NANOMANUFACTURING OF GOLD NANOPARTICLE SUPERSTRUCTURES
FROM THE “BOTTOM-UP”

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NANOMANUFACTURING OF GOLD NANOPARTICLE SUPERSTRUCTURES

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Dissertation

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ABSTRACT

Gold nanoparticles that can generate surface plasmons under appropriate conditions have attracted significant interest for their potential in optics, photonics, data storage and biological sensors. Developing high fidelity fabrication methods that yield gold nanoparticles with well-defined size, shape, composition and self-assembly allows manipulation of surface plasmonic properties for novel applications as well as revealing new aspects of the underlying science. This dissertation demonstrates multiple techniques that describe cost-effective “bottom-up” fabrication methods that yield gold nano-superstructures.

In my initial work, I outline the solution conditions for fabricating Janus nanoparticles composed of one gold nanoparticle per micelle. Poly(ethylene oxide)-b-polystyrene (PEO-b-PS) was synthesized and processed into spherical micelles, which served as the template to induce gold nanoparticles growth within the PEO corona in situ. Organic-inorganic hybrid nanoparticle formation was controlled kinetically by manipulating the concentration of both the micelle and reducing agent (HEPES). We also found that under certain condition, PEO-b-PS yielded micelles with pearl-like morphology, which possessed concentrated PEO domains at the interface between two adjacent PS cores. When treated with gold ions, the junction area can trap more gold ions. Careful manipulation of reaction conditions afforded gold nanoparticles that grew
from the core-shell interface to form 1-dimensional (1-D) periodical gold nanoparticle chains.

Based on similar principles, gold-gold dimers were synthesized by growing a second gold nanoparticle from a gold nanoparticle template surface-functionalized with PEO ligands. Gold dimers fabricated with this method exhibited strong enhancement properties via surface-enhanced Raman scattering (SERS). Instead of kinetic control, the number of newly grown gold nanoparticles on each particle template heavily relied on the PEO density on the nanoparticle template. As the size of the particle template increased from 10 nm to 48 nm, the corresponding number of PEO chains on each particle was estimated to increase proportionally from 6 to 140. Consequently, the structure of the final products could be manipulated from gold dimer to raspberry-like structures.

The third part of my work demonstrated the fabrication of 2-dimensional (2-D) gold nanoparticle arrays using peptide-derivatized block copolymer thin film templates. A triblock polystyrene-b-poly(methyl methacrylate)-b-A3 peptide (PS-b-PMMA-A3) was synthesized and processed into thin film with highly-ordered surface patterns via cold zone annealing (CZA). Gold nanoparticles were selectively immobilized onto PMMA domains due to the binding affinity of A3 peptide located at the PMMA chain end. Gold nanoparticle structures such as hexagonally-packed gold nanoparticle clusters and parallel gold nanoparticle wires have been achieved using this method. GISAXS results indicate that the hexagonal gold-hierarchical structure is constituted of two different structures: a primary structure induced by nanofeatures on the thin film template and a secondary
structure formed through gold nanoparticle packing within each cluster domain.
Selectivity of the thin film template to gold nanoparticles and the nanoparticle aggregation are two competing phenomena that affect resolution of the hierarchical structures.

Figure 1. Gold nanoparticle superstructures fabricated based on the “bottom-up” techniques we have developed: a. gold-micelle Janus nanoparticles; b. gold dimer; c. 1-D periodical gold nanoparticle chain; d. 2-D hexagonal gold nanoparticle arrays; e. 2-D parallel gold nanowires.
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CHAPTER I

INTRODUCTION

Metallic nanomaterials, e.g. gold nanostructures, with at least one dimension on the 1-100 nm scale have attracted intensive attention due to the unique optical and electrical properties. Different from individual atoms and the bulk material, properties of gold nanoparticles are characterized by high surface-volume ratio, quantum size effect and electrodynamic interactions which render them strongly size, shape, composition and structure dependent. Utilization of gold nanoparticle optical properties can be traced back to hundreds of years ago. Gold nanoparticles possessing variable colors were used as the pigment in enamel and glasses early in the 5th BC, leading to the famous art “Lycurgus Cup” exhibiting red in transmitted light and green in reflected light. Mechanism for the phenomenon has been attributed to surface plasmons, which describe the resonant interaction between metallic surface charge oscillations under the excitement of certain electromagnetic field of light wave. Nowadays, a wide spectrum of exciting and emerging applications are expected from surface plasmons of gold nanoparticles, ranging from biomedical diagnostics, molecular sensing to photonic devices. For biomedical diagnostics, gold nanoparticles have shown great potential to be engineered into imaging probe; sensitivity of gold nanoparticles to subtle environmental change offers the opportunity to develop quick and accurate assays for highly sensitive detection;
for molecular sensing, one attractive aspect of surface plasmons lies in the electromagnetic field enhancement which can be used in surface-enhanced Raman scattering (SERS) -- a technique that allows single molecular detection; for photonics, surface plasmons provides us with an opportunity to concentrate and manipulate light with sub-wavelength structures that can give rise to photonic devices in a much smaller size scale than we currently have achieved.

Renewed interested in gold nanoparticles comes from advanced technologies that allow them to be structured, assembled and characterized from the nanometer scale. Growing expertise in synthetic and fabrication methods gives rise to multiple pathways to construct gold nanostructures with well-defined size, shape, composition, structure and assembly, which in turn enables us to control surface plasmon properties to reveal novel applications and new aspects of the underlying science. During the past decade, much attention has been paid to fabricating novel gold nanoparticle structures, gold nanoparticle based hybrid materials, and gold nanoparticle arrays with variable dimensions and morphologies. Given the fact that nanostructures and assemblies are playing a fundamental and central role to all the appealing science and applications, this work is mainly focused on the development of synthetic and fabrication methods for gold nanoparticle superstructures with potential novel applications. Structures we have been developing include gold-micelle Janus nanoparticles, 1-D periodical gold chains, gold dimers and 2-D gold arrays. To serve the growing environmental-benign and energy-conservative demand, the nanomanufacturing we have been working on attaches more importance on the cost-effective perspectives.
1.1 Surface Plasmon Resonance

Methods have long been known to generate beautiful colors by adding gold into glass medium to produce colorful windows and arts. Faraday attributed those colors to very finely divided gold, which is known as gold nanoparticles today.\(^1\) Mie calculated surface electromagnetic field of gold spherical nanoparticles with Maxwell’s equations, which was the first theoretical explanation for gold nanoparticle optical properties published in 1908.\(^2\) The author revealed that electromagnetic field of gold nanoparticle is strongly dependent on particle size, shape, medium, structure, etc. In the mid 20\(^{th}\) century, Rithie conducted the pioneer work searching energy losses of electrons passing through gold thin films. The author accounted for depolarization effect near gold thin film surface and provided the first analysis of surface plasmons which interpret optical properties of gold nanostructures from the electron behavior.\(^3\) Generation of surface plasmons in gold nanoparticles in turn leads to a sharp and intense absorption band in visible light region.

Surface plasmons is a physical concept that describes a collective oscillation of conduction electrons on the surface of a metal stimulated by certain incident light.\(^4\) According to Drude-Lorentz model, metals such as gold with the free electrons (d electron) that can travel throughout the material is treated as plasma, of which there are equal amount of fixed positive ions in the sea of free, mobile electrons. The mean free path of free electrons in gold is ~50 nm, therefore when particles are smaller than 50 nm all the scattering and absorption is expected from the surface. When excited by certain electromagnetic field, electrons which are bounded to a conducting surface set by the
positive charges in the gold nanoparticle can oscillate with an amplitude and phase reminiscent of light waves. A schematic description of the generation of a surface plasmon oscillation is shown in Figure 1.1. As the light front passes, the electric field induces a polarization of the free electrons which oscillate in resonance with respect to the lattice of positive charges. The positive ions are assumed to be immobilized with electrons moving back and forth to create a standing oscillation, which gives rise to a linear restoring force to the system.

