polystyrene resins and polymer crystals can also serve as substrate in the preparation of AuNPs. However, different from glass slides/SiO₂NPs, which only provide steric hindrance to conceal a part of surface of AuNPs, PS resins and polymer crystals used provide chemical modification when AuNPs are attached to their surface. PS resins are chemically functionalized in advance with disulfide containing molecule, through inclusion complexation between crown ether and amine. Thus, when AuNPs are introduced, the bonding of the tethered disulfide molecules with AuNPs provides partial chemical modification of the surface of AuNPs. Subsequently, modified AuNPs are released through dissociation of the supramolecular complex. The work used this strategy to prepare mono-functionalized AuNPs and was done in dichloromethane (Figure 4a)⁷. Another work was reported to use HS-PEO polymer single crystals to prepare Janus AuNPs (Figure 4b)⁸. The experiment was carried out in pentyl acetate and toluene. The composition of solvent in this work needs to be carefully chosen, in order to maintain the crystalline structure of the HS-PEO single crystals. The attachment of AuNPs was also concomitant with the chemical modification of AuNPs at their surfaces facing to the crystals via S-Au bonding. This two works provide examples using materials other than glass slides/SiO₂NPs as substrates, in the preparation of Janus nanoparticles. However, the same with glass slide/silica nanoparticle based method, these two works also have strict requirement on the selection of solvents, to maintain

either the supramolecular complexation or single crystal structure mentioned above.

Figure 4. Preparation of mono-functionalized AuNPs (a) and amphiphilic Janus AuNPs (b) through the use of resins and polymer single crystals as substrate. Reproduced with permission from (a) ref. 7, copyright 2009, American Chemical Society and (b) ref. 8, copyright 2008, American Chemical Society.

A liquid-air interfacial engineering method was developed to asymmetrically modify AuNPs¹⁰. Alkanethiolate stabilized AuNPs were spread onto water surface to form a monolayer of nanoparticles. The monolayer of nanoparticles were then compressed so that ligands on neighboring nanoparticles interdigitated into each other, which prevents the rotation of

AuNPs. Ligands to be modified were then injected into the water solution and thus surfaces of nanoparticles that contact water phase were modified (Figure 5). The interdigitation of ligand layers thus plays a key role that prevents modification of the upper surfaces of AuNPs. The ratio of the two faces of Janus AuNPs prepared in this method can be readily tuned by adjusting the concentration of ligands in the water phase. The advantage of this Langmuir-Blodgett method is obvious: small AuNPs stabilized with hydrophobic alkanethiolates can be used to prepare Janus nanoparticles, and the ratio of the two faces can be tune easily. However, skilled labors are required to operate L-B trough to form a densely packed monolayer of AuNPs to achieve ligand interdigitation. AuNPs synthesized by other methods, such as citrate reduction, may not be used with this technique.

Figure 5. Langmuir-Blodgett technique used to prepare Janus AuNPs. Reproduced with permission from ref. 10, copyright 2007, Wiley interscience.

Apart from the abovementioned methods using either hard solid substrates or soft liquid-air interface to create spatial constraint for the asymmetric chemical modification of nanoparticles, there is also a way to prepare asymmetrically functionalized AuNPs via self-phase separation of ligand layers during chemisorption of ligands. It was reported that phase separation of ligand molecules could lead to formation of ordered sub-nanometer sized domains on the surface of AuNPs¹¹. A phase separation process was also reported to be able to create asymmetric distribution of ligand layers during competitive chemisorption of ligands^{12,13}. The two thiol ligands, one hydrophobic and the other hydrophilic, are not compatible with each other due to large difference in hydrophilicity. Thus, monolayers made of the two ligands on the surface of nanoparticles spontaneously separate to form Janus AuNPs. The assembly of PS-*b*-PAA diblock copolymers on the surface of as formed Janus AuNPs forms eccentrically encapsulation of gold nanoparticle, visualizing the formation of Janus nanoparticles in TEM images.

The potential application of Janus AuNPs include but are not limited to construction of SERS active plasmonic structures for ultrasensitive detection, interfacial stabilizers similar to amphiphilic molecules such as lipid molecules and amphiphilic block copolymers. As mentioned in Chapter One, plasmonic clusters can serve as SERS substrates, since the plasmonic coupling between closely apposed AuNPs leads to generation of strong electromagnetic field in the gaps between them. The anisotropic distribution of surface ligands can

restrict the interaction of nanoparticles spatially and thus avoids large scale aggregation that will precipitate very quickly from the solution. Formation of clusters, such as dimers, can sometimes provide possibility for the tuning of the distance of AuNPs (and so size of the gaps) by growing a shell of noble metals around AuNPs (Figure 6)¹⁴. The fabrication of such dimer (dumbbell) structure was achieved by preparing mono-functionalized AuNPs by DNA. Mono-functionalized nanoparticles can be viewed as an extreme example of Janus nanoparticles. In ref.14, the preparation of mono-functionalized AuNPs was achieved by magnetic separation (Figure 6).

Figure 6. Preparation of dimer (dumbbell) structures of AuNPs with the size of gap tunable by growing a layer of silver on the two AuNPs. Reproduced with permission from ref. 14, copyright 2010, NPG.

The considerably higher interfacial activity of Janus nanoparticles, compared with homogeneous nanoparticles has been studied theoretically^{15,16} and experimentally^{17,18}. This higher interfacial activity can make Janus nanoparticles become an effective stabilizer for emulsions. Based on the interfacial activity of Janus nanoparticles, we anticipate that it is possible to fabricate hollow three dimensional assemblies, such as vesicles and micelles,

based on assembly of nanoparticles at liquid-liquid interfaces. These vesicles/micelles can serve as delivery platform for therapeutics. This aspect of application is similar to the case that uses amphiphilic AuNPs (nanoparticles capped with hydrophilic and hydrophobic polymer brushes) to construct vesicular assemblies.

As mentioned above, today, there are already several methods for the preparation of Janus nanoparticles, including protection-deprotection strategy based on solid substrates such as glass slides/SiO₂NPs, interfacial engineering and spontaneous phase separation of ligand monolayers. Among them, the solid substrate technique appears to be the most popular method. The process begins with the adsorption of nanoparticles onto the substrate surface, so that the surface of nanoparticles in contact with the substrate is concealed while the exposed surface is under chemical modification. Subsequently, nanoparticles are released from the substrate, and the concealed surface is also exposed for the second modification. The key point of this technique lies in that nanoparticles must be adsorbed onto the substrate and the concealed surface of nanoparticles should be readily modifiable after exposure to the second type of ligand. To this end, strong noncovalent interactions (most commonly electrostatic interaction) are usually chosen for the immobilization and dissociation of nanoparticles, while strong covalent bonding (e.g., thiol-Au bonding) may make the second modification difficult to proceed via ligand-exchange. Accordingly, the solid substrate technique is usually

performed in water, and examples using organic solvent as a medium are rare^{7,8}. However, many inorganic nanoparticles (including semiconductors, metal and metal oxide nanoparticles) are synthesized in nonpolar organic solvents, and remain hydrophobic after the synthesis process. In the case of AuNPs, while large sized nanoparticles (12 - 200 nm, normally negatively charged) are obtained via reduction reaction performed in water^{19,20}, small-sized nanoparticles (2-10nm, hydrophobic) are mainly synthesized in nonpolar solvents by Brust method²¹ or others^{22,23}. In order to modify these hydrophobic nanoparticles using the solid substrate technique, nanoparticles need to be coated with charged layers for the immobilization on substrate surface via electrostatic interaction²⁴. In this regard, it would be highly beneficial if the solid substrate technique can be performed not only in water but also in organic solvents to asymmetrically modify nanoparticles that are either charged or hydrophobic in a direct manner. To this end, strong covalent bonding (thiol-Au bonding) will be an ideal choice to anchor AuNPs onto the surface of solid substrates.

In this chapter, we report a novel method for convenient preparation of amine-functionalized Janus AuNPs based on the reversible property of imine bond. By using this method, both charged and hydrophobic AuNPs could be modified into Janus nanoparticles in a direct manner. We relied on SiO₂NPs (SiO₂NPs) as the solid substrate for the immobilization of AuNPs. These SiO₂NPs were grafted with a layer of lipoyl ligands containing imine bond as

the linkage (Scheme 1). They could be dispersed in both aqueous solutions and organic mediums. Therefore, both negatively charged AuNPs (prepared by citrate reduction method) and hydrophobic AuNPs (prepared by Brust method) could be dispersed with SiO₂NPs and be absorbed on their surfaces via S-Au bonds. The contact surfaces of AuNPs were thus functionalized with imine bonds at this step. Subsequently, the surfaces of AuNPs facing to the solution phase could be chemically modified with the second ligand, for example, polyethylene glycol ligand in this case. After removing the excess polyethylene glycol ligands, the imine bonds could be hydrolyzed to amine bonds in acidic conditions, while gold nanoparticles were released from these SiO₂NPs. In this work, we used dilute HF aqueous solution to etch these SiO₂NPs, while the acidity of the HF solution also hydrolyze the imine bonds to generate amine groups that stay on the original surface of AuNPs that is in contact with SiO₂NPs. By this way, Janus AuNPs with a portion of surfaces functionalized with amine groups were prepared.

Scheme 1. Schematic process to prepare amine-functionalized Janus AuNPs via an imine-mediated protection-deprotection strategy.

2.2 Results and Discussion.

For the grafting of SiO₂NPs with lipoyl ligands with imine linkages (Scheme 1), SiO₂NPs were firstly functionalized with azide by reacting pristine SiO₂NPs with 3-azidopropyltrimethoxysilane (Scheme 2). The successful grafting was confirmed by the presence of azido infrared vibration band at 2108 cm⁻¹ (Figure 7a). Thereafter, 4-(propargyloxy)-benzaldehyde moiety was introduced to silica surface by the Cu(I) catalyzed click reaction between azide and alkyne. After the reaction, the azido vibration band disappeared (Figure 7b), confirming the success of the click reaction. The amine containing lipoyl ligand (lipoic-TriEG-amine, Scheme 1) was then conjugated onto silica surface via the formation of imine bond by simply mixing the ligand with benzaldehyde-grafted SiO₂NPs in DMF. The successful conjugation was confirmed by X-ray photoelectron spectroscopy (XPS), which showed 97% reaction efficiency (Figure 8a). To further confirm that the conjugation of the lipoyl groups onto SiO₂NPs was bridged by imine bond, the as-prepared functionalized SiO₂NPs were placed in acidic HCl aqueous solution and stirred for a few hours. Thereafter, functionalized SiO₂NPs were washed several times with water and ethanol followed by drying before characterizing by XPS. The XPS results showed the disappearance of the sulfur element, whereas the nitrogen element still remained on silica surface (Figure 8b). These observations provide concrete evidence that the linkage between SiO₂NPs and the lipoyl functionality was imine bond, which can be hydrolyzed in acidic

aqueous condition.

Scheme 2. Preparation procedure of SiO₂NPs functionalized with lipoyl ligand bridged via imine bond.

Figure 7. (a) FTIR spectrum of SiO₂NPs grafted with 3-azidopropyltrimethoxysilane. The absorption band at 2108 cm⁻¹ (as indicated by the black arrow) proves successful grafting of azide onto the SiO₂NPs. (b) FTIR spectrum of azide functionalized SiO₂NPs after reacting with 4-(propargyloxy)-benzaldehyde. The band at 2108 cm⁻¹ disappeared (as indicated by the black arrow) after the reaction, confirming that the azide functionality reacted with the alkyne group successfully.

Figure 8. (a) XPS spectrum of SiO₂NPs functionalized with lipoic-TriEG-amine via imine bond as the linkage. The theoretical quantity ratio between S and N is 0.400. Experimental obtained ratio is 0.389, showing a reaction efficiency of 97%. (b) XPS spectrum of these SiO₂NPs after treatment with HCl aqueous solution. The signal corresponding to sulfur element disappeared, while the signal of nitrogen element still existed, confirming that the imine bond was cleaved upon the acid treatment.

With the use of lipoyl-containing SiO₂NPs as a substrate, amine partially functionalized Janus AuNPs were prepared via the protection-deprotection strategy (Scheme 1). Asymmetric modification of 40 nm citrate-stabilized AuNPs was first carried out in water. In this case, SiO₂NPs dispersed in DMF were mixed with citrate-stabilized AuNPs dispersed in water under vigorous stirring. The TEM image (Fig. 9a) shows that these AuNPs were successfully adsorbed onto silica surface in a discrete fashion. In this step, the surfaces of AuNPs in contact with SiO₂NPs were decorated with imine. After complete adsorption of AuNPs on SiO₂NPs in solution to obtain the SiO₂@AuNPs composite, the external surfaces of AuNPs were passivated with a PEGylated

lipoyl ligand (lipoic-mPEG₇₅₀, Scheme 1). The SiO₂@AuNPs composite was then purified via typical centrifugation-washing process. Thereafter, the imine linkage between silica and AuNPs was hydrolyzed in HF aqueous solution (ca. 2%, pH~1), where silica substrates were etched completely. Finally, the released Janus AuNPs (Fig. 9b) containing both lipoic-TriEG-amine and lipoic-mPEG₇₅₀ ligands were stably dispersed in HF aqueous solution, and were purified by the centrifugation-washing process and dialysis. The positive zeta-potential (30.4 mV) of as-prepared Janus AuNPs, in contrast to the negative zeta-potential (-33.6 mV) of citrate-stabilized AuNPs, proves the generation of amine functionalities on Janus AuNPs. By using this way, these lipoic-TriEG-amine functionalities were decorated specifically on one patch of the surface of AuNPs, while the rest of surface was passivated by lipoic-mPEG₇₅₀. Smaller citrate-stabilized AuNPs (14 nm in diameter) were also asymmetrically modified using the same procedure (Fig. 9 c, d). Experimentally, smaller 14 nm AuNPs could be captured by SiO₂NPs more readily as compared to the larger 40 nm AuNPs.

Figure 9 (a, c, e) TEM images of AuNPs adsorbed on SiO₂NPs and (b, d, f) free single AuNPs released from SiO₂NPs after etching in 2% HF aqueous solution. Citrate-stabilized AuNPs with a size of (a, b) 40 nm and (c, d) 14 nm, and dodecylamine-stabilized AuNPs with a size of (e, f) 3.6 nm were used respectively for the preparation of Janus NPs.