Characterized by surface plasmons which simultaneously carry electrons and photons, gold nanoparticles can afford extremely high electromagnetic field intensities within a small mode wavelength. For nanoparticles much smaller than the wavelength of incident light, Maxwell’s equations can be solved under a quasi-static approximation to give out the electromagnetic field outside the particle:

\[ E_{\text{out}}(x, y, z) = E_0 \left( \frac{\varepsilon_\text{in} - \varepsilon_\text{out}}{\varepsilon_\text{in} + 2\varepsilon_\text{out}} \right) a^3 \frac{z}{r^3} \left( \frac{3}{r^3} (xx + yy + zz) \right) \]

where

- \( \varepsilon_\text{in} \): Dielectric constant of the metal nanoparticle;
- \( \varepsilon_\text{out} \): Dielectric constant of the external environment;
- \( a \): Particle size.

Because \( \varepsilon_\text{in} \) is strongly dependent on wavelength, the electromagnetic field is enhanced relative to the incident field and the resonance condition for the plasmon absorption is roughly fulfilled when \( \varepsilon_\text{in} \) equals to \( -2\varepsilon_\text{out} \). Gold nanoparticle is interesting because this condition is satisfied in the visible region, which renders it a valuable material for devices that allows optical signals to be controlled at sub-wavelength scale. Absorption
and scattering properties of gold nanoparticles are heavily relying on surface plasmons and can be affected by nanoparticle size, shape and medium. Difference in particle size,

![Diagram](image)

Figure 1.1  a. Schematic illustration of a localized surface plasmons in gold nanoparticles; b. electric field $E$ and magnetic field $H$.

shape and surrounding medium can result in a change of electron density of gold nanoparticles, therefore leading to a change of plasmon resonance. The above equation illustrates that nanoparticle size (a) has a strong influence on the electromagnetic field of the nanoparticle too. A more significant effect has been found when the shape of gold
nanoparticles changes. Take gold rods for instance, as the shape-anisotropy is introduced, plasmons split into two modes, transverse and longitudinal.\textsuperscript{5} Absorption spectrum of different gold nanorods is shown in Figure 1.2.\textsuperscript{6} The transverse mode

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{absorption_spectrum.png}
\caption{Absorption spectrum of gold nanoparticles with different sizes and aspect ratios. Increasing the size of gold nanospheres results in the red-shifting of absorption peak. Enhanced aspect ratio of nanorods leads to sharp plasmon splitting (Ref. 6).}
\end{figure}
shows a resonance peak ~ 520 nm, which is consistent with the absorption peak of gold nanospheres. Behavior of longitudinal mode is different, of which the absorption peak is at a much higher wavelength and is red-shifting as the aspect ratio increases. Development in calculation method of discrete dipole approximation (DDA) currently allows estimation of surface plasmon resonance for gold nanoparticles with arbitrary geometries.\textsuperscript{7} Besides size and shape, changing the medium surrounding gold nanoparticles can make a change on the surface plasmons.\textsuperscript{8} The equation below can explain the absorption change when gold nanoparticles are subjected to a different local environment:

\[ \lambda_{\text{max}} = m \Delta n \left[ 1 - \exp \left( \frac{-2d}{l_d} \right) \right] \]

where

- $\lambda_{\text{max}}$: Shift of absorption peak;
- $m$: Bulk refractive-index response of the nanoparticle;
- $\Delta n$: Change in refractive index;
- $d$: Effective adsorbent layer thickness;
- $l_d$: Electromagnetic field decay length.

When medium changes, refractive index surrounding the particles will change and make a direct effect on the maximum absorption peak. Above all, manipulation of nanoparticle size, shape and medium can result in a change of surface plasmons, which allows application in developing highly sensitive probes and spectroscopic techniques.

Dimensional and structural variation of gold nanoparticles introduces more complexities to surface plasmons, which leads to tremendous interesting and useful
applications. Gold dimer, one of the simplest gold nanoparticle superstructures, possesses “hot spots” in the junction between the two adjacent gold nanoparticles where

**Bio-sensing & Plasmonics**

![Gold nanoparticle and its assemblies](image)

**Figure 1.3** Gold nanoparticle and its assemblies have important implication ranging from biosensing to plasmonics. Single gold nanoparticles can be used as imaging agent with superior sensitivity while gold dimers can provide repeatable SERS measurement. In addition to sensing applications, 1-D and 2-D gold nanoparticle arrays allow plasmonic waveguide with sub-wavelength scale.
electromagnetic field can be orders of magnitude higher. The ability of gold dimers to enhance the local electric fields has facilitated the development of surface-enhanced spectroscopy, typically, surface enhance Raman scattering (SERS) which allows single molecular detection. Up to now, mechanism of “hot spots” is still under extensive debates and requires further investigation. Besides gold dimers, 1D gold nanoparticle chains, 2D gold nanoparticle surface and 3D gold nanoparticle lattice has attracted huge attention due to their appealing collective plasmon properties for waveguide, transistor and metamaterials. It opens the avenue for scientists to explore surface plasmons in a much broader area to reveal fascinating application as well as the fundamental science (Figure 1.3).

1.2 Synthesis of Spherical Gold Nanoparticles

The first scientific report for gold nanoparticle synthesis can be traced back early to 1857, when Faraday prepared “fine particles” through reducing chloroaurate (AuCl₄⁻) with phosphorous in aqueous solution in the presence of carbon disulfide (CS₂) as stabilizer.¹ The principle to reduce gold salt in the presence of stabilizing ligands to control particle size and to prevent aggregation has greatly stimulated the development of synthetic methods during the 20th century. Among the conventional methods, the one introduced by Turkevitch in 1951 to use citrate to reduce HAuCl₄ in water has made a significant impact.⁹ Citrate here is working as reducing agent as well as to stabilize gold nanoparticle from aggregation. With the invention of Electron Microscope technique, Turkevitch et. al. successfully observed gold nanoparticles of different particle diameters.
Almost at the same time, mechanism of gold nanoparticle formation was widely investigated in order to better control nanoparticle synthesis. One of the explanations is

Figure 1.4  Effect of sodium citrate concentration on gold nanoparticle size. Reduced concentrated resulted in increased gold nanoparticle size (Ref. 11).
termed LaMer growth model, which suggests that nucleation and growth of gold nanoparticles are two independent processes that could be manipulated by changing relative amount of reactants. Frens later investigated the effect of different amount of citrate on gold nanoparticle size and found that higher citrate concentration resulted in smaller particle size (Figure 1.4). The author concluded that size of mono-disperse gold nanoparticle suspensions was governed by the number of nuclei which finally grew into particles. With given gold source, larger population of nuclei can result in smaller particle size, which is in accordance with the LaMer model. Many efforts have been made to explore synthetic methods allowing well-defined gold nanoparticle fabrication since then. The breakthrough occurred in 1981, when Schmid et al. reported the synthetic strategy to prepare the “molecular” cluster [Au55(PPh3)12Cl6] by reducing PPh3AuCl with diborane in benzene. This reaction yielded particles with well-defined formula weight which were unique for the narrow size distribution (1.4 ± 0.4 nm). Some research groups focused on improving synthetic method through using different stabilizing ligands. In 1993, Mulvaney et.al started to use alkanethiols as the stabilizer for gold nanoparticle synthesis. The year after, Brust reported the famous two-phase synthetic method for gold nanoparticles with narrow size distribution and higher stability. This method employed a phase transfer agent (tetraoctylammonium bromide) to transfer gold salts from water into toluene and to be reduced with NaBH4 in the presence of alkanethiol as the stabilizer. Suspensions of 1-3 nm gold nanoparticles surface-functionalized with thiol-derivatized molecules were obtained. This method opened the door towards synthesizing gold nanoparticles in organic solution with facile
chemical procedures and high stability. Based on the above principle, synthesis of gold nanoparticles with a wide variety of functional thiol ligands was performed by many other research groups. Ratio of thiol/gold was studied, which revealed the general trend that higher thiol/gold ratio resulted in smaller nanoparticle size, vice versa.