In order to show the versatility of this technique in the preparation of Janus NPs, we also demonstrated the applicability of this technique in organic solvents such as DMF and toluene for direct asymmetric modification of hydrophobic NPs. For this purpose, dodecylamine-stabilized AuNPs (3.6 nm in size) were adsorbed onto SiO₂NPs upon mixing in DMF or toluene (Fig. 9e). After the external faces were passivated by lipoic-mPEG₇₅₀, the SiO₂@AuNPs composite was purified by the centrifugation-washing process. The obtained SiO₂@AuNPs composite became dispersible in water, indicating successful passivation by lipoic-mPEG₇₅₀. After hydrolysis and etching in acidic HF aqueous solution, the released AuNPs (Fig. 9f) could be stably dispersed in water and purified by dialysis. This study confirms the successful modification

of hydrophobic AuNPs with both lipoic-TriEG-amine and lipoic-mPEG₇₅₀. Thus, it was concluded that the imine-based solid substrate method could be extended for direct asymmetric modification of hydrophobic NPs in organic medium, an added advantage to the solid substrate methods reported in literature.

On top of the obtained results showing the successful conjugation of two different lipoyl ligands onto Janus AuNPs, asymmetric distribution of these two types of ligands on NP surface could be confirmed through the formation of colloidal clusters. When negatively charged citrate-stabilized AuNPs were mixed with the as-prepared amine functionalized positively charged Janus AuNPs, they bound together through electrostatic interaction. Thus, upon mixing 40nm Janus AuNPs with 14 nm citrate-stabilized AuNPs, we observed that the smaller AuNPs bound to the larger Janus NPs in a side-specific manner, forming dimers or other cluster structures (Figure 10a-c). This side-specific binding is consistent with the regioselective modification of 40 nm AuNPs. UV-vis absorption spectrum shows enhanced absorption in the range above 600 nm (Figure 11a), which confirms the assembly of these two types of AuNPs in solution phase. Particularly, based on measurement on Figure 10c, we estimate the percentage of amine passivated area to be ca. 22%, in agreement with the fact that only a small patch of NP surface is in contact with silica particles and thus modified with amine groups (Figure 9a). In the case of 14 nm Janus AuNPs, upon mixing with 14 nm citrate-stabilized AuNPs, we observed the

formation of discrete clusters including dimers, trimers, and tetramers (Figure 10d-f). Similarly, UV-vis absorption spectrum exhibits enhanced absorption in the range above 600 nm (Figure 11b). We also mixed 14 nm Janus AuNPs with citrate-stabilized AgNPs (about 56 nm in size). From TEM (Figure 10g, h) and SEM (Figure 12) images, the formation of discrete core-satellite structures with AgNPs as cores and Janus AuNPs as satellites was observed. The bimetallic structures were confirmed by energy-dispersive X-ray spectroscopy (EDX) (Figure 13). In this case, we observed a red shift in the plasmonic resonance band of the AgNPs from original 415 nm to 433 nm, and also a red shift in the plasmonic band of the AuNPs from 525 nm to 569 nm (Figure 11c). The as-prepared 3.6 nm Janus AuNPs were also assembled with citrate-stabilized AgNPs (about 56 nm in size) to form the core-satellite structures (Figure 10i). The spectral change (Figure 11d) of 3.6 nm Janus AuNPs/AgNPs is similar to that of 14 nm Janus AuNPs/AgNPs (Figure 11c), but is of much smaller magnitude, presumably due to the small size of Janus AuNPs used. Taken together, the assembly of the three types of Janus AuNPs with citrate-stabilized NPs all leads to the formation of discrete and regular cluster superstructures. As it is well known that the assembly of two types of regular NPs with opposite charges usually leads to the formation of random and irregular aggregates (Figure 14), the formation of colloidal clusters proves the successful preparation and application of these Janus NPs.

Figure 10. TEM images of NP clusters assembled from Janus AuNPs with citrate-stabilized NPs. (a-c) Clusters assembled from 40nm Janus AuNPs with 14 nm citrate-stabilized AuNPs; (d-f) clusters assembled from 14nm Janus AuNPs with 14 nm citrate-stabilized AuNPs; (g, h) core-satellites assembled from 14 nm Janus AuNPs with citrate-stabilized AgNPs (about 56 nm in size); (i) core-satellites assembled from 3.6 nm Janus AuNPs with citrate-stabilized AgNPs (about 56 nm in size). Insets: illustrations of the NP arrangements within clusters. Blue color represents the surfaces of AuNPs modified with amines.

Figure 11. (a) UV-vis spectra of as-prepared 40 nm Janus AuNPs (black curve), 14 nm citrate-stabilized AuNPs (red curve) and clusters from the assembly of these two types of AuNPs (blue curve); (b) UV-vis spectra of as-prepared 14 nm Janus AuNPs (red curve), 14 nm citrate-stabilized AuNPs (black curve), and clusters from the assembly of these two types of AuNPs (blue curve); (c) UV-vis spectra of as-prepared 14 nm Janus AuNPs (red curve), 56 nm citrate-stabilized AgNPs (black curve), and core-satellites from the assembly of these two types of NPs (pink curve); (d) UV-vis spectra of as-prepared 3.6 nm Janus AuNPs (black curve), 56 nm citrate-stabilized AgNPs (red curve), and core-satellites from the assembly of these two types of NPs (pink curve). Water was used as the solvent for all the samples.

Figure 12. SEM images of core-satellite structures assembled from 14 nm Janus AuNPs and AgNPs (average size 56 nm). The structures have AgNPs as cores and AuNPs as satellites covered on AgNPs surfaces.

Figure 13. SEM-EDS analysis showing both Au and Ag elements at the fringe (up) and central (down) sites, while the percentage of Ag is larger than Au at the central site, indicating that it is a satellite structure with an AgNP center surrounded by AuNPs.

Figure 14. TEM image of a large irregular aggregate formed by mixing citrate-stabilized AgNPs and 14 nm AuNPs stabilized only with lipoic-TriEG-amine. The concentrations of AgNPs and AuNPs were kept similar to those in Fig. 2g, h and Fig. S6 via the adjustment by UV-vis absorption intensity.

2.3 Conclusion

In conclusion, we have prepared amine functionalized Janus AuNPs by taking advantage of reversible formation and dissociation of imine bonds. The Janus structure of as-prepared AuNPs has been demonstrated through the formation of discrete nanoclusters. In common solid substrate techniques, immobilization of NPs sterically protects one side of NPs from chemical modification. Differently, in the approach developed here, immobilization of NPs is accompanied with modification of chemical functionalities (amine groups in this case). This process is similar to the “solid-state grafting-to” technique. Compared to the reported solid substrate methods where electrostatic attraction is commonly chosen as the force to immobilize NPs and thus they are usually applicable only in aqueous solutions, our approach demonstrated in this work uses strong covalent bonding to immobilize NPs, which enables direct modification of NPs in diverse media. This is an advantage especially when modification of hydrophobic NPs and application of hydrophobic ligands are desired.

In addition, we propose that the number of amine groups modified on the patch of nanoparticle surface can be readily adjusted by simply changing the grafting density of the lipoic-TriEG-amine molecules on the silica particles. This might enable the obtained Janus nanoparticles having only a few or even only one amine group attached, and such kind of Janus nanoparticles would be useful to construct hot-spots in applications for surface enhanced Raman

scattering. It was also reported recently that Janus AuNPs conjugated with probe DNA could be used in colorimetric detection of target DNA molecules⁵. The sensor developed with the use of Janus AuNPs was reported to possess wider dynamic range of detection compared to sensors using uniformly conjugated probe DNA molecules. So, improving the performance of sensors is also a possible direction for the application of Janus NPs.

2.4 Experimental Section

2.4.1 Materials and instruments

Compounds AgNO₃, 3-chloropropyltrimethoxysilane, 4-(dimethylamino)pyridine, dodecylamine, 2,2'-(ethylenedioxy)bis(ethylamine), 4-hydroxybenzaldehyde, poly(ethylene glycol) methyl ether (average Mn 750), (+)-sodium L-ascorbate, sodium citrate dihydrate, and tetraethyl orthosilicate (TEOS) were purchased from Sigma Aldrich. Compounds CuSO₄·5H₂O, di-*tert*-butyl dicarbonate, H₂AuCl₄·3H₂O, *N*-hydroxysuccinimide (NHS, >98%), lipoic acid, propargyl bromide solution, sodium azide, sodium borohydride, tetraoctylammonium bromide (TOAB), and trifluoroacetic acid were purchased from Alfa Aesar. *N*, *N'*-dicyclohexylcarbodiimide (DCC) was purchased from Merck. All chemicals were used as received.

¹H NMR and ¹³C NMR spectra were measured on a Bruker BBFO-400 spectrometer. Mass spectrometry was measured on a ThermoFinnigan LCQ Fleet MS spectrometer. Transmission electron microscopy (TEM) characterization was carried out on a JEM-1400 (JEOL) operating at 100kV.

Scanning electron microscopy (SEM) characterization and energy dispersive X-ray spectroscopy were carried out on a field emission JSM-6700F (JEOL). X-ray photoelectron spectroscopy (XPS) analysis was carried out on a SPECS HSA3500 plus spectrometer with Mg X-ray source. FT-IR spectra were recorded using Perkin-Elmer 1760X FT-IR spectrometer with SiO₂ powder diluted in KBr. UV-vis spectra were recorded using a Shimadzu UV-3600 UV-vis-NIR spectrophotometer. Zeta-potential value was measured by Mavern Nanosizer.

2.4.2 Synthesis of organic compounds and nanoparticles

Scheme 3. Preparation of the ligand lipoic-TriEG-amine.

2.4.2.1 Synthesis of 3-azidopropyltrimethoxysilane

Compounds 3-Chloropropyltrimethoxysilane (0.86g, 4.3 mmol) and sodium azide (1.10 g, 17 mmol) were loaded into anhydrous DMF (10 mL). The reaction was carried out at 90 °C under nitrogen atmosphere for 24 hours. Then, the reaction solution was cooled to room temperature and filtered quickly to remove any solids. The filtrate was subjected to vacuum at 40 °C to remove all DMF solvent, affording the crude product. Yield: 69 %. ¹H NMR (400 MHz,

CDCl₃): δ 3.55 (s, 9H), 3.25 (t, J=8Hz, 2H), 1.69 (m, 2H), 0.68(t, J=8Hz, 2H).

¹³C NMR (100 MHz, CDCl₃): δ 53.69, 50.29, 22.42, 6.30.

2.4.2.2 Synthesis of 4-(propargyloxy)-benzaldehyde

Compound 4-Hydroxybenzaldehyde (3.05 g, 25 mmol) was dissolved in acetone (125 mL). Potassium carbonate (4.85 g, 35 mmol) was then added and the mixture was refluxed under N₂ for 30 min. Thereafter, propargylbromide (5.55 mL, 50 mmol) was added and the mixture was further refluxed for 2 hours. After cooling, acetone was removed under reduced pressure and the residue was dissolved in ethyl acetate (150 mL). The solution was washed with water and brine, dried over MgSO₄. After filtration, it was concentrated under reduced pressure. The crude mixture was purified through chromatography (hexane: EtOAc, 5: 1) to obtain product as white solids. Yield: 90%. ¹H NMR (400 MHz, CDCl₃): δ 9.90 (s, 1H), 7.86 (t, 2H), 7.10 (t, 2H), 4.78 (s, 2H), 2.59 (s, 1H). ¹³C NMR (100 MHz, CDCl₃): 190.75, 162.37, 131.88, 130.60, 115.19, 77.58, 76.41, 55.95. ESI-MS: m/z calcd for C₁₀H₈O₂:160.05, found: 160.98 [M + H]⁺.

2.4.2.3 Synthesis of lipoic-TriEG-amine-Boc

Lipoic acid (0.74 g, 3.6 mmol) and *N*-hydroxysuccinimide (0.46 g, 3.96 mmol) were dissolved in tetrahydrofuran (14 mL). *N,N'*-Dicyclohexylcarbodiimide (0.82 g, 3.96 mmol) dissolved in tetrahydrofuran (7 mL) was then added. The mixture was stirred for 4 hours and the generated precipitate was removed by filtration. The filtrate was then added into

tetrahydrofuran solution (14 mL) of {2-[2-(2-amino-ethoxy)-ethoxy]-ethyl}-carbamic acid tert-butyl ester (amine-TriEG-amine-Boc in Scheme 3, 0.90 g, 3.60 mmol). The mixture was stirred overnight at room temperature. After removing the generated solids, the crude product was purified through chromatography (hexane: EtOAc, 1: 1 and then EtOAc: MeOH, 10: 1) to obtain yellow viscous oil. Yield: 88%. ¹H NMR (400 MHz, MeOH-d₄): δ 3.53-3.65 (m, 9H), 3.38 (t, 2H), 3.11-3.26 (m, 4H), 2.45-2.53 (m, 1H), 2.24 (t, 2H), 1.88-1.96 (m, 1H), 1.60-1.80 (m, 4H), 1.46 (s, 11H). ¹³C NMR (100 MHz, MeOH-d₄): δ 174.62, 156.94, 78.73, 69.95, 69.75, 69.41, 56.29, 40.03, 38.98, 38.09, 35.50, 34.41, 33.44, 28.54, 27.75, 25.44, 25.38. ESI-MS: m/z calcd for C₁₉H₃₆N₂O₅S₂: 436.21, found: 437.28 [M + H]⁺.

2.4.2.4 Synthesis of lipoic-TriEG-amine

Lipoic-TriEG-amine-Boc (6.46 g, 14.8 mmol) was dissolved in DCM (50 mL). Then, trifluoroacetic acid (TFA, 15 mL) was added under stirring for overnight. Both DCM and TFA were removed under vacuum. The crude product was purified via chromatography (hexane: EtOAc, 1: 1 to 1: 10) to obtain yellow viscous oil. Yield: 60%. ¹H NMR (400 MHz, MeOH-d₄): δ 3.66-3.75 (m, 9H), 3.38-3.41 (m, 2H), 3.13-3.18 (m, 4H), 2.45-2.50 (m, 1H), 2.21-2.28 (m, 2H), 2.00-2.10 (m, 2H), 1.85-1.95 (m, 1H), 1.63-1.72 (m, 4H), 1.47-1.55 (m, 2H). ¹³C NMR (100 MHz, MeOH-d₄): δ 174.83, 70.00, 69.32, 66.56, 56.23, 39.95, 39.27, 38.87, 37.96, 35.59, 34.33, 28.46, 25.49, 25.02. ESI-MS: m/z calcd for C₁₄H₂₈N₂O₃S₂: 336.15, found: 337.21 [M + H]⁺.