Figure 1.5 Synthesis of biocompatible and aqueous-stabilized gold nanoparticles with controlled size and shape using A3 peptide. A3 peptide provides a simple one-pot pathway to synthesize water-stabilized, monodisperse gold nanospheres coated with biomolecular motifs on the surface. TEM bright field image shows gold nanoparticles synthesized by the reduction of HAuCl₄ with A3 (a and b). The scale bar in (a) is 100 nm and 20 nm in (b) (Ref. 20b)
Despite of the great achievement, neither the gold nanoparticle produced by Turkevitch method nor Brust method satisfies the requirement for biomedical application due to toxicity of the ligands employed. Motivated by increasing biomedical demand for gold nanoparticle, synthetic protocols based on environmentally benign polymers, e. g. polyethylene oxide (PEO) have been come up with. In 1994, Longenberger et. al. investigated the formation of gold nanoparticles in PEO solution, where generation of the particle rates increased significantly with increasing polymer molar mass. Later, PEO melts and PEO derivatized block copolymers were also incorporated for gold nanoparticle synthesis. However, limited control of nanoparticle size distribution sets challenges to this method. As discrete and well-defined polymers, dendrimers are well-suited for hosting nanoparticles with the interior cavities. They have been utilized as templates to control the size, stability and solubility of gold nanoparticles with the diameter ranging from less than 1 nm up to 5 nm. Recently, the use of proteins and peptides to direct synthesis of inorganic materials has attracted intensive attention due to the mild reaction condition including room temperature, aqueous solution and at or near neutral pH. A3 peptide (AYSSGAPPSPPF), identified through phase display peptide library, exhibit selective affinity to gold and is able to induce gold nanoparticles with narrow size distribution (12.8 ± 2.9 nm), as shown in Figure 1.5. To better understand the role of A3 peptide during mineralization, Stanley et. al. investigated the nucleation and growth behavior of gold nanocrystals mediated by A3 peptide and revealed that: (1) A3 peptide inhibits nucleation and growth of gold nanocrystals; (2) HEPES and HAuCl₄ accelerate the kinetics of nanoparticle nucleation and growth. Empirical rate laws for nucleation
and growth of gold nanocrystals were proposed as below:

\[
\frac{1}{t_{inc}} \approx [\text{HEPES}]^{1.7} \sqrt{[\text{HAuCl}_4]/[\text{peptide}]},
\]

\[
g_{\text{init}} \approx [\text{HAuCl}_4]^{2.55-6.34}[\text{HEPES}]^{0.13-1.16} \exp(-c[\text{peptide}])
\]

Becker et al. further studied the effect of A3 peptide on gold nanoparticle formation from molecular level. Through analyzing peptide morphological and binding affinity change for different particle sizes, the author suggested the stability-limited mechanism that peptide can adjust the curvature to bind to gold nanoparticles with diameter below 6 nm. Beyond 6 nm, peptide gradually loses the control ability and results in a dramatic reduction in the growth kinetics of the nanoparticle.\(^{23}\)

1.3 Shape Control of Gold Nanoparticles

Shape-controlled synthesis of gold nanostructures has attracted extensive attention due to the fascinating properties and intriguing applications implemented by the corresponding morphological and dimensional properties. Synthesis of gold nanorods, falling in the field of shape-controlled nanoparticles, is one of the most established protocols for anisotropic nanoparticles. Early in 1991, Martin developed a “microtubules” based template to synthesize rod-like gold nanoparticles by means of electrodeposition in nanoporous aluminum oxide membrane.\(^ {24}\) Inspired by the electrochemical principle, Wang et al. successfully prepared gold nanorods within normal micelles in aqueous solution and greatly enhanced the yields.\(^ {25}\) In the system, formation of gold nanorods was based on electrochemical oxidation of gold metal plate, which was used as the anode, in the presence of a cationic surfactant,
hexadecyltrimethylammonium bromide (C₁₆TAB), and a rod-inducing cosurfactant, tetraoctylammonium bromide (TC₈AB). Absorption spectra showed a dominant surface plasmon band corresponding to the longitudinal resonance.

![TEM images of gold nanorods with varying aspect ratios.](image)

Figure 1.6  Effect of gold salt/seed ratio on the aspect ratio of nanorods. TEM images show the gold nanorods with average aspect ratios of 1.5 (a), 2.4 (b), 6.1(c), 8.0 (d), and 10.0 (e and f). With constant gold salt, reducing the amount of seeds resulted in an increase of aspect ratio. (Ref. 25)
Compared with above electrochemical deposition, seed-mediated growth has been so far the most efficient and popular method for gold nanorods synthesis. Shape control is conventionally achieved through two steps. The first step is to rapidly form small and uniform seed particles. In the second step, more gold ions and a mild reducing agent are used to control the seeds growth in the presence of certain surfactant. The surfactant is functioning as a “shape-inducing” agent which has selective binding affinity to certain gold crystal facet to appropriately induce anisotropy. It’s also working as a stabilizer to provide colloidal stability to gold nanorods. In the first report of making gold nanorods through seed mediated approach, Murphy et. al. synthesized spherical borohydride-reduced and citrate-capped gold nanoparticle seeds (3 and 4 nm) and mixed them with a growth solution containing gold salt, cetyltrimethylammonium bromide (CTAB), ascorbic acid and small amount of silver ions. Ascorbic acid was used as a mild reducing agent to selectively reduce gold (III) to gold (I). Gold (I) can be further reduced upon the addition of gold seeds. With this method, the authors successfully produced rod-like gold nanoparticle exhibiting a pentagonal cross section with five-fold twinning. Particle aspect ratio was varied from 1 to 10 through decreasing the amount of gold seeds under constant gold salt dosing (Figure 1.6). To achieve shape control, slow rate of nanoparticle growth is essential. Fast rate can result in an overall crystal growth instead of preferential accumulation. Mulvaney et. al. showed that gold (I) was strongly bound to CTAB that the rate of nanorods growth was reduced. By reducing the reaction temperature and ionic strength, yield of gold nanorods can be enhanced. However, using Murphy’s method, population of nanoparticles with other structures still
occupies a large overall fraction of the whole product. It has been found that the citrate-capped gold seeds which possess multiply-twinned structures are playing an important role in determining structures of the final products. To overcome the above limitation, El-Sayed et al. slightly modified Murphy’s approach by using CTAB-capped seed instead of citrate caped one.\textsuperscript{28} Using this method, single crystalline gold nanorods \textit{ca.} 10 – 20 nm in diameter and up to 300 nm in length were obtained in high yield (> 90%). The ratio between additional gold ions and gold seed is also important in determine aspect ratio of nanorods. Higher ratio can result in higher aspect ratio. In addition, the role of silver ions in directing anisotropic growth of gold nanorods is critical but remains a point of contention. In Murphy’s method, they found that without silver ions, the aspect ratio couldn’t be controlled by varying the seed-to-metal salt ratio.\textsuperscript{26} Another interesting observation is that, despite of using the single crystalline seeds, incubation solution without AgNO\textsubscript{3} as an additive gave rise to gold nanoparticles with wide range of structures (Figure 1.7). Conversely, when silver ions were added, yield of gold nanorods can be achieved as high as 99%. The aspect ratio can also be adjusted by simply changing the silver ion concentration. Generally, higher silver ion concentration can lead to higher aspect ratio. There is no conclusion for mechanism of silver ions. It has been hypothesized that the formation of AgBr (Ag\textsuperscript{+} combined with Br\textsuperscript{-} from CTAB) can protect crystal facet from continuously growing. Gold salt can be selectively reduced at the two ends of nanoparticle to introduce anisotropy into the nanostructure.\textsuperscript{29, 30} Even though precise mechanism of nanorod growth has been remaining in intensive debate, especially confronting the use of additives such as AgNO\textsubscript{3}, the CTAB-capped
seed-mediated synthesis has become the most popular method for gold nanorods formation.

Figure 1.7 Effect of silver and the structure of gold seeds on the growth of gold nanoparticles. TEM images show gold nanoparticles grown from either single crystal (d) or multiply twinned (e) seeds, in the presence (a–c) and absence (f–h) of silver nitrate. (Ref. 27)
Many gold nanostructures, other than gold nanorods, have been obtained due to advanced synthetic technology, which has greatly expanded the scope of plasmonic properties. Halas et. al. synthesized gold nanoshells consisting of a dielectric silica spherical core with a gold shell of nanometer thickness.\textsuperscript{31} By varying the thickness ratio and dimensions of the core and the shell, optical properties of the nanoparticles could be manipulated. Xia et. al. has developed the method by making use of a galvanic replacement reaction between silver nanostructures and gold salts to synthesize gold nanocages with hollow interiors and porous walls.\textsuperscript{32} Plasmon absorption peak could be continuously adjusted from 425 nm to 1200 nm by changing the silver and gold ratio. Recently, Mirkin et. al. have described a seed-mediated synthetic route for concave cubic gold nanoparticles.\textsuperscript{33} In a typical synthesis, gold seeds were prepared using cetyltrimethylammonium chloride (CTAC) instead of CTAB, of which the Cl\textsuperscript{-} counterion played an essential role in controlling the concave morphology. By adjusting experimental parameters, structure of those reluctant nanomaterials can be easily varied over a wide range, which in turn has enabled us to control plasmonic properties to reveal new aspects of the underlying science.

1.4 Gold-Based Janus nanoparticles

Since De Gennes introduced the concept of “Janus Grains”, named after the mythological figure Janus who possesses two distinct faces, efforts to synthesize anisotropic Janus particles have been pursued widely with varying levels of success.\textsuperscript{34,35} With well-defined structures and asymmetric functionalities, Janus particles exhibit novel
physical and chemical properties, which have attracted significant attention due to their diverse potential applications. For instance, Crossley et. al. synthesized amphiphilic Janus nanoparticles by growing carbon nanotubes on metal oxide nanoparticles, of which the oxide surface preferred to be in water while carbon nanotubes preferred to be in oil. Addition of palladium to the nanoparticles created catalytic sites allowing biofuel upgrade reactions at the water/oil interface. Gao et. al. has demonstrated that by incorporating magnetic nanoparticles in to a Janus particle, it can be used broadly from cell imaging, orientation control of cell behavior to innovative magnetolytic therapy on tumor cells. Sun et. al. synthesized monodisperse dumbbell-like Pt-Fe₃O₄ nanoparticles by epitaxial growth of iron (Fe) nanoparticle onto platinum (Pt) nanoparticles followed by oxidation of Fe. Through controlling the Janus nanoparticle parameters such as Pt particle size and shape as well as the interaction between Pt and Fe₃O₄, catalytic capability of the nanoparticles can be manipulated.

Besides the appealing collective functionalities, Janus nanoparticles are one of the basic building blocks used to meet the challenging goal in materials chemistry and physics to spontaneously form different superstructures. Cheng et. al. reported a series of precisely defined, nonspherical, polyhedral oligomeric silsesquioxane (POSS)-based molecular Janus particles which can self-organize into hierarchically ordered supermolecular structures in the bulk. In addition to the self-assembly from molecular level, design of functional materials usually involves building blocks of colloidal-sized particles. Asymmetric surface properties of colloidal Janus particles provide a pathway for such exploration. Making use of vapor deposition, Granick et. al. synthesized
amphiphilic Janus particles with an anionic surface on the silica side owing to native silanol groups on the silica and cationic surface on the wax side owing to deposition of amine-terminated self-assembled monolayer (SAM). These dipolar Janus particles self-assembled into chains commonly consisting of three to five particles in aqueous solution. Recently, a more complex colloidal kagome lattice has been made through decorating particle surfaces with hydrophobic domains. The building blocks, named “triblock Janus”, were micrometer-sized spheres with electrostatic repulsion in the middle, hydrophobic attraction at the poles, allowing self-assembly by short-range hydrophobic attraction through screening electrostatic repulsion.

Nowadays, many research groups have been working on incorporating gold nanoparticles into the scope in order to take advantage of their optical and catalytic properties. Composto et. al. demonstrated that by casting silica particles onto a copolymer thin film, one side of the particles can be shielded so that only the other side can be functionalized with gold nanoparticles to form Janus particles. Through measuring the plasmon absorption behaviors when gold nanoparticles are wetting and dewetting on silica surface, the particles can perform as an optical probe to detect the surface energy between gold and silica substrate underneath. Properties of gold nanoparticles can be affected by the other component material of the Janus particle. Sönnichsen et. al. synthesized metal-semiconductor hybrid nanoparticles (CdS-Au and CdSe-Au) and systematically studied their optical properties. It has been found that the CdS-Au Janus particles essentially maintained the optical properties of their original components while CdSe-Au Janus nanoparticles exhibited strong interference in the
optical properties. A challenging goal in therapeutic strategy and diagnostic accuracy is the ability to sense and detect signals from target inside and surrounding a living cell at an early stage. Driving by the motivation to fabricate small probes at nanometer scale that can be used to precisely detect the targeted regions, Lee et. al. developed the Janus particles with a polystyrene body half surface shielded with gold nanoparticles and half

Figure 1.8  Gold-polystyrene Janus particles as multifunctional probes for targeting, sensing, and drug delivery; SEM images shows the Janus particle composed of gold and PS surface; in the bottom right inset the PS template has been etched. (Ref. 41)
with target recognition agent. This device shows strong target-sensing ability for breast cancer cells (Figure 1.8). With further refinement, it can perform as drug delivery system. Another important application of gold nanoparticle is to be used as the catalyst. Even though the structure cannot be strictly included in the Janus category, polymer-encapsulated gold nanoparticle dimers developed by Chen et. al. for catalyzing ZnO-nanowire growth suggests the great potential for gold-derivatized Janus particle to be used as catalyst.

Quantitative integration of functionalities implemented through the specific Janus structure is of considerable interest and have boomed the development of Janus particle fabrication methods. The key challenge for developing such methods relies on how to precisely combine individual components together without sacrifice their individual properties. In order to satisfy the requirement, various methods have been developed, e. g. microfluidics, protecting-deprotecting, surface-nucleation, etc. Several research groups have been working on using a microfluidic approach to synthesize Janus beads on a large scale. This method usually relies on a Y-shape channel (geometry is variable) to form a two phase monomer steam, which permits regular creation of monodisperse droplets bearing spherical or non-spherical shapes and spatially segregated chemical properties over a wide range of flow rates. This method is sufficiently effective in synthesizing micrometer-sized polymer particles. However, very few efforts have been made for gold-derivatized Janus nanoparticles with microfluidics, mainly due to the limited control over particle size on nanometer scale and inorganic materials. Instead, much work has been done under the principle of “protecting and deprotecting”, which
usually requires partially shielding of the targeted particle for intended decoration with a “mask” followed by removal of the “mask” to either expose the surface or to further functionalize it. In the work done by Xu et. al., they assembled gold nanoparticles at a liquid-liquid interface between organic droplets and aqueous bulk solution to partially protect the nanoparticle surface, leaving the heterogeneous reaction taking place on the surface exposed to aqueous solution. With this method, hetero-dimers composed of two distinct nanospheres (Au-Ag Janus nanoparticle) have been fabricated. Lei et. al. deposited a monolayer of polystyrene spheres onto a substrate for surface protection, followed by a gold thin film coating on the exposed surface. The non-close-packed Janus particles with symmetry breaking were further decorated with silver nanoparticles above gold layer to show combined plasmonic properties. Chen et. al. has developed a scalable route to fabricate Janus Au-SiO$_2$ and Ag-Au-SiO$_2$ nanoparticles. In this method, surface of gold nanoparticles was partially covered with silica shell in the presence of the ligand mixture consisting of 4-mercaptophenylacetic acid (4-MPAA) and poly(acrylic acid) (PAA), which can bind to the gold nanoparticle surface too. The competition effect between silica and ligands produced Au-SiO$_2$ Janus nanoparticle, of which silver nanoparticle can be further induced from the gold nanoparticle surface without silica shell (Figure 1.9). With the same principle to form Janus nanoparticle through “protecting and deprotecting”, Li et. al. recently has utilized a single crystal formed by thiol-capped PEO chains as the mask. When crystal formed, the thiol end groups were excluded out onto the surface of the crystal substrate, which were able to immobilize gold nanoparticles and partially shield the surface. Gold nanoparticle
surface exposed to solution could undergo further decoration to conjugate platinum nanoparticles to form Pt-Au Janus nanostructures after crystal melted. Surface nucleation is another commonly used method for gold-derivatized Janus nanoparticle fabrication. It usually requires a limited region of the original nanoparticle accessible to