2.4.2.5 Synthesis of lipoic-mPEG₇₅₀

Lipoic acid (3.10 g, 15.0 mmol), mPEG₇₅₀ (13.50 g, 18.0 mmol), 4-(dimethylamino)pyridine (0.55 g, 4.5 mmol) were dissolved in DCM (120 mL). Then, *N, N'*-dicyclohexylcarbodiimide (3.40 g, 16.5 mmol) dissolved in DCM (30 mL) was added. After stirring at room temperature overnight, the generated solids were removed by filtration. The filtrate was then washed with dilute HCl aqueous solution, brine and water, dried over MgSO₄. After concentrating under reduced pressure, the crude product was purified through chromatography (hexane: EtOAc, 1: 1, then EtOAc: MeOH, 6:1) to obtain yellow viscous oil. Yield: 72%. ¹H NMR (400 MHz, CDCl₃): δ 3.98 (t, 2H), 3.30-3.58 (m, 57H), 2.84-2.97 (m, 2H), 2.19-2.27 (m, 1H), 2.10-2.13 (m, 2H), 1.63-1.71 (m, 1H), 1.39-1.49 (m, 4H), 1.21-1.32 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ 173.00, 71.74, 70.38, 68.93, 63.22, 58.77, 56.08, 40.02, 38.29, 34.41, 33.70, 28.49, 24.43.

2.4.2.6 Synthesis of pristine SiO₂NPs.

Under vigorous stirring, TEOS (9 mL) was added dropwise into ethanol (300 mL) containing ammonia aqueous solution (ca. 25 %, 21 mL). The reaction was stirred overnight. Thereafter, the generated SiO₂NPs were washed with ethanol for 5 times and finally dispersed in ethanol for storage.

2.4.2.7 Preparation of azide functionalized SiO₂NPs.

Pristine SiO₂NPs (ca. 1 g) were dispersed in toluene (100 mL) and the mixture was then heated to 100 °C under N₂ atmosphere and with vigorous

stirring. Then, 3-azidopropyltrimethoxysilane (1 mL) was injected. The reaction was maintained at 110 °C for 24 hours. After the temperature of reaction solution was lowered to room temperature, SiO₂NPs were purified via repeated centrifugation-washing process with the use of ethanol to finally obtain azide functionalized SiO₂NPs.

2.4.2.8 Preparation of benzaldehyde functionalized SiO₂NPs.

Azide functionalized SiO₂NPs (0.16 g) and 4-(propargyloxy)-benzaldehyde (0.14 g, 0.88 mmol) were loaded into DMF (5 mL). The mixture was ultra-sonicated to make a clear suspension. Then, CuSO₄·5H₂O (0.32 g, 1.29 mmol) and (+)-sodium *L*-ascorbate (1.28 g, 6.45 mmol) were added successively. The mixture was stirred at room temperature for 24 hours. After that, HCl aqueous solution was added to make insoluble organic by-products dissolved, before the mixture was subjected to centrifugation to obtain benzaldehyde functionalized SiO₂NPs. The obtained SiO₂NPs were washed few times with DMF and water, and finally stored in DMF.

2.4.2.9 Conjugation of lipoic-TriEG-amine onto SiO₂NPs.

For the conjugation of lipoic-TriEG-amine onto SiO₂NPs, the obtained benzaldehyde functionalized SiO₂NPs (ca. 0.1 g) were dispersed in DMF (4 mL), to which a methanol solution of lipoic-TriEG-amine (0.5 M, 1 mL) was then added. The reaction mixture was stirred at room temperature overnight. After that, SiO₂NPs were washed with DMF for 3 times to remove the excess lipoic-TriEG-amine, which were finally dispersed in DMF. For XPS analysis,

ethanol was used as solvent in place of DMF to avoid interference on nitrogen analysis.

2.4.2.10 Synthesis of AuNPs.

Citrate-stabilized AuNPs with a size of 14 nm were synthesized as follows. $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (0.07 g, 0.17 mmol) was dissolved in deionized water (386 mL). The solution was heated to about 140 °C. Under vigorous stirring, sodium citrate dihydrate (0.23 g, 0.79 mmol) dissolved in deionized water (4 mL) was injected. The reaction was maintained at 140 °C for 15 min before it was slowly cooled down to room temperature.

Citrate-stabilized AuNPs with a size of 40 nm were synthesized under a similar process. Compound $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (0.05 g, 0.12 mmol) was dissolved in deionized water (400 mL) and the mixture was heated to 140 °C. Under vigorous stirring, sodium citrate dihydrate (0.05 g, 0.15 mmol) dissolved in deionized water (4 mL) was injected. The reaction was kept for 5 min before cooling to room temperature.

Dodecylamine-capped AuNPs were synthesized as follows. Compound $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (0.06 g, 0.15 mmol) was dissolved in deionized water (6 mL), to which a toluene solution (16 mL) of tetraoctylammonium bromide (0.43 g, 0.79 mmol) was then added. The two-phase mixture was vigorously stirred at 0 °C for 10 min before a freshly prepared water solution of sodium borohydride (0.07 g, 1.78 mmol) was added dropwise. After 2 hours, the toluene phase was separated and washed with H_2SO_4 water solution (1 M) and water successively.

Thereafter, dodecylamine (0.15 g, 0.79 mmol) was added into the toluene solution and the mixture was stirred at room temperature overnight. After that, methanol was added to precipitate these dodecylamine-capped AuNPs. These AuNPs were collected after centrifugation and re-dispersed in toluene for storage.

2.4.2.11 Synthesis of citrate-stabilized AgNPs.

Compound AgNO_3 (90.5 mg, 0.53 mmol) was dissolved in deionized water (500 mL) and the mixture was heated to 140 °C. Sodium citrate dihydrate (0.1 g, 0.34 mmol) dissolved in water (10 mL) was then injected to the reaction mixture. The reaction was kept at 140 °C for 1 hour before it was cooled to room temperature. The obtained AgNPs were polydisperse, including some non-spherical crystals such as rods, triangular bricks and long wires. After standing for a few days, some large crystals were settled down and thereafter, AgNPs on the uppermost level were collected for further use.

2.4.2.12 Preparation of Janus AuNPs.

Under vigorous stirring, the lipoic-TriEG-amine conjugated SiO_2NPs in DMF were added into water solution (50 mL) of as-prepared citrate-stabilized AuNPs. The density of AuNPs on SiO_2NPs surface could be adjusted by changing the quantity of SiO_2NPs added. Similarly, for adsorption of dodecylamine-capped AuNPs, SiO_2NPs in DMF were added into toluene or DMF solution of dodecylamine-capped AuNPs. To passivate the external surface of the adsorbed AuNPs, an excess amount of lipoic-mPEG₇₅₀ dissolved

in MeOH was added to the above NPs dispersions, and the reaction was kept overnight. Thereafter, the obtained SiO₂NPs@AuNPs were isolated through centrifugation by removing the excess lipoic-mPEG₇₅₀. The obtained SiO₂NPs@AuNPs were then washed with water for three times before they were dispersed in 2% HF water solution (pH ~ 1). After a few hours, the adsorbed AuNPs were released completely, while the solution was further kept in the acidic solution overnight. Finally, the obtained AuNPs were purified by either the centrifugation-washing process or dialysis against water.

2.5 References

- (1) Walther, A.; Müller, A. H. E. *Chem. Rev.* **2013**, *113*, 5194.
- (2) Lattuada, M.; Hatton, T. A. *Nano Today* **2011**, *6*, 286.
- (3) Sardar, R.; Heap, T. B.; Shumaker-Parry, J. S. *J. Am. Chem. Soc.* **2007**, *129*, 5356.
- (4) Li, Z.; Cheng, E.; Huang, W.; Zhang, T.; Yang, Z.; Liu, D.; Tang, Z. *J. Am. Chem. Soc.* **2011**, *133*, 15284.
- (5) Guo, L.; Xu, Y.; Ferhan, A. R.; Chen, G.; Kim, D.-H. *J. Am. Chem. Soc.* **2013**, *135*, 12338.
- (6) Hu, Y.; Sun, Y. *J. Am. Chem. Soc.* **2013**, *135*, 2213.
- (7) Chak, C.-P.; Xuan, S.; Mendes, P. M.; Yu, J. C.; Cheng, C. H. K.; Leung, K. C.-F. *ACS Nano* **2009**, *3*, 2129.
- (8) Wang, B.; Li, B.; Zhao, B.; Li, C. Y. *J. Am. Chem. Soc.* **2008**, *130*, 11594.

- (9) Yoon, J. H.; Lim, J.; Yoon, S. *ACS Nano* **2012**, *6*, 7199.
- (10) Pradhan, S.; Xu, L.; Chen, S. *Adv. Funct. Mater.* **2007**, *17*, 2385.
- (11) Jackson, A. M.; Myerson, J. W.; Stellacci, F. *Nat. Mater.* **2004**, *3*, 330.
- (12) Chen, T.; Yang, M.; Wang, X.; Tan, L. H.; Chen, H. *J. Am. Chem. Soc.* **2008**, *130*, 11858.
- (13) Chen, T.; Chen, G.; Xing, S.; Wu, T.; Chen, H. *Chem. Mater.* **2010**, *22*, 3826.
- (14) Lim, D.-K.; Jeon, K.-S.; Kim, H. M.; Nam, J.-M.; Suh, Y. D. *Nat Mater* **2010**, *9*, 60.
- (15) Binks, B. P.; Fletcher, P. D. I. *Langmuir* **2001**, *17*, 4708.
- (16) Komura, S.; Hirose, Y.; Nonomura, Y. *J. Chem. Phys.* **2006**, *124*, 241104.
- (17) Glaser, N.; Adams, D. J.; Böker, A.; Krausch, G. *Langmuir* **2006**, *22*, 5227.
- (18) Fernandez-Rodriguez, M. A.; Song, Y.; Rodríguez-Valverde, M. Á.; Chen, S.; Cabrerizo-Vilchez, M. A.; Hidalgo-Alvarez, R. *Langmuir* **2014**, *30*, 1799.
- (19) Jana, N. R.; Gearheart, L.; Murphy, C. J. *Langmuir* **2001**, *17*, 6782.
- (20) Perrault, S. D.; Chan, W. C. W. *J. Am. Chem. Soc.* **2009**, *131*, 17042.
- (21) Brust, M.; Walker, M.; Bethell, D.; Schiffrin, D. J.; Whyman, R. J. *J. Chem. Soc., Chem. Commun.* **1994**, 801.

- (22) Jana, N. R.; Peng, X. *J. Am. Chem. Soc.* **2003**, *125*, 14280.
- (23) Zheng, N.; Fan, J.; Stucky, G. D. *J. Am. Chem. Soc.* **2006**, *128*, 6550.
- (24) Lattuada, M.; Hatton, T. A. *J. Am. Chem. Soc.* **2007**, *129*, 12878.

Chapter 3

Byproduct-Induced In-Situ Formation of Monodispersed Gold Colloidal Supraparticles

3.1 Introduction

Supraparticles (SPs) are colloidal assemblies of nanoparticles with dimensions in the sub-micrometer scale. As discussed in section 1.5, chapter 1, there are already several methods to fabricate SPs, such as emulsification-evaporation method, controlled induction of solvophobic interaction, multidentate molecules induced spherical assembly, high-temperature hydrolysis reaction, etc. These SPs are different in structure, influenced by the conditions by which they are fabricated. The most significant differences are the internal structures of SPs and their stability in solution. SPs fabricated by the two methods, emulsification-evaporation¹ and controlled induction of solvophobic interaction², possess supercrystalline structures or multiple supercrystalline domains since the forces driving their assembly into SPs are hydrophobic/solvophobic interactions, which allows them to reconfigure within the assemblies to achieve the energetically optimal positions. The structure of SPs corresponding to the lowest overall energy usually has the most number of closest contacts between constituent nanoparticles, so nanoparticles inside the SPs are closely packed and exhibit supercrystallinity. The introduction of anisotropic nanoparticles, such as nanorods, greatly enriches the structure of SPs. SPs with needle shapes or with multiple

well-defined supercrystalline domains could be obtained with the use of nanorods as building blocks^{3,4}.

Multidentate organic molecules or polymers were also used as organic mediators to assemble nanoparticles into SPs. The obtained SPs do not exhibit supercrystallinity in this case. Though there is lack of supercrystallinity, which may lower their significance in fundamental research of physical properties, the introduction of organic mediator can bring some advantages. For example, insertion of cleavable bonds or responsive groups could provide SPs the capability to undergo dissociation in response to environmental stimuli, such as change of pH, temperature, the existence of certain kinds of enzymes, the concentration of biological thiols or reactive oxidative species, etc. Controlled dissociation of SPs is useful in scenarios such as drug delivery and release. Apart from the intrinsic physical properties of the inorganic nanoparticles that can be harnessed to obtain some functionalities such as photothermal/photoacoustic effect for therapy/imaging, the chemical structure of the organic mediators can make the SPs chemically smart materials.

Different from the abovementioned SPs whose constituent nanoparticles are still crystallographically discrete, SPs synthesized from high-temperature hydrolysis reactions do not have such individuality for their constituent nanoparticles. They are crystallographically merged in their contact zones. Such structural features make these SPs much more stable than the abovementioned SPs, while maintaining the physical properties of

nanoparticles.

The value for practical application of SPs comes from the colloidal size and the mesoporous structures of SPs. SPs are usually a few hundreds of nanometers in size. This dimension allows membranes made of SPs possess some effects that do not exist with membranes made of nanoparticles. For example, when used as anode materials of lithium ion batteries, the formation of excessive solid-electrolyte interphase (SEI) layers could be controlled to be only at the external surface of SPs. At the same time, the mesoporous structure of SPs facilitates the diffusion of lithium ions within the supraparticles^{5,6}. Another example is about SPs made of zinc oxide nanoparticles as photoelectrodes. The dimension of SPs (about 300 nm) as building blocks for constructing membranes promotes light scattering within the membranes, which provides more photon absorption and electron-hole generation and thus enhanced energy conversion efficiency⁷.