Figure 1.9 Protecting-deprotecting strategy for Janus nanoparticle fabrication  

a. Schematic illustration of the competition effect between ligands and SiO$_2$ that led to the formation of Janus Au-SiO$_2$; (b) TEM image of Janus Au-SiO$_2$; inset is the digital photo showing the product of a scaled-up synthesis; TEM image of the AgNS-Au-SiO$_2$. (Ref. 47)
the second particle, or high surface tension between two adjacent particles to prevent them merging together during incubation. Sun et. al. synthesized dumbbell-like Au-Fe₃O₄ nanoparticles through decomposition of Fe(CO)₅ on the gold nanoparticle surface followed by oxidation in air. The driving force for epitaxial growth of Fe₃O₄

![Diagram](attachment:image.png)

Figure 1.10 Surface nucleation strategy for Janus nanoparticle fabrication. a. Schematic illustration of the synthetic strategy to grow Au-Fe₃O₄ Janus nanoparticles with varied size ratios; b. TEM bright field image of the 3-14 nm Au-Fe₃O₄ Janus nanoparticles; c. TEM bright field image of the 8-14 nm Au-Fe₃O₄ Janus nanoparticles; d. HAADF-STEM image of the 8-9 nm Au-Fe₃O₄ Janus nanoparticles; and e. HRTEM image of one 8-12 nm Au-Fe₃O₄ Janus nanoparticles. (Ref. 49)
on gold nanoparticle was attributed to electron deficiency of the gold nanoparticles resulted from the fact that free electrons from gold must compensate for the charge induced by the polarized plane at the interface, which caused the lack of multi-nucleation (Figure 1.10). Ji et al. have demonstrated the synthesis of PbS-Au Janus nanoparticle utilizing the differences in polarity of crystal facets that led to the selective growth of metal on the semiconductor surface.\textsuperscript{54} Deposition of gold took place preferentially on PbS facets with the highest reactivity. Number of the newly grown gold nanoparticles was controlled through the addition of HAuCl\textsubscript{4}. With the similar mechanism, Pellegrino et al. reported the formation of heterogeneous CoPt\textsubscript{3}-Au dimers through selectively growing gold nanoparticles on the surface of CoPt\textsubscript{3} nanoparticle.\textsuperscript{55} Xia et al. has reported a tadpole-like Janus nanostructure consisting of a gold head and a palladium tail.\textsuperscript{56} The synthesis was based on the galvanic replacement reaction between palladium nanorods and HAuCl\textsubscript{4}. Instead of forming a core-shell structure, AuCl\textsubscript{4}\textsuperscript{-} was preferentially reduced at the end of rods. A transition from two-end to one-end growth to form Janus nanoparticle was observed but the mechanism was still undetermined.

Fabrication methods for gold-derivatized Janus nanoparticles are not limited to the above categories. Many efforts have been devoted to broadening the synthesis of asymmetric, hybrid colloidal particles. Xia et al. generated the synthetic method for hybrid Janus particles consisting of metallic nanoparticles and polymer beads through initiating polymerization of polystyrene from gold nanoparticle surface.\textsuperscript{57} Chen et al. introduced a method for fabricating the hybrid Janus nanoparticle with a bimetallic core-shell (Au-Ag) and a polyaniline bead.\textsuperscript{58} The eccentric partial polyaniline
polymer shell was utilized to protect gold particles and to control the epitaxial overgrowth of silver from the void space of gold nanorods. Recently, Becker et. al. has developed a

Figure 1.11  a. Schematic illustration of the fabrication strategy of gold-micelle Janus nanoparticles; gold nanoparticles are incubated from the PEO corona of the spherical micelles; b. TEM bright field image of gold-micelle Janus nanoparticles. (Ref. 55)
facile, scalable method for micelle-gold hybrid Janus nanoparticle synthesis. Hydrophilic polyethylene oxide (PEO) was covalently conjugated with hydrophobic polystyrene (PS) and processed into well-defined micelle in water solution with PEO as the corona and PS as the core. Gold nanoparticles were initiated from the PEO corona due to its mineralization ability. Through controlling micelle and HAuCl$_4$ concentrations, high fidelity of Janus nanoparticles were fabricated with each particle composed of one micelle and one gold nanoparticle (Figure 1.11).

1.5 Gold Dimer

Noble metallic nanocrystals, e.g. gold nanoparticles, have generated significant interest due to their enhanced optical properties. Certain gold nanostructures, identified by their distinct chemical and dimensional properties, can give rise to plasmon resonances affording intense optical frequency fields known as surface enhanced Raman scattering (SERS) under certain conditions. When the specific gold nanostructures are used as substrates for SERS measurement, many orders of magnitude higher enhancement can be achieved, which can give rise to single molecular detection. In general, compared to isolated symmetric gold nanoparticles, rough or asymmetric aggregates have yielded a much stronger SERS signal. This dramatic variation has been attributed to the random formation of localized plasmons or “hot spots” within nanoparticle junctions. However, formation of well-defined gold aggregates is difficult leaving investigators to rely on broadly distributed aggregate populations that are not uniform, which gives rise to poor SERS signal reproducibility. Therefore scattering active nanoparticle populations
that can harness the structure dependent local fields of the junction plasmons are urgently needed to advance the SERS imaging modality.

Among the various established superstructures, a pair of closely spaced gold nanoparticles supporting “dimer” plasmons has shown great promise in this context due to the significant electromagnetic field enhancement occurring in the junction once surface plasmons are excited.\textsuperscript{64, 65} While the gold nanoparticle dimers may not be the optimal choice for most intense electromagnetic field enhancement, the single, narrow gap in between two particles serve as a simple model to provide repeatable measurement. Many efforts have been devoted into investigating the plasmonic properties of gold nanoparticle dimers. Early in 2003, Halas \textit{et. al.} came up with the hybridization model which treated plasmon response of metal-based nanostructures as the collection of plasmons arising from simpler geometries to form an interacting system.\textsuperscript{66} In this model, plasmons of the metallic nanostructures was determined by the electromagnetic interaction between “free” plasmons.\textsuperscript{66} Nordlander \textit{et. al.} has further applied the plasmon hybridization method to nanoparticle dimers and found that the dimer plasmons can be viewed as the bonding and anti-bonding combination, which is a hybridization of individual nanoparticle plasmons in analogy with that of the homo-nuclear diatomic molecules.\textsuperscript{67} A few theoretical and experimental reports have examined the relationship between SERS enhancement and the corresponding far field spectral position.\textsuperscript{68, 69} Schatz \textit{et. al.} reported the general trend that SERS enhancement factor in the gap between two spheres was increased as the exciting energy moved towards near-IR.\textsuperscript{68} Recently, using a correlated LSPR-transmission electron microscopy (TEM) surface-enhanced
Raman excitation spectroscopy (SERES) technique, Duyne et al. have suggested that little dependence has been found between the hot spot dominated systems and the far-field scattering properties of the gold nanoparticle dimers.\textsuperscript{69}
Generation of intense and repeatable SERS signals through gold dimers requires the fabrication of highly uniform nanostructures. The “top-down” methods, e.g. photo-lithography, have been employed for gold dimers engineering with controlled particle size and interparticle spacing. However, manipulation of matters on nanometer scale is limited by resolution of the laser and the manufacturing becomes extremely expensive as nano-feature is brought down to below 100 nm. The “top-down” methods have been thoroughly reviewed before and are not the focus of this work. On the other hand, multiple methods have been developed for gold dimer fabrication using the “bottom-up” chemical techniques to bring down the cost as well as to achieve smaller nanofeatures. During the past decade, one of the most often used methodologies is to form molecular “bridges” to link two individual particles. Programmable DNA and synthetic rigid molecules with defined architectures and end groups have been investigated as linkers to couple gold nanoparticles since years ago. In 1996, Alivisatos et. al. reported that gold nanocrystals functionalized with single-stranded DNA oligonucleotides of defined sequence could self-assemble into gold dimers in the presence of the complementary single-stranded DNA template (Figure 1.12). Using molecules as the bridge, in 1999, Feldheim et. al. synthesized the phenylacetylene with a rigid backbone and two thiol end groups to serve as the bridge connecting two individual gold nanoparticles. Later, the similar molecule was also applied to silver nanoparticle assembly. Development of solid state synthesis has facilitated quantitative control of ligands modification on particle surface, which allows assembly of well-defined nanostructures through regiospecific reaction. In the study from Jacobson et. al., gold
nanoparticles \((d \approx 2 \text{ nm})\) mono-functionalized with 11-mercaptopoundecanoic acid \((\text{HO}_2\text{C(CH}_2\text{)}_{10}\text{SH}, \text{MUA})\) was synthesized through solid-phase reaction using PS Wang.

Figure 1.13  Gold dimer fabrication based on solid-state synthesis to form single molecular linkage between particles.  a. Schematic illustration of the synthetic route for mono-functional gold nanoparticles; b. coupling of two individual gold nanoparticles to form the gold dimer; c and d. HRTEM images of gold nanoparticle dimers. (Ref. 67)
resin as a transferring agent. With the single carboxylic acid group on each nanoparticle, every two gold nanoparticles were conjugated in the presence of the molecular linker ethylenediamine (Figure 1.13).\textsuperscript{77} Similar work was done by Huo et. al. through taking advantage of Wang resin for gold dimer synthesis.\textsuperscript{74} Later, Mirkin et. al. applied solid phase synthetic strategy to perform site-specific modification of gold nanoparticles with single-stranded DNA chains, which provided a better structure control during particle hybridization.\textsuperscript{78} Shumaker-Parry et. al. deposited a monolayer of gold nanoparticles onto a silanized glass surface and protected the exposed nanoparticle surface with 11-mercaptop-1-undecanol (MUOH). To release the nanoparticles from the substrate, the MUOH functionalized particles were sonicated in ethanol in the presence of 16-mercaptohexadecanoic acid (MHA) or mecaptoethylamine (MEA). MHA or MEA molecules were expected to be asymmetrically bound only to the surface area of gold nanoparticles attached to the silane layer due to strong binding affinity of thiol group to gold. Finally, through forming covalent bonding between MHA functionalized gold nanoparticle possessing carboxylic groups and MEA functionalized gold nanoparticles possessing amine groups, gold dimers were generated.\textsuperscript{79} Li et. al. later simplified the method by utilizing a PEO single crystal of which the thiol end groups were excluded onto the surface as the solid phase substrate to immobilize gold nanoparticles. By attaching additional gold nanoparticle on the exposed surface, gold dimers could be fabricated.\textsuperscript{80} Recently, “click” chemistry has been utilized to facilitate the formation of spherical gold dimers as well as gold nanorods coupling.\textsuperscript{78, 79, 81} Even though, methods employing molecular linkers to form gold nanoparticle dimers often suffer from
complication in quantitative surface modification and require gold nanoparticle size to be small enough, which usually sacrifice their optical properties. To reduce the burden on

Figure 1.14  Gold dimer fabrication through polymer encapsulation. a. Aggregation of gold nanoparticles upon addition of HCl; b. encapsulation of gold nanoparticle aggregates through PS-b-PAA; c. enrichment of gold dimers by centrifugation; d. schematic illustration of the interactions between gold nanoparticle, hydrophobic ligands and PS-b-PAA; e, TEM bright field image of gold dimers; f. Histogram of the dimer percentage. (Ref. 73)
gold nanoparticle size, Chen et. al. developed a large-scale fabrication method that allowed gold nanoparticles with diameter ~5 nm to be segregated into dimers under the protection of polystyrene-block-poly(acrylic acid) (PS-b-PAA) shell. In this method,

Figure 1.15  Gold dimer fabrication through surface nucleation  a. Schematic illustration of gold dimer synthesis; b. TEM bright field image of Au-Fe3O4 seeding nanoparticles; c. TEM bright field image of Au2-Au1-Fe3O4 nanoparticles after 1 h incubation; d. TEM bright field image of Au2-Au1-Fe3O4 nanoparticles after 3 h incubation; e. TEM bright field image of Au2-Au1-Fe3O4 nanoparticles after 6 h incubation. (Ref. 75)
a hydrophobic thiol-capped ligand, 2-dipalmitoyl-sn-glycero-3-phosphothiethanol, was utilized to modify the gold nanoparticle surface. Addition of HCl triggered aggregation of gold nanoparticles, which were subsequently encapsulated into PS-b-PAA shell to yield gold dimers (Figure 1.14). They also developed a differential centrifugation method to purify the product and enhance yield of gold nanoparticle dimer.\textsuperscript{53}

![Figure 1.16](image.png)

Figure 1.16  a. Schematic illustration of gold dimer fabrication through growing gold nanoparticle from template gold nanoparticle tethered with PEO ligands; b. TEM bright field image of template gold nanoparticles; c. TEM bright field image of gold dimers. (Ref. 76)
Recently, a pioneering study was conducted by Sun et. al. to surface nucleate the second gold nanoparticle (d ≈ 7 nm) from the gold nanoparticle template (d ≈ 5 nm) which was partially capped with Fe₃O₄. In this study, Au₂-Au₁-Fe₃O₄ nanoparticles were prepared by overgrowing Au₂ onto Au₁-Fe₃O₄ nanoparticles. It was interesting to notice that Au₂ was trying to extract Au₁ out of conjugation with Fe₃O₄ during growth and won over Fe₃O₄ on binding to Au₁ at longer incubation time to finally form gold nanoparticle dimers (Figure 1.15).

Currently, more and more efforts have been devoted to developing methods with simplified operation and large scale fabrication to yield well-defined and SERS active gold nanoparticle dimers. To achieve this goal, recently, Becker et. al. reported a one-pot surface nucleation method to incubate SERS active gold dimers in water solution. It utilized polyethylene oxide (PEO) functionalized gold nanoparticles as the template to initiate additional gold nanoparticles growing from the PEO corona. Gold dimers were achieved through carefully controlling the reducing environment as well as payload of PEO chains through controlling size of template nanoparticles. Strong SERS activity has been measured (Figure 1.16).