Nowadays, the formation pathways of SPs are still under exploration by researchers, since the understanding about them is not complete yet. Herein, we report a new phenomenon where monodispersed gold SPs (AuSPs, Scheme 1) were formed *in-situ* in the synthetic solution of gold NPs (AuNPs) when a special type of thiol ligands was used. These ligands feature a structure consisting of a tetraethylene glycol chain with the two terminal hydroxyl groups replaced by a thiol group and a hydrocarbon segment respectively (HS-TEG-hydrocarbon, Scheme 1). Briefly, to a tetrahydrofuran (THF) solution

containing the ligand and gold(III) chloride was injected a reducing agent *tert*-butylamine borane (TBAB) that induced the formation of monodispersed AuNPs. Then, these AuNPs spontaneously aggregated to form AuSPs. Further investigation revealed that a byproduct, *tert*-butylamine hydrochloride, generated from the redox reaction played a key role in the formation of AuSPs.

Scheme 1. Schematic Representation of the In-Situ formation process of Gold Supraparticles and Comparison of AuNPs' Aggregation Behaviors When Different Thiol Ligands Were Used.

3.2 Results and discussion

THF was used as a solvent, which can dissolve both HAuCl_4 and TBAB, so the reaction occurred in a single-phase system. HS-TEG-adamantane was the first ligand we used. Upon the injection of TBAB/THF solution, monodispersed AuNPs (sample obtained after 30 second reaction, 8.0 nm in diameter, see Figure 1a, b) formed quickly in the nucleation process, a phenomenon which is similar to other synthetic systems. However, the difference was that these AuNPs began to aggregate in solution in the following few minutes if no purification step was applied, as indicated by the UV-vis spectra (Figure 1a).

Transmission electron microscopy (TEM, Figure 1c, d) and scanning electron microscopy (SEM, Figure 1e) images showed that these AuNP aggregates (i.e., AuSPs) adopted a spherical morphology and were relatively uniform in size. Within these AuSPs, single AuNPs packed closely, the crystalline structure of which was confirmed by selected area electron diffraction (SAED, Figure 1f). The formation of spherical assemblies is consistent with the emergence of two absorption bands located at 549 nm and 630 nm in UV-vis spectrum of the 30 min sample⁸. These AuSPs could further grow from $137 \pm 15 \text{ nm}$ to $159 \pm 19 \text{ nm}$ from 5 min to 30 min and maintained monodispersity (size standard deviation 11% and 12% respectively (Figure 2). Given that the size of AuNPs is about 8 nm, this small size growth (22 nm) of AuSPs equates to only one to two layers of AuNPs, implying that the finished state for the formation of AuSPs is nearly at the point of 5 min. We couldn't obtain TEM images of AuSPs with smaller sizes (for example, the nuclei of AuSPs), because the aggregation of AuNPs was a fast process where AuSPs with sizes above 100 nm could be reached in a very short time. Importantly, these AuSPs could be disassembled to free single AuNPs (8.8 nm in diameter) after the addition of 1-dodecanethiol, indicating that the constituent AuNPs were separated from neighboring ones by the ligand layer, and they were loosely held together by certain kinds of weak forces, rather than being crystallographically coalesced.

Figure 1. (a) UV-vis spectra for temporal evolution of the AuNP solution starting from the injection of TBAB solution at 30 seconds, 5 minutes, and 30 minutes; (b-d) TEM images of AuNPs initially formed at 30s after injection of the reducing agent (b), AuSPs obtained after 30 min reaction (c, d) and corresponding SEM (e) and SAED pattern (f) of AuSPs. Ligand: HS-TEG-adamantane.

Figure 2. TEM images of AuSPs (a, b) obtained after 5min reaction, mean size $137 \pm 15 \text{ nm}$, standard deviation $\delta = 11\%$, measured from 96 particles (c); TEM images of AuSPs (d, e) obtained after 30 min reaction, mean size $159 \pm 19 \text{ nm}$, standard deviation $\delta = 12\%$, measured from 158 particles (f).

In addition to HS-TEG-adamantane, we also tested ligands with similar molecular structures to prove that this phenomenon was not exclusive to HS-TEG-adamantane. Firstly, we used 2, 6-diisopropylbenzene in place of adamantane as the terminal segment to afford HS-TEG-2, 6-diisopropylbenzene,

since both 2, 6-diisopropylbenzene and adamantane are bulky groups. As expected, we observed the formation of AuSPs (Figure 3a, b). In order to demonstrate that the formation of AuSPs was also not exclusive to ligands with bulky terminal groups, we further employed one of the simplest hydrocarbon units, hexyl group, to replace adamantane for achieving HS-TEG-hexane. Again, we observed the formation of AuSPs (Figure 3c, d), though their size homogeneity seems to be not as good as those AuSPs using ligands with bulky terminals. Based on these results, it was supposed that the formation of AuSPs could be generalized to some other ligands with the molecular structure of HS-TEG-hydrocarbon.

Figure 3. TEM images of AuSPs with (a, b) HS-TEG-2, 6-diisopropylbenzene as a ligand, and (c, d) HS-TEG-hexane as a ligand.

Since these ligands consist of two segments, i.e., TEG and hydrocarbon units, we then investigated the roles of these two segments in the formation of AuSPs. When we removed the TEG segment, for example using 1-dodecanethiol as the ligand, stable AuNP solution was obtained (Figure 4). Thus, the middle TEG segment is needed for the aggregation to occur. When we removed the hydrocarbon segment, for example using HS-TEG-OH as the ligand, the formed AuNPs aggregated immediately and completely upon the injection of TBAB to become precipitates, leaving a colorless supernatant. However, these AuNP precipitates were large pieces of random aggregates with no regular morphology (Figure 5a). Thus, the terminal hydrocarbon segment is indispensable for AuSPs to be monodispersed and regular in morphology. Since hydroxyl group is usually associated with hydrogen bonding interaction, the fast aggregation may result from strong hydrogen bonding between AuNPs in THF. When THF was replaced with water, a solvent with stronger hydrogen bonding ability than THF, these aggregates were disassembled completely to form free single AuNPs (Figure 5b). In fact, changing the hydroxyl group to the shortest methyl group (i.e. HS-TEG-methane) significantly reduced the rate of aggregation in THF, while the obtained aggregates were slightly more regular (Figure 5c). Therefore, the role of a hydrocarbon terminal group should lie in the elimination of strong hydrogen bonding interaction between AuNPs in THF, which may enable AuNPs to rearrange themselves to energetically most favorable positions, thus leading the aggregates to adopt the energetically most

favorable spherical morphology^{4,9}.

Figure 4. (a) TEM image of single AuNPs with 1-dodecanethiol as ligand, and (b) UV-vis spectra showing that after stored for 5 weeks, the obtained AuNPs solution was still stable, without obvious aggregation.

Figure 5. TEM images of (a) AuNPs aggregates with HS-TEG-OH as ligand, and (b) free single AuNPs obtained from the dissociation of these AuNPs aggregates after replacing THF with water. (c) AuNPs aggregates with HS-TEG-methane as ligand.

Then, we need to explain why the aggregation occurred in the synthetic

solution of AuNPs when these HS-TEG-hydrocarbon ligands were used. One possible explanation is that the ligand layers are not compatible with the solvent. We noticed that THF was reported to be not a good solvent for polyethylene glycols while a good solvent for hydrocarbons^{10,11}. However, this explanation was excluded by the observation that AuNPs coated with HS-TEG-adamantane (denoted as AuNPs@HS-TEG-adamantane) could in fact be stably dispersed in THF after being purified from the synthetic solution. This observation is reasonable, as the solubility of AuNPs is largely determined by the chemical structure of the terminal groups of ligands. The inherent solubility of AuNPs@HS-TEG-adamantane in THF suggests that chemical species remained in the synthetic solution may play a role in the AuNP aggregation. Experimentally, we found that, accompanied with AuNP aggregation, some precipitates were separated out from the synthetic solution. Based on ¹H NMR (Figure 6) and X-ray photoelectron spectroscopy (XPS, Figure 7) analysis, we identified these precipitates as *tert*-butylamine hydrochloride, the ionicity of which explained why they were separated out from THF, a moderately polar solvent. Thus, the chemical species remained in the solution phase were identified as mainly unreacted HS-TEG-adamantane and *tert*-butylamine (Figure 8). *tert*-Butylamine hydrochloride is a byproduct of the redox reaction between HAuCl₄ and TBAB. During the reaction, HCl liberated from HAuCl₄ and *tert*-butylamine liberated from TBAB could undergo acid-base reaction to form *tert*-butylamine hydrochloride.

Figure 6. ^1H NMR (methanol- d_4) spectrum of precipitates collected after centrifugation. Estimate based on integration of respective NMR peaks indicates that the ratio of *tert*-butylamine to HS-TEG-adamantane is about 20. Inset: enlargement of the region where NMR peaks of HS-TEG-adamantane are located. The arrows indicate NMR peaks belonging to HS-TEG-adamantane.

Figure 7. XPS spectrum of precipitates collected after centrifugation. The composition is N (32.5%), Cl (44.8%), B (22.7%).

Figure 8. ^1H NMR (THF-d_8) of the species remained in THF-d_8 after removing both AuSPs and the precipitates immediately after reaction. Estimate based on integration of respective NMR peaks indicates that the ratio of HS-TEG-Ad to *tert*-butylamine is about 3. The dash arrows indicate NMR peaks belonging to HS-TEG-adamantane and *tert*-butylamine respectively.

Based on the composition analysis, we believe that *tert*-butylamine was a necessary component for the AuNP aggregation to occur, as it existed in both the solution and precipitate phases. To prove this statement, we carried out a control experiment using BH_3 in place of TBAB as the reducing agent. AuNPs were synthesized without any aggregation at any time points in this case (Figure 9a). However, when certain amount of *tert*-butylamine was added to the above solution, these AuNPs aggregated immediately. At the same time, we also observed the generation of some precipitates, a phenomenon which was similar to the original reaction using TBAB. TEM images of the formed aggregates showed the presence of spherical subdomains consisting of AuNPs packed together to form large pieces of aggregates (Figure 9b and Figure 10).

These spherical subdomains have a striking resemblance to AuSPs (Figure 1-3). Thus, this control experiment identified *tert*-butylamine as the key component in the formation of AuSPs. It is worth noting that when triethylamine was added in place of *tert*-butylamine, a similar phenomenon was observed, indicating that it is the amino group, instead of the *tert*-butyl structure, plays the key role.

Figure 9. TEM images of (a) free single AuNPs obtained by using BH_3 in place of TBAB as a reducing agent, and (b) AuNP aggregates obtained after adding *tert*-butylamine into the AuNP solution.

Figure 10. TEM images of two pieces of nanoparticle aggregates obtained by adding *tert*-butylamine into AuNPs solution prepared from the control experiment using BH_3 as the reducing reagent in place of TBAB.

Next, we need to understand how *tert*-butylamine works to induce the AuNP aggregation. Since the added quantity of thiol ligands exceeds that of either *tert*-butylamine (calculated based on the quantity of TBAB) or HAuCl_4 in the synthetic solution and the affinity of thiols towards AuNPs is much stronger than that of amines, direct bonding of *tert*-butylamine on AuNP surface is not reasonable. We then performed a control experiment. To a THF solution of AuNPs@HS-TEG-adamantane (synthesized with BH_3 as reducing agent and then purified for use) was added 200 μL of *tert*-butylamine. No noticeable aggregation occurred at this step, proving that the aggregation of AuNPs was not resulted from direct bonding of *tert*-butylamine. However,

when dry HCl gas was bubbled into the solution, there was a significant drop of AuNP concentration (Figure 11) and simultaneous generation of some precipitates (a mixture of AuNP aggregates and *tert*-butylamine hydrochloride). Without the addition of *tert*-butylamine, bubbling HCl gas to the AuNP solution caused no obvious change. Although these AuNP aggregates were random ones without any regular morphology in this case, their formation did suggest that reacting with HCl to form *tert*-butylamine hydrochloride was the way that *tert*-butylamine could induce the AuNP aggregation and thus AuSP formation.

Figure 11. UV-vis spectra of AuNP/THF solution with only *tert*-butylamine added (curve a) and then HCl gas introduced (curve b). Inset: photographs of the corresponding AuNP solution before (cuvette a) and after (cuvette b) introducing HCl gas.

From the above discussions, we could identify the two key ingredients for the formation of AuSPs, i.e., chemical structure of the ligands (HS-TEG-hydrocarbon) and generation of the byproduct (*tert*-butylamine hydrochloride). Thus, AuSP formation should be a result of the interaction

between the ligand monolayers of AuNPs and the *in-situ* generated *tert*-butylamine hydrochloride, denoted as NP-byproduct interaction. As abovementioned, AuNPs did not aggregate when 1-dodecanethiol was used as a ligand, meaning that there was no NP-byproduct interaction in this case. This is understandable, since 1-dodecanethiol monolayer is nearly zero in terms of polarity. For comparison, *tert*-butylamine hydrochloride is a salt with relatively high ionicity, which can aggregate and separate out from THF. On the other hand, HS-TEG-hydrocarbon has a TEG segment with a certain degree of polarity. The NP-byproduct interaction in this case should be an electrostatic one deriving from the polarity of the HS-TEG-hydrocarbon monolayer and the ionicity of *tert*-butylamine hydrochloride. This interaction might make some *tert*-butylamine hydrochloride molecules (or their small aggregates) adsorbed onto the surface of AuNPs, rendering AuNPs solvophobic in THF and ultimately forming AuSPs through the aggregation.

3.3 Conclusion

In conclusion, we have developed a methodology for *in-situ* formation of AuSPs. The generation of AuSPs requires the use of a certain type of ligands with the structure of HS-TEG-hydrocarbon, THF as solvent, and the generation of ionic byproduct *tert*-butylamine hydrochloride. Based on the experimental observations, solvophobic effect induced from electrostatic adsorption of the ionic byproduct onto the AuNP surface was proposed to be the driving force for the formation of AuSPs. As SPs are an important member of functional

nanomaterials, research on their formation pathways is of great importance, and thus present work may lead to a better understanding of the SP formation.