1.6 One Dimensional (1-D) Gold Nanoparticle Chain

Currently, photonic devices such as optical lenses, fibers and integrated circuits are performing with the light wavelength on the order of 1000 nm. When dimension of photonics is approaching the wavelength of light, propagation of light within the devices will be obstructed by optical diffraction. In order to achieve control over photonic
devices at nanometer scale, dimensions of the structures that guide electromagnetic energy propagation should be laterally confined below the diffraction limit of light. It has been suggested that a linear chain of spherical gold nanoparticles could be utilized to guild light below the diffraction limit by the electrodynamic interparticle coupling, which can give rise to coherent propagation of energy along the array (Figure 1.17).\textsuperscript{86}

Figure 1.17  Schematic illustration of the excitation and detection of energy transport in plasmon waveguide made of gold nanoparticle arrays. Light emanating from the tip of an illumination-mode near-field scanning optical microscope locally excites a plasmon waveguide. Electromagnetic energy is transported by the waveguide to a fluorescent device, of which the fluorescence can be collected in the far-field. (Ref. 77a)
Motivated by the exciting potential application to miniaturize photonics, a variety of methods from the “top-down” and the “bottom-up” have been used to construct gold nanoparticle plasmon waveguides. It’s well known that the “top-down” method is efficient for nanostructure constructions regardless of its high cost. Techniques such as electron beam (E-Beam) lithography and focused ion beam (FIB) lithography have progressively developed “top-down” engineering for metallic surface towards higher integration density and smaller size.\(^{72}\) Aimed to bring down the cost as nanofeatures becoming smaller, review of the fabrication methods will focus on development of the “bottom-up” techniques.

Efforts devoted to the “bottom-up” can be generally divided into two categories: “template-assisted fabrication” and “polymerization of nanoparticles”. Materials presenting fibril nanostructures that have specific interactions with gold nanoparticles can be utilized as the template for self-assembly of gold nanoparticles into 1-D chain. The use of DNA as to template gold nanoparticle wires is of great interest for its base-pairing programmability for predesigned shapes, lengths and composition.\(^{90}\) In 2004, Willner et. al. fabricated telomeres (single-stranded DNA present in cancer cell) into 1-D template to direct gold nanoparticle self-assembly through base-pairing interaction. The author also covalently conjugated ester-modified gold nanoparticles to the amine units on the DNA template to yield gold nanoparticle wires.\(^{91}\) Other than using base-pairing DNA, Pochan et. al. employed an alanine-rich polypeptide which could self-assemble into highly-ordered β-sheet fibrils to template gold nanoparticle arrangement through electrostatic interactions.\(^{92}\) Later, Hsieh et. al. found that insulin fibrils with multiple
amide groups could perform strong interactions with gold nanoparticles and were able to induce them into 1-D wires.\textsuperscript{93} Recently, Voit \textit{et. al.} have demonstrated the use of poly(propylene imine) (PPI) to direct 1-D gold nanoparticle chains.\textsuperscript{94} In this method,

![Diagram of 1-D gold nanowire fabrication based on “soft” polymeric fibril template.](image)

Figure 1.18 1-D gold nanowire fabrication based on “soft” polymeric fibril template.  a. Schematic illustration of the formation of 1-D gold nanoparticle chains; there are three steps: self-assembly of 1-D PPI dendrimer triggered by PH and addition of Cd ions, formation of CdSe nanoparticles and formation of gold nanoparticles based on the 1-D CdSe-dendrimer template; b. TEM bright field image of 1-D gold nanoparticle arrays; inset is the HRTEM image of one gold nanoparticle. (Ref. 82)
careful manipulation of PH gave rise to selective protonation of dendrimer surface which could form complex compound with Cd ion to direct the self-assembly of the dendrimer into 1-D nanofibrils. Upon addition of NaHSe, Cd ions were reduced into CdSe nanoparticles \textit{in situ}, which in turn fixed the fibril morphology. These nanofibrils were used as the scaffold to induce gold nanoparticle synthesis within each dendrimer along the chain (Figure 1.18). Besides biomolecules, polymeric fibril is another type of “soft” template for gold nanoparticle arrangement. Park \textit{et. al.} synthesized hyaluronic acid polymer chain with thiol side groups along the backbone to assemble gold nanoparticles into 1-D array.\textsuperscript{95} Huo \textit{et. al.} fabricated gold nanoparticle rings through forming covalent bonding between mono-functionalized gold nanoparticle and ring-like polymer backbones.\textsuperscript{96} Similarly, Greiner \textit{et. al.} grafted a single polymer chain with a mono-carboxylic end group from each gold nanoparticle and conjugated the carboxylic group with each amine group along a polymer backbone to induce 1-D gold nanoparticle arrangement.\textsuperscript{97} Using solid phase synthesis, Shumaker-Parry \textit{et. al.} fabricated 1-D gold nanoparticle chains through polymerizing asymmetrically functionalized gold nanoshperes immobilized on glass substrate followed by a release step.\textsuperscript{98}

Different from the above “soft” molecular templates, Giersig \textit{et. al.} used “hard” templates like carbon nanotube to induce gold spherical nanoparticles and nanorods into 1-D wires through electrostatic interaction between particle and carbon nanotube surface.\textsuperscript{99, 100} In 2011, Patolsky \textit{et. al.} utilized silicon nanotubes to guide gold nanoparticles.\textsuperscript{101} They developed the method to selectively functionalize the inner and outer walls of silicon nanotubes with organic layers which show either hydrophilic or
hydrophobic chemical nature. Gold nanoparticles were allowed to be preferentially wetting either inside or outside of the nanotubes (Figure 1.19). The use of functional

Figure 1.19 1-D gold nanowire fabrication based on “hard” tubular template. a. Schematic illustration of the strategy used for the selective wall-wetting of gold nanoparticle at the outer and inner surfaces of SiNTs by varying silian-derivative molecular layers; b. TEM bright field image of gold nanoparticle located on the inner wall; c. TEM bright field image of gold nanoparticle located on the outer wall. (Ref. 88)
metallic nanoparticles as templates has also been introduced as a useful way for nanoparticle assembly. Liz-Marzán et al. showed that gold nanowires were able to align gold nanospheres and nanorods along the long-axis of the wire template to form 1-D structures. Pyun et al. used magnetic nanoparticles as the core buried inside gold shells to form dipolar core-shell colloids. Stimulated by magnetic field, gold nanoshells could self-assemble into 1-D chains.

Analogy has been made between functionalized gold nanoparticles and monomers in polymer. Without using fibril template to assist assembly, gold nanoparticles can be triggered for polymerization under appropriate condition. The most common example is the end-to-end “conjugation” of gold rods into 1-D chains. Unlike forming covalent bonding between neighboring monomers when synthesizing polymers, polymerization of gold nanoparticles usually requires some specific interactions including hydrogen bonding, metallic complex, electrostatic interaction, etc. Pioneer work was done by Murphy et al. to selectively functionalize tips of gold nanorods with biotin. The functional building blocks could be assembled in an end-to-end pattern using streptavidin as the interparticle linker (Figure 1.20). Several years later, Banin et al. applied the same strategy to assemble gold-tipped CdSe nanorods into 1-D nanoparticle chains. Knecht et al. anchored carboxylic acid group on ends of the nanorods to take advantage of the cooperative intermolecular hydrogen bonding for linear assembly. As the chain of nanorods was growing, the longitudinal plasmon absorption of the nanoparticle chain continuously changed. Formation of metallic complex is another type of interaction that can be used to generate particle self-assembly. Ren et al. synthesized a series of
Ru$_2$(DMBA)$_4$(oligo(phenyleneethyne))$_2$ compounds bearing sulfide end groups which allowed readily assemble of gold nanoparticles into chains with well-defined interparticle