3.4 Experimental Section

3.4.1 Materials and Instruments

All chemical reagents were used as received. Anhydrous DMF, borane tetrahydrofuran complex solution 1.0 M in THF, 1-bromoadamantane, *tert*-butylamine, *tert*-butylamine borane complex, 18-crown-6, gold(III) chloride trihydrate, sodium hydride, tetraethylene glycol, and tetraethyleneglycol monomethyl ether were purchased from Sigma Aldrich. 1-Bromohexane, 2, 6-diisopropylphenol, lithium bromide, and triethylamine, thiourea were purchased from Alfa Aesar. *p*-Toluenesulfonyl chloride was purchased from Merck. CDCl₃ was purchased from Cambridge Isotope Laboratories, Inc.

¹H NMR spectra were measured on a Bruker BBFO-400 spectrometer. Mass spectrometry was measured on a ThermoFinnigan LCQ Fleet MS spectrometer. Transmission electron microscopy (TEM) characterization was carried out on a JEM-1400 (JEOL) operating at 100 kV. Selected area electron diffraction (SAED) was carried out on a FEG-TEM JEM-2100F (JEOL) operating at 200 kV. Scanning electron microscopy (SEM) characterization was carried out on a field emission JSM-6700F (JEOL). X-ray photoelectron spectroscopy (XPS) analysis was carried out on a SPECS HSA3500 plus spectrometer with Mg X-ray source. UV-vis spectra were recorded using a

3.4.2 Synthetic procedures of thiol ligands

Compound 1 (HO-TEG-OTs)

To dichloromethane (DCM, 47 mL) was added tetraethylene glycol (20.4 g, 105 mmol) and triethylamine (1.6 g, 15.7 mmol). The solution was cooled by ice water for a few minutes. *p*-Toluenesulfonyl chloride (2.0 g, 10.5 mmol) was then added slowly under vigorous stirring. After being stirred in an ice bath for 2 hours, the solution was stirred at room temperature for another 16 hours. Thereafter, the solution was washed with water (40 mL) 3 times and then acetic acid/water (2%, 40 mL) 2 times. The organic layer was dried over Na_2SO_4 and then concentrated under reduced pressure to afford the product as colorless oil (3.5 g). Yield: 95%. 1H NMR (400 MHz, $CDCl_3$): 7.71-7.79 (t, 2H), 7.32-7.34

(t, 2H), 3.49-3.86 (m, 16H), 2.43 (s, 3H). ESI-MS: m/z calcd for $C_{15}H_{24}O_7S$: 348.12, Found: 348.99 $[M + H]^+$.

Compound 2 (HS-TEG-OH)

Compounds HO-TEG-OTs (3.0 g, 8.6 mmol) and thiourea (1.0 g, 13 mmol) were dissolved in ethanol (20 mL). Under nitrogen atmosphere, the solution was heated to 80 °C and kept at this temperature overnight. After cooling down to room temperature, NaOH aqueous solution (3 M, 34 mL) was added. Under nitrogen atmosphere, the solution was heated to 100 °C for 4 hours. After cooling down to room temperature, the solution was neutralized with HCl/water solution. Water and ethanol were then removed under reduced pressure. DCM was added to dissolve the residue and the solution was dried over Na_2SO_4 . The crude product was purified via chromatography (hexane/ethyl acetate 1: 1) to afford the pure product (0.4 g). Yield: 23%. 1H NMR (400 MHz, $DMSO-d_6$): 3.27-3.32 (m, 12H), 3.22 (t, 2H), 2.70 (t, 2H). ESI-MS: m/z calcd for $C_8H_{18}O_4S$: 210.09, Found: 233.10 $[M + Na]^+$.

Compound 3 (HO-TEG-adamantane)

A mixture of 1-bromoadamantane (1.5 g, 7.0 mmol), triethylamine (3 mL, 21.6 mmol) and tetraethylene glycol (25 mL, 145 mmol) was heated to 180 °C under nitrogen atmosphere and vigorous stirring. The solution was kept at this temperature overnight. After cooling down to room temperature, DCM (25 mL) was added into the solution. The solution was washed with HCl/water solution (2 M, 10 mL) 4 times and then brine (10 mL) once. The organic layer was then

dried over MgSO_4 and concentrated to afford the product as yellow brown oil (3.5 g). Yield: 95%. ^1H NMR (400 MHz, CDCl_3): 3.55-3.72 (m, 16H), 2.11 (s, 3H), 1.72 (s, 6H), 1.54-1.61 (m, 6H). ESI-MS: m/z calcd for $\text{C}_{18}\text{H}_{32}\text{O}_5$: 328.44, Found: 329.31 $[\text{M} + \text{H}]^+$, 351.41 $[\text{M} + \text{Na}]^+$.

Compound 4 (TsO-TEG-adamantane)

Compound HO-TEG-adamantane (3.0 g, 9.2 mmol) was dissolved in THF (9 mL), to which NaOH (1.1 g) dissolved in water (10 mL) was added. The solution was cooled in an ice bath. *p*-Toluenesulfonyl chloride (2.9 g, 15.5 mmol) dissolved in THF (15 mL) was then added slowly. The obtained solution was stirred in ice bath for 2 hours and then raised to room temperature to react overnight. Thereafter, DCM (150 mL) was added and the solution was washed with water. The DCM solution was then dried over Na_2SO_4 and concentrated to afford crude product. The crude product was purified via chromatography (hexane/ethyl acetate 1: 1) to afford the pure product (4.0 g). Yield: 90%. ^1H NMR (400 MHz, CDCl_3): 7.79 (t, 2H), 7.35 (t, 2H), 4.16 (t, 2H), 3.55-3.68 (m, 14H), 2.43 (s, 3H), 2.12 (s, 3H), 1.72 (s, 6H), 1.61 (m, 6H). ESI-MS: m/z calcd for $\text{C}_{25}\text{H}_{38}\text{O}_7\text{S}$: 482.23, Found: 482.94 $[\text{M} + \text{H}]^+$, 505.22 $[\text{M} + \text{Na}]^+$.

Compound 5 (HS-TEG-adamantane)

Compounds TsO-TEG-adamantane (4.4 g, 9.2 mmol) and thiourea (1.4 g, 18.2 mmol) were dissolved in ethanol (22 mL). Under nitrogen atmosphere, the solution was heated to 80 °C and maintained at this temperature overnight. After cooling down to room temperature, NaOH/water solution (10%, 34 mL)

was added. The obtained solution was again heated to 100 °C and kept at this temperature for 4 hours. After cooling down to room temperature, the solution was neutralized with HCl/water solution. Water and ethanol were then removed under reduced pressure. Thereafter, DCM was added to dissolve the residue and the solution was dried over Na₂SO₄. The crude product was purified via chromatography (hexane/ethyl acetate 6: 1) to afford the pure product (1.8 g). Yield: 58%. ¹H NMR (400 MHz, CDCl₃): 3.57-3.67 (m, 14H), 2.65-2.72 (m, 2H), 2.13 (s, 3H), 1.90 (s, 1H), 1.73 (s, 6H), 1.56-1.64 (m, 6H). ESI-MS: m/z calcd for C₁₈H₃₂O₄S: 344.20, Found: 367.29 [M + Na]⁺.

Compound 6 (HO-TEG-hexane)

To anhydrous DMF (15 mL) was added tetraethylene glycol (9.8 g, 50.2 mmol), to which sodium hydride (0.6 g, 15.3 mmol) dispersed in anhydrous DMF (8 mL) was slowly added under nitrogen atmosphere in ice bath. The reaction mixture was stirred for 1h, and then 1-bromohexane (1.7 g, 10 mmol) was added dropwise. The reaction mixture was stirred at room temperature overnight. Thereafter, DMF was removed under reduced pressure and then DCM (100 mL) was added. The solution was washed with water (100 mL) 3 times to remove excess tetraethylene glycol, and the organic layer was dried over Na₂SO₄. After removing DCM under reduced pressure, the crude product was purified via chromatography (hexane: ethyl acetate 1: 5) to afford pure product (1.8 g). Yield: 63%. ¹H NMR (400 MHz, CDCl₃): 3.53-3.78 (m, 16H), 3.39-3.42 (t, 2H), 1.50-1.55 (m, 2H), 1.21-1.29 (m, 6H), 0.82-0.85 (m, 3H).

ESI-MS: m/z calcd for $C_{14}H_{30}O_5$: 278.21, Found: 279.07 $[M + H]^+$.

Compound 7 (TsO-TEG-hexane)

Compound HO-TEG-hexane (1.6 g, 5.9 mmol) was dissolved in THF (6 mL), to which NaOH (0.7 g) dissolved in water (6.6 mL) was added. The solution was cooled in an ice bath. Then, *p*-toluenesulfonyl chloride (1.9 g, 9.9 mmol) dissolved in THF (9 mL) was added. The solution was stirred in ice bath for 2 hours and raised to room temperature for reaction overnight. The solution was separated to two layers. The upper THF layer was collected, while the lower water layer was extracted with DCM. The DCM and THF solution were then combined and concentrated under reduced pressure. DCM was added to dissolve the residue and the solution was dried over $MgSO_4$. After removing DCM under reduced pressure, the crude product was purified via chromatography (hexane/ethyl acetate 6: 1) to afford the pure product as colorless oil (2.4 g). Yield: 93%. 1H NMR (400 MHz, $CDCl_3$): 7.79 (t, 2H), 7.35 (t, 2H), 4.16 (t, 2H), 3.56-3.70 (m, 14H), 3.44 (t, 2H), 2.45 (s, 3H), 1.55-1.59 (m, 2H), 1.26-1.34 (m, 6H), 0.88 (t, 3H). ESI-MS: m/z calcd for $C_{21}H_{36}O_7S$: 432.57, Found: 433.50 $[M + H]^+$.

Compound 8 (HS-TEG-hexane)

Compounds TsO-TEG-hexane (2.4 g, 5.5 mmol) and thiourea (0.9 g, 11.8 mmol) were added into ethanol (13 mL). Under nitrogen atmosphere, the solution was heated to 80 °C and kept at this temperature for 24 hours. After cooling down to room temperature, NaOH (2.7 g) dissolved in a water/ethanol

(5 mL/16 mL) mixture was then added. Thereafter, the reaction mixture was heated to 100 °C under nitrogen atmosphere for overnight. After cooling down to room temperature once again, the solution was neutralized with HCl/water solution. Water and ethanol were then removed under reduced pressure. DCM was added to dissolve the residue and the solution was dried over MgSO₄. After removing DCM under reduced pressure, the crude product was purified via chromatography (hexane/ethyl acetate 2: 1) to afford the pure product (1.0 g). Yield: 62%. ¹H NMR (400 MHz, CDCl₃): 3.56-3.68 (m, 14H), 3.42-3.46 (m, 2H), 2.67-2.72 (m, 2H), 1.55-1.61 (m, 2H), 1.26-1.34 (m, 6H), 0.86-0.90 (t, 3H). ESI-MS: m/z calcd for C₁₄H₃₀O₄S: 294.19, Found: 294.90 [M + H]⁺, 312.06 [M + NH₄]⁺.

Compound 9 (HO-TEG-2, 6-diisopropylbenzene)

To a round bottom flask was added HO-TEG-OTs (4.2 g, 12.0 mmol), 2, 6-diisopropylphenol (3.2 g, 18.0 mmol), K₂CO₃ (4.2 g, 30.0 mmol), LiBr (0.2 g, 2.0 mmol), 18-crown-6 (0.1 g, 0.4 mmol) and acetonitrile (360 mL). The reaction mixture was heated to reflux under stirring for 24 hours. After cooling down to room temperature, it was filtered and the filtrate was collected. The solid was washed with acetonitrile and the filtrate was combined. After removing the solvent under reduced pressure, the crude product was purified via chromatography (hexane/ethyl acetate 4: 1) to afford pure product as light yellow liquid (2.5 g). Yield: 58%. ¹H NMR (400 MHz, CDCl₃): 7.09 (s, 3H), 3.60-3.93 (m, 16H), 3.34-3.39 (m, 2H), 1.21-1.22 (d, 12H). ESI-MS: m/z calcd

for $C_{20}H_{34}O_5$: 354.24, Found: 355.09 $[M + H]^+$, 372.04 $[M + NH_4]^+$.

Compound 10 (TsO-TEG-2, 6-diisopropylbenzene)

Compound HO-TEG-2, 6-diisopropylbenzene (2.4 g, 6.7 mmol) was dissolved in THF (6.7 mL). NaOH (0.8 g) dissolved in water (7.7 mL) was then added. The solution was cooled with an ice bath, to which *p*-toluenesulfonyl chloride (2.6 g, 13.5 mmol) dissolved in THF (12 mL) was added. The solution was then stirred overnight. The solution was separated to two layers. The upper THF layer was collected, while the lower water layer was extracted with DCM. The THF and DCM solution were combined and concentrated. Then, DCM was added to dissolve the residue. The solution was dried over $MgSO_4$ and concentrated. The crude product was purified via chromatography (hexane/ethyl acetate 6: 1 and 3: 1) to afford pure product as colorless oil (3.1 g). Yield: 90%. 1H NMR (400 MHz, $CDCl_3$): 7.79 (t, 2H), 7.34 (t, 2H), 7.09 (s, 3H), 4.16 (t, 2H), 3.59-3.92 (m, 14H), 3.38 (m, 2H), 2.43 (s, 3H), 1.20 (d, 12H). ESI-MS: m/z calcd for $C_{27}H_{40}O_7S$: 508.25, Found: 509.00 $[M + H]^+$, 526.28 $[M + NH_4]^+$.

Compound 11 (HS-TEG-2, 6-diisopropylbenzene)

Compounds TsO-TEG-2, 6-diisopropylbenzene (2.6 g, 5.2 mmol) and thiourea (0.8 g, 10.3 mmol) were dissolved in ethanol (11 mL). Under nitrogen atmosphere, the solution was heated to 80 °C for 24 hours. After cooling down to room temperature, NaOH (2.3 g) dissolved in a water/ethanol (4.3 mL/14 mL) mixture was added. Under nitrogen atmosphere, the solution was again heated

to 100 °C and kept at this temperature overnight. After cooling down to room temperature, the solution was neutralized with HCl/water solution. Water and ethanol were then removed under reduced pressure. DCM was added to dissolve the residue and the solution was dried over MgSO₄. After removing DCM under reduced pressure, the crude product was purified via chromatography (hexane/ethyl acetate 5: 1 and 2: 1) to afford the pure product as colorless oil (1.0 g). Yield: 52%. ¹H NMR (400 MHz, CDCl₃): 7.09 (s, 3H), 3.63-3.92 (m, 14H), 3.38 (m, 2H), 2.69-2.83 (m, 2H), 1.58 (t, 1H), 1.21-1.23 (d, 12H). ESI-MS: m/z calcd for C₂₀H₃₄O₄S: 370.55, Found: 371.73 [M + H]⁺.

Compound 12 (TsO-TEG-methane)

Tetraethyleneglycol monomethyl ether (1.3 g, 6.0 mmol) was dissolved in THF (6 mL), to which NaOH (0.7 g) dissolved in water (7 mL) was added. The solution was cooled with an ice bath. Then, *p*-toluenesulfonyl chloride (2.3 g, 12.0 mmol) dissolved in THF (10 mL) was added. The solution was stirred overnight. The solution was separated to two layers. The upper THF layer was collected, while the lower water layer was extracted with DCM. The THF and DCM solutions were combined and concentrated. Then, DCM was added to dissolve the residue. The solution was then dried over MgSO₄ and concentrated. The crude product was purified via chromatography (hexane/ethyl acetate 1: 1 and 1: 4) to afford pure product as colorless oil (1.8 g). Yield: 83%. ¹H NMR (400 MHz, CDCl₃): 7.79 (t, 2H), 7.35 (t, 2H), 4.16 (t, 2H), 3.53-3.70 (m, 14H), 3.37 (s, 3H), 2.45 (s, 3H). ESI-MS: m/z calcd for C₁₆H₂₆O₇S: 362.44, Found:

363.46 $[M + H]^+$.

Compound 13 (HS-TEG-methane)

Compounds TsO-TEG-methane (1.7 g, 4.7 mmol) and thiourea (0.7 g, 9.3 mmol) were added into ethanol (10 mL). Under nitrogen atmosphere, the solution was heated to 80 °C for 24 hours. After cooling down to room temperature, NaOH (2.1 g) dissolved in a water/ethanol (4 mL/12 mL) mixture was added. Under nitrogen atmosphere, the solution was again heated to 100 °C overnight. After cooling down to room temperature once again, the solution was neutralized using HCl/water solution. Thereafter, water and ethanol were removed under reduced pressure. DCM was added to dissolve the residue. The solution was dried over $MgSO_4$ and then concentrated. The crude product was purified via chromatography (hexane/ethyl acetate 3:1, 2:1 and 1:1 sequentially) to afford pure product as colorless oil (0.5 g). Yield: 51%. 1H NMR (400 MHz, $CDCl_3$): 3.51-3.62 (m, 14H), 3.33-3.35 (m, 3H), 2.63-2.68 (m, 2H), 1.56 (t, 1H). ESI-MS: m/z calcd for $C_9H_{20}O_4S$: 224.32, Found: 242.35 $[M + NH_4]^+$.

3.4.3 Formation procedure of gold supraparticles

The procedure was depicted in Scheme 1. HS-TEG-adamantane (0.021 g, 0.060 mmol) was mixed with $HAuCl_4 \cdot 3H_2O$ in THF (0.015 M, 1.95 mL, 0.030 mmol). The solution was stirred at room temperature for 3 hours and then heated to 55 °C. *tert*-Butylamine borane complex/THF solution (0.065 M, 0.70 mL, 0.046 mmol) was then injected into the above solution. Aliquots were obtained for UV-vis absorption measurement and TEM characterization. Other

thiol molecules were weighed by the same moles to that of HS-TEG-adamantane when they were used as ligands, while keeping other parameters unchanged. The concentration of borane tetrahydrofuran complex solution was adjusted to that of *tert*-butylamine borane complex/THF solution by dilution with THF, when it was used in place of *tert*-butylamine borane complex (TBAB) as the reducing reagent in the control experiment. For HCl bubbling experiment, the obtained NPs were purified by adding some MeOH to induce slight aggregation and then subjected to centrifugation and finally redispersed in THF. The HCl gas was obtained by bubbling concentrated HCl aqueous solution with N₂ gas. The gas was dried by going through a long CaCl₂ tube, before being introduced into AuNPs THF solution.

For composition analysis, the reaction was carried out in THF-d₈. After the reaction was completed, the solution was centrifuged to separate the solution phase and precipitate phase. The solution phase was subjected to ¹H NMR characterization directly. The precipitate was dissolved in MeOH-d₄. While AuNPs coated with HS-TEG-adamantane could not be dissolved in MeOH-d₄, the generated salt, *tert*-butylamine hydrochloride, could be dissolved well in MeOH-d₄ for ¹H NMR characterization.

3.5 References

(1) Bai, F.; Wang, D.; Huo, Z.; Chen, W.; Liu, L.; Liang, X.; Chen, C.; Wang, X.; Peng, Q.; Li, Y. *Angew. Chem., Int. Ed.* **2007**, *46*, 6650.

(2) Zhuang, J.; Wu, H.; Yang, Y.; Cao, Y. C. *J. Am. Chem. Soc.* **2007**, *129*,

14166.

(3) Zhuang, J.; Shaller, A. D.; Lynch, J.; Wu, H.; Chen, O.; Li, A. D. Q.; Cao, Y. C. *J. Am. Chem. Soc.* **2009**, *131*, 6084.

(4) Wang, T.; Zhuang, J.; Lynch, J.; Chen, O.; Wang, Z.; Wang, X.; LaMontagne, D.; Wu, H.; Wang, Z.; Cao, Y. C. *Science* **2012**, *338*, 358.

(5) Lee, S. H.; Yu, S.-H.; Lee, J. E.; Jin, A.; Lee, D. J.; Lee, N.; Jo, H.; Shin, K.; Ahn, T.-Y.; Kim, Y.-W.; Choe, H.; Sung, Y.-E.; Hyeon, T. *Nano Lett.* **2013**, *13*, 4249.

(6) Liu, N.; Lu, Z.; Zhao, J.; McDowell, M. T.; Lee, H.-W.; Zhao, W.; Cui, Y. *Nat. Nanotechnol.* **2014**, *9*, 187.

(7) Chou, T. P.; Zhang, Q.; Fryxell, G. E.; Cao, G. Z. *Adv. Mater.* **2007**, *19*, 2588.

(8) Maye, M. M.; Chun, S. C.; Han, L.; Rabinovich, D.; Zhong, C.-J. *J. Am. Chem. Soc.* **2002**, *124*, 4958.

(9) Wang, T.; Wang, X.; LaMontagne, D.; Wang, Z.; Wang, Z.; Cao, Y. C. *J. Am. Chem. Soc.* **2012**, *134*, 18225.

(10) Gitsov, I.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1996**, *118*, 3785.

(11) Niikura, K.; Iyo, N.; Higuchi, T.; Nishio, T.; Jinnai, H.; Fujitani, N.; Ijio, K. *J. Am. Chem. Soc.* **2012**, *134*, 7632.

Chapter 4

Oxidation-Triggered Hydrophilicity Change of Gold Nanoparticles for Naked Eye Detection of Hydrogen Peroxide

4.1 Introduction

Hydrogen peroxide (H_2O_2) is an important biologically relevant small molecule. It is the substrate/product of some intracellular enzymes^{1,2} and antibodies^{3,4}. One direction of research today has been focusing on the fundamental study of the complex roles of H_2O_2 that have been associated with some diseases⁵⁻⁷. Another direction of research aims to detect disease-related proteins through detection of hydrogen peroxide, relying on techniques like enzyme-linked immunosorbent assay (ELISA)⁸⁻¹⁰. In the ELISA technique, H_2O_2 -generating/consuming enzymes like glucose oxidase or horseradish peroxidase are frequently selected as the enzyme labels^{9,10}, and subsequent measurement of H_2O_2 concentration could be carried out by colorimetric observation⁹⁻¹², plasmonic absorption spectroscopy¹³ or electrochemical detection^{14,15}. The colorimetric method, especially, is instrument-free and in some cases can provide ultralow sensitivity that rivals the standard but costly methods based on nucleic acids⁹.

Figure 1 Structure of sandwich-type ELISA and the two methods that generate colors to manifest the quantity of label enzymes and target proteins. Reproduced with permission from ref. 9, copyright 2012, NPG.

The ELISA technique comprises antibodies to capture or recognize disease biomarker proteins (capture antibody & primary antibody, Figure 1a). Biotin conjugated secondary antibodies that recognized primary antibodies were used to connect primary antibodies and label enzymes conjugated with streptavidin. This sandwich-structure ELISA thus related the concentration of target proteins to the quantity of label enzymes. Conventionally, substrates of the label enzymes were used to generate colored products after the enzyme catalyzed reaction. These substrates are usually organic molecules that were converted to colored species by reaction catalyzed by the label enzymes. Another method based on color change of gold nanoparticle aggregation was developed to manifest the existence of label enzymes and biomarkers, and it was called “plasmonic ELISA”. This method utilized the very narrow H_2O_2 concentration range (20 μM) across which gold nanoparticles exhibited different aggregation behaviors and thus different colors were developed. This method used a solution with a specific recipe. The performance of the as-developed plasmonic

ELISA technique was reported to be better than the commercial nucleic acid based tests which are very costly.

Figure 2 Different nucleation and growth pathways of silver ions at different concentration of GOx and H₂O₂. Reproduced with permission from ref. 13, copyright 2012, NPG.

H₂O₂ can also reduce some noble metal salts to zero valence. This chemistry was used to design a nanosensor based on the change of absorbance wavelength of gold nanostars upon the nucleation of silver ions on their surface (called enzyme-guided crystal growth)¹³. When the concentration of GOx (glucose oxidase) is low, the concentration of H₂O₂ is also low, leading to a slow nucleation rate of silver ions which favors the conformal growth of a silver coating on the surface of gold nanostars. The conformal growth of a silver shell causes large blue-shift of the absorbance of gold nanostars. When the concentration of GOx is high, the concentration of H₂O₂ is also high, leading to a fast nucleation of silver ions which favors independent crystallization of silver nanocrystals. Thus, in this case, the absorbance of gold nanostars stays constant. The larger change of absorbance wavelength at lower concentration of GOx endows the nanosensors with inverse sensitivity that can

detect disease biomarkers at the ultralow detection limit.

Figure 3 ELISA platform with signal generation based on the nucleation of gold ions on the surface of small gold seeds after reduced by H_2O_2 . Reproduced with permission from ref. 10, copyright 2014, American Chemical Society.

The same chemistry was employed to design an ELISA platform for the detection of prostate specific antigen (PSA) (Figure 3)¹⁰. In this case, Gold ions, instead of silver ions were reduced by H_2O_2 . In the presence of gold nanoparticle seeds with 5nm in size, the nucleation of gold ions took place on the surface of gold seeds. As the size of gold nanoparticle grew, the color of the solution became deeper. So, in contrary to the above mentioned nanostar sensors with inverse relationship between the intensity of signal and the concentration of target protein, this ELISA technique has a direct relationship between the two. The application of magnetic beads to integrate a large number of label enzymes significantly strengthens the detection capability of the ELISA platform.

The above three examples demonstrate the importance of H_2O_2 in immunoassay applications, which derives from the H_2O_2 -related enzymes, such

as glucose oxidase or horseradish peroxidase that are among the most frequently used label enzymes in ELISA¹⁶. In this work, we developed a nanoparticle-based signal generation system by engineering the surface of AuNPs to be H₂O₂-responsive: from being hydrophilic to more hydrophobic upon H₂O₂ treatment, so that these AuNPs aggregate in the aqueous solution with color change from red to blue (Scheme 1). The system we developed can demonstrate the concentration of H₂O₂ by the color change of AuNP solution. The lowest concentration of H₂O₂ that can be clearly distinguished is 10 μM.

Scheme 1 Schematic representation of the H₂O₂ triggered hydrophilicity change and subsequent aggregation of AuNPs.

4.2 Results and discussion

As illustrated in Scheme 1, AuNPs were initially modified with two ligands: 1-hexanethiol and the H₂O₂ responsive ligand, lipoic-phenylboronate-mPEG₅₀₀₀. 1-Hexanethiols are hydrophobic ligands with short length, and they form a

hydrophobic layer (indicated as yellow shells in Scheme 1) around AuNPs. Lipoic-phenylboronate-mPEG₅₀₀₀ is a ligand with a long hydrophilic polyethylene glycol tail. Though the lipoyl and phenylboronate segments are hydrophobic, the long polyethylene glycol tail provides excellent water solubility and steric hindrance that protects AuNPs from aggregation in water solution. The phenylboronate segment in the middle of the ligand serves as the H₂O₂ responsive structure: they react with H₂O₂ to generate a phenyl oxide which initiates quinone methide rearrangement around the aromatic structure and causes fragmentation of the cresol structures¹⁷⁻²¹. As a result, the polyethylene glycol tail is finally cut away from the ligand. The detachment of the polyethylene glycol tails from the surface of AuNPs decreases their water solubility, which eventually leads to their aggregation and a red-to-blue color change distinguishable with naked eyes. The color change is determined by the extent of AuNP aggregation which is associated with the concentration of H₂O₂, therefore different concentrations of H₂O₂ can be discriminated by naked eyes.

Figure 1 ^1H NMR spectra of compound **6** without H_2O_2 (a) and after reaction with H_2O_2 for 70 hours at 37°C (b). (a) compound **6**, 9.76mM, in DMSO-d_6 456 μL and deuterated PBS 7.4 solution 130 μL ; (b) compound **6**, 9.76 mM, in DMSO-d_6 456 μL and deuterated PBS 7.4 solution 124 μL and H_2O_2 (H_2O solution, 10M) 6 μL , H_2O_2 concentration 96.8 mM, compound **6**: $\text{H}_2\text{O}_2 = 1: 10$.

Figure 2 Evolution of ^1H NMR spectra of compound **6** in the reaction with H_2O_2 . Compound **6**, 9.76 mM, in DMSO-d_6 456 μL and deuterated PBS 7.4 solution 124 μL and H_2O_2 (H_2O solution, 10 M) 6 μL , H_2O_2 concentration 96.8 mM, compound **6**: $\text{H}_2\text{O}_2 = 1: 10$. Inset: enlargement of the peak at 4.52 ppm.

Firstly, the fact that the as-synthesized lipoic-phenylboronate-mPEG₅₀₀₀ molecules could undergo fragmentation in the presence of H_2O_2 was proved by ^1H NMR characterization. For simplicity of spectrum, we used compound **6** (Figure 1), synthetic precursor of the final lipoic-phenylboronate-mPEG₅₀₀₀ ligand, to react with H_2O_2 . As shown in Figure 1a, in the absence of H_2O_2 , there were no peaks from 4.10 to 4.80 ppm, indicating that there were no methylene

protons from benzyl alcohols. After reacting with H_2O_2 for 70 hours, we observed the appearance of two singlet peaks at 4.52 ppm and 4.36 ppm (Figure 1b). These two peaks belong to methylene protons of the two benzyl alcohols generated from the fragmentation of the middle phenylboronate segment. Correspondently, abundance of the two peaks at 4.90 ppm and 5.04/5.05 ppm (belonging to methylene protons of the benzyl ether and benzyl esters of compound 6, respectively) decreased almost completely, which proved the hydrolysis of the benzyl ester bonds in the presence of H_2O_2 . It is noteworthy to mention that, the reaction between phenylboronate and H_2O_2 to generate phenol completed within 30 min, and hydrolysis of benzyl ether and benzyl ester bonds started at this time point and then progressed slowly (Figure S2). Under the specific condition used in this ^1H NMR experiment, only after 2 hours of reaction could the hydrolysis of benzyl ester bonds be detected with ^1H NMR and after 23 hours of reaction could the hydrolysis reach a significant extent (Figure S2 inset). However, we need to emphasize that the rate of hydrolysis is depending on the concentration of H_2O_2 , the quantity of water, pH and temperature, so this ^1H NMR experiment is just for proving the hydrolysis of the as-synthesized ligand in the presence of H_2O_2 . The condition of the AuNPs experiment (described in latter paragraphs) is vastly different from that of this ^1H NMR experiment.

Figure 3 Aggregation of AuNPs with different concentration of H_2O_2 at different time points. B: blank. 1-14: 10 μM , 20 μM , 40 μM , 70 μM , 100 μM , 120 μM , 160 μM , 200 μM , 300 μM , 400 μM , 600 μM , 800 μM , 1 mM, 2 mM H_2O_2 . Time: a 0h, b 3h45min, c 6h31min, d 9h11min, e 16h24min, f 20h16min, g 24h, h 28h, i 43h33min, j 69h. The incubation temperature is 37 $^\circ\text{C}$.

Figure 4 Comparison of the extent of AuNP aggregation with low concentrations of H_2O_2 . H_2O_2 concentration: B, blank; 1, 10 μM ; 2, 20 μM ; 3, 40 μM . Time: a, 20h; b, 24h; c, 28h; d, 43.5h. The incubation temperature is 37 $^\circ\text{C}$.

Then, the prepared AuNPs were incubated with different concentrations of H_2O_2 at PBS 7.4 buffer at 37 $^\circ\text{C}$. The concentrations of H_2O_2 were set to be from 10 μM to 2 mM. Initially, all nanoparticle samples were red in color

(Figure 3a) upon the addition of H_2O_2 . After about 4 hours, the samples with the highest concentrations of H_2O_2 began to aggregate (Figure 3b). In the following time, these nanoparticle solutions slowly aggregate in a sequential manner, and samples with high concentrations of H_2O_2 showed sedimentation of AuNPs at the bottom of vials, leaving colorless supernatants (Figure 3c-j). The prepared AuNPs kept stable within at least 28 hours and started to aggregate after around 43 hours (Figure 3h and Figure 4). Within this period of time, all the samples with added H_2O_2 had either aggregated or exhibited sedimentation. H_2O_2 as low as 10 μM to 40 μM in concentration could be clearly distinguished in the period of 20 to 28 hours (Figure 4).

The extent of aggregation of AuNPs under different concentrations of H_2O_2 could be probed more accurately through UV-vis spectroscopy. When AuNPs aggregate, the UV-vis spectrum will show the increase of absorption at the wavelength above 600nm (Figure 5a, d, g). Thus, we measured the degree of AuNP aggregation by using the ratio of absorption between 650nm and 527 nm. The larger this ratio, the larger the extent of aggregation it would be. At 4 hours, only the last 4 samples with H_2O_2 concentrations above 600 μM exhibited aggregation that could be distinguished by a naked eye (Figure 3b). However, the UV-vis spectra showed that the two samples with H_2O_2 concentration of 300 μM and 400 μM also exhibited slight aggregation (Figure 5b). The aggregation extent of AuNPs at this case exhibited linear profile as the concentration of H_2O_2 increased (Figure 5b), especially within the range of 200

μM and $800\ \mu\text{M}$ with a R^2 above 99% (Figure 5c).

Figure 5 UV-vis spectra of AuNPs solutions incubated with different concentrations of H_2O_2 and the plotting of data of the ratio of absorption between 650 nm and 527 nm at different concentrations of H_2O_2 at the corresponding incubation time: a, b and c, 3h45min; d, e and f, 6h31min; g and h, 16h24min. The incubation temperature is $37\ ^\circ\text{C}$.

The aggregation proceeded with time and occurred on the samples with lower concentrations of H_2O_2 . At 6.5 hours, the sample with $100\ \mu\text{M}$ H_2O_2

exhibited eye-distinguishable aggregation (Figure 3c). The absorption spectra of all the fifteen samples were recorded (Figure 5d). Plotting of the ratio of absorption between 650 nm and 527 nm against H_2O_2 concentration exhibited a curve with an “S” shape. The aggregation extent of AuNPs also showed a good linearity from 40 μM to 600 μM with a R^2 above 99% (Figure 5f). Those samples with H_2O_2 concentration below 40 μM did not aggregate at this time point, so the corresponding data were horizontal. Only at 16.5 hours did the aggregation take place on these samples and the corresponding ratios exhibited a curving shape that could be fitted with a quadratic equation (Figure 3e and Figure 5h).

Figure 6 Aggregation of AuNPs with different concentration of H_2O_2 at different time points. B: blank. 1-13: 10 μM , 20 μM , 40 μM , 70 μM , 100 μM , 120 μM , 160 μM , 200 μM , 300 μM , 400 μM , 600 μM , 800 μM , 2 mM H_2O_2 . Time: a 0h; b, 19h; c, 34h; d, 46h; e, 62h; f, 83h; g, 140h. The incubation temperature is 25 $^{\circ}\text{C}$.

Figure 7 UV-vis spectra of AuNPs solutions incubated with different concentrations of H_2O_2 and the plotting of data of the ratio of absorption between 650 nm and 527 nm at different concentrations of H_2O_2 at the corresponding incubation time: a, b and c, 34h; d, e and f, 46h. The incubation temperature is 25 °C.

Temperature is a major factor that influences the kinetic of the hydrolysis of the benzyl ester bonds of the phenylboronate segment. Thus it should also have a large effect on the rate of aggregation of AuNPs. We decreased the temperature from 37 °C to 25 °C for the colorimetric detection of H_2O_2 at different concentrations. As mentioned above, at 37 °C, the aggregation of AuNPs were eye-distinguishable at about 4 hours in the samples with the highest concentration of H_2O_2 . At 25 °C, the aggregation occurred at the time of

19 hours in the samples with the highest concentration of H_2O_2 (Figure 6b). As with the case of 37°C , the aggregation also proceeded slowly with time and when the samples with lower concentrations of H_2O_2 started to aggregate, those samples with higher concentrations of H_2O_2 had already showed sedimentation. At 34 hours to 46 hours, the aggregation proceeded to the middle samples (Figure 6 c and d), and UV-vis spectra were recorded (Figure 7a and d). The ratio of the absorption between 650 nm and 527 nm were also plotted against the concentration of H_2O_2 (Figure 7b and e). The same with the case of 37°C , the curves also exhibited an “S” shape. The two curves possessed good linearity from $40\ \mu\text{M}$ to $200\ \mu\text{M}$ and $20\ \mu\text{M}$ to $100\ \mu\text{M}$, respectively (Figure 7 c and f). Though the test suffered from slow rate at lower temperature, as-prepared AuNPs had better stability at lower temperature, and when time is sufficient, the discrimination of the blank sample and the sample with $10\ \mu\text{M}$ H_2O_2 could also be distinguished clearly (Figure 6g).

Figure 8 UV-vis spectra of AuNPs modified with different concentrations of lipoic-phenylboronate-mPEG₅₀₀₀, after incubations with $100\ \mu\text{M}$ and $600\ \mu\text{M}$ of H_2O_2 for 3 hours at 37°C .

Except for incubation temperature, the ratio of

lipoic-phenylboronate-mPEG₅₀₀₀ to 1-hexanethiol on the surface of AuNPs is also an important parameter in the detection of H₂O₂. In principle, the sensitivity of AuNPs in the detection of H₂O₂ should be enhanced by decreasing the ratio of the lipoic-phenylboronate-mPEG₅₀₀₀ ligand on the surface monolayer of AuNPs. However, the stability of these AuNPs would be low in water solution if the ratio is too low. Thus, an optimal ratio should be found to balance the two considerations. The exact measurement on the ratio of two ligands on the mix monolayers of nanoparticle is very difficult to achieve. Thus, we determined the concentrations of the two ligands by sequential adjustment of the ratio of the two ligands used in experiment. We used 0.0185 mM, 5 μ L of the lipoic-phenylboronate-mPEG₅₀₀₀ ligand and 91% (v/v), 100 μ L of 1-hexanethiol to modify 2 mL AuNPs in DMF. We didn't know the exact ratio of the two ligands on the surface of AuNPs, but we proposed that the ratio should be altered once the starting ratio of the two ligands was changed. To demonstrate the influence of the ratio of the two ligands on the detection of H₂O₂, we increased the concentration of lipoic-phenylboronate-mPEG₅₀₀₀ from 0.0185 mM to 0.074 mM (4 folds) while kept other parameters constant. The results showed that after about 3 hours of incubation at two concentrations of H₂O₂, AuNPs with 0.0185 mM lipoic-phenylboronate-mPEG₅₀₀₀ added exhibited aggregation, while AuNPs with 0.074 mM lipoic-phenylboronate-mPEG₅₀₀₀ added showed no sign of aggregation (Figure 8a, b). This result is consistent with the conjecture that increase in the ratio of

lipoic-phenylboronate-mPEG₅₀₀₀ on surface of AuNPs makes these NPs more stable and aggregate more slowly in H₂O₂, as larger amounts of the PEG ligands must be cleaved from NP surfaces before AuNPs can aggregate.

4.3 Conclusions

In conclusion, we have designed a ligand that undergoes fragmentation in the presence of H₂O₂. The phenylboronate moiety reacts with H₂O₂ to generate phenol, which initiates quinone methide rearrangement around the aromatic structure of the ligand. The polyethylene glycol tail was cut away after the fragmentation, which reduces the stability of the modified AuNPs in water solution and causes their aggregation, a process accompanied with a color change from wine red to blue. The rate of NP aggregation and color change is associated with the concentration of H₂O₂. Thus, the concentration of H₂O₂ could be estimated by a naked eye. The platform we designed in this report has relatively high performance in the detection of H₂O₂. It has wide dynamic range of detection and high resolution in the micro molar concentration of H₂O₂. The lowest distinguishable concentration is 10 μ M. The dynamic range of detection of our system (from 10 μ M to 2 mM at different incubation times) is wider than those of two recently reported methods based on AuNPs (10-100 μ M and 20-120 μ M, respectively), while the limit of detection, 10 μ M, is comparable to theirs (10-20 μ M and 20 μ M, respectively)^{9,10}.

As H₂O₂ is an important biologically relevant small molecule that is associated with some commonly used label enzymes in ELISA technique, the

platform we designed in this work could be used as the signal transducer in the detection of disease biomarker. The very low concentration of H₂O₂ that could be distinguished by our platform promises great potential in this application.

4.4 Experimental section

4.4.1 Materials and instrument

Compounds 2, 6-bis-(hydroxymethyl)-*p*-cresol, imidazole, anhydrous DMF, *tert*-butyldimethylsilyl chloride, potassium carbonate, 4-bromomethylphenylboronic acid pinacol ester, ammonium chloride, *p*-toluenesulfonic acid monohydrate, lipoic acid, 4-(dimethylamino)pyridine (DMAP), *N*, *N*'-dicyclohexylcarbodiimide (DCC), anhydrous DCM, 1,1'-Carbonyldiimidazole, propargylamine, poly(ethylene glycol) methyl ether (M.W. 5000), *p*-toluenesulfonyl chloride, sodium azide, gold(III) chloride trihydrate, sodium citrate, copper(II) sulfate pentahydrate, sodium ascorbate, hydrogen peroxide solution were purchased from Sigma Aldrich and used without further purification.

¹H NMR and ¹³C NMR spectra were measured on a JEOL ECASL400 spectrometer. Mass spectrometry was measured on a ThermoFinnigan LCQ Fleet MS spectrometer. UV-vis spectra were recorded using a Shimadzu UV-3600 UV–vis–NIR spectrophotometer.

4.4.2 Organic synthesis

Compound 5

Compound 6

Compound 1

Compounds 2, 6-bis-(hydroxymethyl)-*p*-cresol (1.68 g, 10 mmol) and imidazole (1.54 g, 22.65 mmol) were dissolved in anhydrous DMF (7 mL). The solution was stirred at 0 °C, to which *tert*-butyldimethylsilyl chloride (3.31 g, 22 mmol) dissolved in anhydrous DMF (5 mL) was added. The solution was stirred at room temperature for 2 hours, and then it was diluted with ether and washed with water 3 times. Thereafter, it was dried with sodium sulfate, and ether was removed. The residue was purified through chromatography (Hex: EA = 95: 5) to afford colorless oil (3.65 g, Yield 93%). ¹H NMR (400 MHz, CDCl₃): 8.07 (s, 1H), 6.97 (s, 2H), 4.89 (s, 4H), 2.32 (s, 3H), 1.01 (s, 18H),

0.19 (s, 12H). ^{13}C NMR (400 MHz, CDCl_3): 150.97, 128.28, 126.24, 125.80, 63.06, 25.93, 20.72, 18.35, -5.37. ESI-MS: m/z calcd for $\text{C}_{21}\text{H}_{40}\text{O}_3\text{Si}_2$: 396.25, found: 395.05 $[\text{M} - \text{H}]^+$.

Compound 2

Compound **1** (0.83 g, 2 mmol) was dissolved in anhydrous DMF (4 mL), to which potassium carbonate (0.35 g, 2.5 mmol) was added and the solution was stirred at 0 °C for 10 min. 4-Bromomethylphenylboronic acid pinacol ester (0.65 g, 2 mmol) was added to the solution and the reaction solution was stirred at room temperature overnight. Thereafter, the solution was diluted with ether and washed with saturated ammonium chloride aqueous solution and brine solution. The organic phase was collected and dried over magnesium sulfate. The raw product was purified through chromatography (Hex: EA = 95: 5) to afford slightly yellow oil (1.15 g, Yield 80%). ^1H NMR (400 MHz, CDCl_3): 7.847 (d, 2H), 7.434 (d, 2H), 7.196 (s, 2H), 4.912 (s, 2H), 4.722 (s, 4H), 2.361 (s, 3H), 1.375 (s, 12H), 0.936 (s, 18H), 0.087 (s, 12H). ^{13}C NMR (400 MHz, CDCl_3): 151.10, 140.78, 135.03, 133.80, 133.66, 127.90, 126.95, 83.82, 76.15, 60.36, 26.02, 24.89, 21.21, 18.44, -5.23. ESI-MS: m/z calcd for $\text{C}_{34}\text{H}_{57}\text{BO}_5\text{Si}_2$: 612.38, found: 611.27 $[\text{M} - \text{H}]^+$.

Compound 3

Compound **2** (1.15 g, 1.88 mmol) was dissolved in MeOH (6 mL), and then *p*-toluenesulfonic acid monohydrate (0.072 g, 0.35 mmol) was added. The reaction solution was stirred at room temperature overnight, after which MeOH

was removed. The residue was subjected to chromatography (Hex: EA = 1: 1) to afford colorless oil (0.62 g, Yield 85.6%). ^1H NMR (400 MHz, CDCl_3): 7.732 (d, 2H), 7.295 (d, 2H), 7.047 (s, 2H), 4.719 (s, 2H), 4.511 (s, 4H), 2.171 (s, 3H), 1.285 (s, 12H). ^{13}C NMR (400 MHz, CDCl_3): 151.96, 140.26, 135.02, 133.98, 133.84, 129.01, 127.06, 83.86, 76.44, 59.95, 24.79, 24.62. ESI-MS: m/z calcd for $\text{C}_{22}\text{H}_{29}\text{BO}_5$: 384.27, found: 790.96 $[2\text{M} + \text{Na}]^+$.

Compound 4

Compound **3** (0.61 g, 1.58 mmol), lipoic acid (0.16 g, 0.79 mmol), DMAP (0.03 g, 0.24 mmol) were dissolved in DCM (7 mL). The solution was stirred at 0 °C for 10 min, and then DCC (0.18 g, 0.87 mmol) dissolved in DCM (1.6 mL) was added dropwise. The reaction solution was then stirred at room temperature overnight. After removing the generated solids, DCM was removed and the residue was purified through chromatography (Hex: EA = 5: 1) to afford yellow oil (0.23 g, Yield 50%). ^1H NMR (400 MHz, CDCl_3): 7.801 (d, 2H), 7.397 (d, 2H), 7.190 (s, 1H), 7.107 (s, 1H), 5.126 (s, 2H), 4.910 (s, 2H), 4.623 (s, 2H), 3.441-3.493 (m, 1H), 3.017-3.145 (m, 2H), 2.280-2.417 (m, 7H), 1.786-1.869 (m, 2H), 1.329-1.432 (m, 16H). ^{13}C NMR (400 MHz, CDCl_3): 173.30, 153.16, 140.05, 135.10, 134.30, 130.66, 130.37, 129.03, 126.96, 83.89, 76.90, 61.60, 60.51, 56.26, 40.17, 38.47, 34.54, 34.09, 28.68, 24.90, 24.81, 24.64. ESI-MS: m/z calcd for $\text{C}_{30}\text{H}_{41}\text{BO}_6\text{S}_2$: 572.58, found: 573.08 $[\text{M} + \text{H}]^+$, 590.12 $[\text{M} + \text{NH}_4]^+$.

Compound 5

Compound **4** (0.207 g, 0.36 mmol) was dissolved in anhydrous DCM (2 mL), and then, 1, 1'-carbonyldiimidazole (0.18 g, 1.11 mmol) was added. The reaction solution was stirred at room temperature overnight. Thereafter, the solution was diluted with DCM and washed with brine 3 times and dried over magnesium sulfate. After removing DCM, the residue was purified through chromatography (Hex: EA = 2: 1) to afford yellow oil (0.123 g, Yield 50%). ^1H NMR (400 MHz, CDCl_3): 8.094 (s, 1H), 7.821 (d, 2H), 7.409 (d, 2H), 7.373 (s, 1H), 7.253 (s, 2H), 7.039 (s, 1H), 5.403 (s, 2H), 5.186 (s, 2H), 4.994 (s, 2H), 3.495-3.529 (m, 1H), 3.064-3.147 (m, 2H), 2.335-2.440 (m, 6H), 1.838-1.891 (m, 1H), 1.622-1.663 (m, 4H), 1.355-1.471 (m, 14H). ^{13}C NMR (400 MHz, CDCl_3): 173.22, 154.23, 148.57, 139.60, 137.14, 135.21, 134.63, 132.72, 131.88, 130.67, 129.84, 127.71, 126.76, 117.21, 83.94, 74.77, 65.20, 61.35, 60.44, 56.31, 40.24, 38.52, 34.61, 34.12, 28.76, 24.95, 21.10. $\text{C}_{34}\text{H}_{43}\text{BN}_2\text{O}_7\text{S}_2$: 666.26, found: 666.93 $[\text{M} + \text{H}]^+$, 689.21 $[\text{M} + \text{Na}]^+$.

Compound 6

To synthesize compound **6**, compound **5** was used without purification by chromatography. Compound **4** (0.51 g, 0.89 mmol) was dissolved in anhydrous DCM (4 mL), and 1, 1'-carbonyldiimidazole (0.29 g, 1.78 mmol) was added. The solution was stirred at room temperature overnight, and then washed with brine 3 times after diluted with DCM. The DCM solution was then dried over magnesium sulfate and DCM was removed under reduced pressure. Thereafter,

anhydrous DCM (4 mL) was added to dissolve the residue (compound **5**), to which propargylamine (1.11 mL, 17.8 mmol) was added, and the solution was stirred at room temperature overnight. The solution was then washed with water and brine, and then dried over magnesium sulfate. The raw product was purified through chromatography to afford yellow oil (0.32 g, 0.49 mmol, Yield 55%). ^1H NMR (400 MHz, DMSO- d_6): 7.739 (d, 2H), 7.493 (d, 2H), 7.236 (d, 2H), 5.096 (d, 4H), 4.951 (s, 2H), 3.814 (s, 2H), 3.555-3.592 (m, 1H), 3.115-3.202 (m, 3H), 2.325-2.421 (m, 6H), 1.831-1.880 (m, 1H), 1.510-1.642 (m, 4H), 1.275-1.401 (m, 14H). ^{13}C NMR (400 MHz, CDCl_3): 173.2703, 155.8341, 153.9084, 140.0074, 135.1789, 134.3071, 131.5959, 129.7660, 129.4499, 126.9973, 83.9434, 71.7764, 62.3494, 61.5542, 56.3329, 53.5259, 40.2380, 38.5423, 34.6622, 34.1545, 28.7703, 24.9957, 24.9574, 24.7083, 22.4378, 20.9049. $\text{C}_{34}\text{H}_{44}\text{BNO}_7\text{S}_2$: 653.27, found: 654.20 $[\text{M} + \text{H}]^+$.

4.4.3 Preparation of H_2O_2 -responsive AuNPs.

Citrate-stabilized AuNPs were prepared by the following way: $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (0.0685 g, 0.174 mmol) was first dissolved in 386 ml deionized water, after which the solution was heated to boiling at $140\text{ }^\circ\text{C}$. Under vigorous stirring, sodium citrate dihydrate (0.231 g, 0.785 mmol) dissolved in 4 ml deionized water was injected into the above solution. Thereafter, the reaction was maintained at reflux for 15 min at $140\text{ }^\circ\text{C}$ before it was cooled down to room temperature.

The H_2O_2 -responsive AuNPs were prepared in the following way: 3 mL of

as-synthesized citrate-stabilized AuNPs was centrifuged and the supernatant was removed and AuNPs were then dissolved in 2 mL DMF. Then, a DMF solution of lipoic-phenylboronate-mPEG₅₀₀₀ (5 µL, 0.0185 mM) was added to the above DMF solution of AuNPs and subsequently, a DMF solution of 1-hexanethiol (100 µL, 91% v/v) was added to the AuNPs solution. The AuNPs solution was stirred at room temperature for a few hours and subjected to centrifugation to collect AuNPs. These AuNPs were washed by centrifugation-washing process and finally dissolved in PBS 7.4 solution for incubation with H₂O₂.

4.5 References

- (1) Wong, C.; Wong, K.; Chen, X. *Appl. Microbiol. Biot.* **2008**, 78, 927.
- (2) Chelikani, P.; Fita, I.; Loewen, P. C. *Cell Mol. Life Sci.* **2004**, 61, 192.
- (3) *Science* **2001**, 293, 1806.
- (4) Zhu, X.; Wentworth, P.; Wentworth, A. D.; Eschenmoser, A.; Lerner, R. A.; Wilson, I. A. *Proc. Natl. Acad. Sci. USA* **2004**, 101, 2247.
- (5) Veal, E. A.; Day, A. M.; Morgan, B. A. *Mol. Cell*, 26, 1.
- (6) Jonsson, T. J.; Johnson, L. C.; Lowther, W. T. *Nature* **2008**, 451, 98.
- (7) Gough, D. R.; Cotter, T. G. *Cell Death Dis.* **2011**, 2, e213.
- (8) Jin, H.; Heller, D. A.; Kalbacova, M.; Kim, J.-H.; Zhang, J.; Boghossian, A. A.; Maheshri, N.; Strano, M. S. *Nat. Nanotechnol.* **2010**, 5, 302.
- (9) de la Rica, R.; Stevens, M. M. *Nat. Nanotechnol.* **2012**, 7, 821.
- (10) Liu, D.; Yang, J.; Wang, H.-F.; Wang, Z.; Huang, X.; Wang, Z.; Niu,

G.; Hight Walker, A. R.; Chen, X. *Anal. Chem.* **2014**, 86, 5800.

(11) Gao, Z.; Xu, M.; Hou, L.; Chen, G.; Tang, D. *Anal. Chem.* **2013**, 85, 6945.

(12) Lai, W.; Tang, D.; Zhuang, J.; Chen, G.; Yang, H. *Anal. Chem.* **2014**, 86, 5061.

(13) Rodríguez-Lorenzo, L.; de la Rica, R.; Álvarez-Puebla, R. A.; Liz-Marzán, L. M.; Stevens, M. M. *Nat. Mater.* **2012**, 11, 604.

(14) Lyon, J. L.; Stevenson, K. J. *Anal. Chem.* **2006**, 78, 8518.

(15) Yu, A.; Caruso, F. *Anal. Chem.* **2003**, 75, 3031.

(16) Deshpande, S. S. *Enzyme Immunoassays: From Concept to Product Development; Chapman & Hall: New York* **1996**.

(17) Gnaim, S.; Shabat, D. *Acc. Chem. Res.* **2014**, 47, 2970.

(18) Sella, E.; Lubelski, A.; Klafater, J.; Shabat, D. *J. Am. Chem. Soc.* **2010**, 132, 3945.

(19) de Gracia Lux, C.; Joshi-Barr, S.; Nguyen, T.; Mahmoud, E.; Schopf, E.; Fomina, N.; Almutairi, A. *J. Am. Chem. Soc.* **2012**, 134, 15758.

(20) Kumar, R.; Han, J.; Lim, H.-J.; Ren, W. X.; Lim, J.-Y.; Kim, J.-H.; Kim, J. S. *J. Am. Chem. Soc.* **2014**, 136, 17836.

(21) Yoshii, T.; Onogi, S.; Shigemitsu, H.; Hamachi, I. *J. Am. Chem. Soc.* **2015**, 137, 3360.

List of Publications

Shaojue Wu, Si Yu Tan, Chung Yen Ang, Kim Truc Nguyen, Menghuan Li and Yanli Zhao*. An imine-based approach to prepare amine-functionalized Janus gold nanoparticles. *Chem. Commun.*, 2015, 51, 11622-11625.

Shaojue Wu, Chung Yen Ang, Zhong Luo, Si Yu Tan, Kim Truc Nguyen and Yanli Zhao*. Byproduct-induced *in-situ* formation of monodisperse gold colloidal superparticles. *SCIENCE CHINA Materials*, 2015, Accepted.

Shaojue Wu and Yanli Zhao*. Oxidation-triggered hydrophilicity change of gold nanoparticles for naked eye detection of hydrogen peroxide. Manuscript submitted.

Chung Yen Ang, Si Yu Tan, **Shaojue Wu**, Qiuyu Qu, Mun Fei Eddy Wong, Zhong Luo, Pei-Zhou Li, Subramanian Tamil Selvan* and Yanli Zhao*. A dual responsive “turn-on” fluorophore for orthogonal selective sensing of biological thiols and hydrogen peroxide. *J. Mater. Chem. C*, 2015, Advance Article.

Zhong Luo, Xingwei Ding, Yan Hu, **Shaojue Wu**, Yang Xiang, Yongfei Zeng, Beilu Zhang, Hong Yan, Huacheng Zhang, Liangliang Zhu, Junjie Liu, Jinghua Li, Kaiyong Cai* and Yanli Zhao*. Engineering a hollow nanocontainer platform with multifunctional molecular machines for tumor-targeted therapy in vitro and in vivo” *ACS Nano* 2013, 7, 10271-10284.

Liangliang Zhu, Xin Li, **Shaojue Wu**, Kim Truc Nguyen, Hong Yan, Hans Ågren and Yanli Zhao*. Chirality control in *in-situ* preparation of gold nanoparticle superstructures directed by a coordinatable organogelator. *J. Am.*

Chem. Soc. 2013, 135, 9174-9180.

Linyi Bai, Liangliang Zhu, Chung Yen Ang, Xin Li, **Shaojue Wu**, Yongfei Zeng, Hans Ågren and Yanli Zhao*. Fe(III) quantity-dependent aggregation-dispersion conversion of functionalized gold nanoparticles. *Chem. Eur. J.* 2014, 20, 4032-4037.

Jingting Song, **Shaojue Wu** and Yanli Zhao*. Encapsulation of CdSe/ZnS nanocrystals within mesoporous silica spheres. *Mater. Res. Bull.* 2013, 48, 1530-1535.