Figure 1.20 1-D gold nanowire fabrication based on end-to-end recognition of nanorods. a. Schematic illustration of gold nanorods self-assembly through surface functionalization of biotin disulfide (red) and addition of streptavidin (blue); b. bright field TEM image of 1-D arrays of gold nanorods; inset is the magnification of the joint point. (Ref. 91)
With similar principle, Newcome et al. later synthesized [(disulfide-terminated tpy)$_2$-M$^{II}$] complexes and used them as the linker for end-to-end attachment of gold nanorods. Electrostatic interaction has also been incorporated into this scope. Yan et al. used glutathione and cysteine, which were thiol species with both carboxylic and amino groups, to functionalize gold nanoparticles. It was found out that when the molecules were in their zwitterionic form, gold nanoparticles could be induced
into 1-D arrays through intermolecular electrostatic interaction. On the other hand, amphiphilic polymers have been employed to guide gold nanoparticle arrangement. Taton et. al. reported that gold nanoparticles which were encapsulated into cross-linked poly(styrene-b-acrylic acid) (PS-b-PAA) spherical micelles could be induced into 1-D chains by increasing salt concentration in the aqueous solution. Gibson et. al. covalently conjugated an amphiphilic polystyrene-b-poly(ethylene oxide) molecule to the surface of gold nanoparticles and drove their assembly in water into 1-D arrays. Even though the amphiphilicity-driven self-assembly doesn’t require any molecular-recognition or hydrogen bonding, the driving force is not strong enough for nanoparticles with larger sizes (>5 nm). Recently, instead of using amphiphilic macromolecular ligands to assist nanoparticle self-assembly, Rubinstein et. al. synthesized the amphiphilic gold nanorods with the longitudinal side tethered with hydrophilic CTAB and two ends tethered with hydrophobic polystyrene. The author directed gold nanorods into well-defined nanochains by making a striking analogy between amphiphilic ABA triblock copolymers and the amphiphilic nanorods. Through carefully manipulating solvent quality, self-assembly of the gold nanorods can be varied ranging from clusters, rings and chains (Figure 1.21).

1.7 Two Dimensional (2-D) Gold Nanoparticle Substrate

Controlled organization of gold nanoparticles into multi-dimensional and morphologically addressable arrays is the foundation for emerging applications as diverse as in single molecule surface-enhanced spectroscopy, colorimetric sensing, high
Among various types of nanostructures, 2-D highly-ordered gold nanoparticle arrays have attracted significant attention due to their great potential to miniaturize photonic devices to a much smaller scale than can be currently attained, which is termed plasmonics. Concentrating light with highly-ordered 2-D gold nanoparticle arrays also leads to significantly enhanced electromagnetic field that allows construction of plasmonic sensors for molecular information decoding. Through massive signal enhancement, well-defined gold nanoparticle assembly allows significantly enhanced signal-to-noise ratio to achieve single molecular detection. Therefore, fabrication of gold substrate with highly-ordered nanotextures becomes central to the development of emerging and exiting applications.

Advancement in chemical synthetic techniques leads to rapid progress in the development of “bottom-up” fabrication methods for 2-D gold nanoparticle arrays with various patterned features. As an alternative method of the “top-down” technique, the “bottom-up” fabrication does not rely on high energy laser for nanostructure construction, which greatly reduces the burden on cost especially when feature size becomes small. Duyen et. al. have come up with the principle of nanosphere lithography (NSL), which is capable of producing large scale and well-defined 2-D nanoparticle arrays. During the processing, monodisperse spherical polymer colloids were homogenously casted on a flat substrate to form a single layer of particles with hexagonal close-packing. Periodic arrays of metallic nanoparticles were produced by evaporating metals into the void space among neighboring colloids followed by removal of the colloidal substrate. Lateral
dimension of the nanoparticle arrays can be manipulated through changing the colloidal particle size, shape and stacking (Figure 1.22). Within the “bottom-up” techniques, thermodynamically driven self-assembly is another appealing pathway for 2-D gold nanoparticle substrate fabrication. This method usually requires gold nanoparticles with

Figure 1.22  Schematic illustration of nanosphere lithography (NSL) and the tunable 2-D periodic particle arrays.  a. Single layer mask; b. single layer particles; c. AFM image of single layer particles; d. double layer mask; e. double layer particles; f.  AFM image of double layer particles. (Ref. 103)
specific surface modification to facilitate nanoparticle arrangement during processing. Beyer et al. induced 2-D gold nanoparticle arrangement by slowly immobilizing the gold nanoparticles onto a polymer-modified surface. Self-assembly was performed through the acid-base reaction by dipping a poly(ethylene imine)-covered, carbon coated copper grid into a \( \text{[Au}_{55}(\text{Ph}_{2}\text{PC}_{6}\text{SO}_{3}\text{H})_{12}\text{Cl}_{6}] \) cluster aqueous solution. Gold nanoparticles gradually attached to the substrate and yielded 2-D hexagonal and cubic arrays. Wei et al. synthesized resorcinarene tetrathiol as the ligand to functionalize gold nanoparticle surface. The single layer molecular coating implemented repulsion among nanoparticles at close range but was thin enough to maintain minimal interparticle separations. This specific interaction gave rise to the formation of 2-D gold nanoparticle hexagonal close-packed arrays with periodicities up to 170 nm (Figure 1.23). Teranishi et al. reported a protective agent, bis-4,4’-(4,4’-dithiobutylbenzyl)-N,N,N’,N’-tetraethylamine, to functionalize gold nanoparticles. Amino groups formed ammonium salts with organic acids to control the 2-D structures ranging from quasi-honeycomb to square structures through slow evaporation of solvent. In addition, length of ligands on gold nanoparticle surface has played an important role in determining structures and the corresponding properties. Based on the previous work, Teranishi et al. later synthesized gold nanoparticles functionalized with a series of bidentate ligands. Through controlling chain length of the ligands, gold nanoparticles presented hexagonal close-packing with tunable interparticle spacing. Similarly, through changing the alkyl chain length of surface ligands, Gwo et al. fabricated 2-D gold nanoparticle substrate with tunable lattice...
Different interparticle spacing gave rise to different near-field coupling modes, thereby allowing tunable collective surface plasmon resonance. However, highly

Figure 1.23 Thermodynamically driven self-assembly of 2-D gold nanoparticle arrays.

a. Structure of resorcinarene tetrathiol which was used to functionalize gold nanoparticles;
b. TEM bright field image of 2-D gold nanoparticle (d = 70 ± 5 nm) arrays. (Ref. 105)
ordered surface patterns are difficult to be achieved due to notable defects left in the 2-D structures during processing with the above methods. Instead, programmable DNA

Figure 1.24 2-D gold nanoparticle arrays induced by DNA template. a. Schematic illustration of DNA scaffold used to assemble 2-D gold nanoparticle arrays; b. AFM image of DNA scaffolding after hybridization with gold nanoparticles; c. TEM bright field image of DNA scaffolding after hybridization with gold nanoparticles. (Ref. 109)
chains are more effective in guiding well-defined nanoparticle assemblies with tunable morphologies and dimensionalities in buffered aqueous solution. Kiehl et. al. and Yan et. al. demonstrated that functionalized with single-stranded DNA chains, gold nanoparticles can be specifically assembled onto 2-D DNA scaffold through base-pairing interaction to show well-defined nanoarrays. Active sites to immobilize gold nanoparticles within DNA substrate are programmable, which can lead to tunable and well-defined structure and domain spacing of gold nanoparticle arrays (Figure 1.24). Biomaterials such as proteins have also been used to fabricate gold nanoarrays. Trent et. al. fabricated highly-ordered gold nanoparticle arrays by binding the particles onto crystalline protein template made from hollow double-ring structured chaperonins with either 3 nm or 9 nm apical pores surrounded with thiol groups. Through crystallization, chaperonins can be assembled into 2-D templates to selectively bind and direct gold nanoparticles into 2-D arrays, of which the order was defined by the lattice of the underlying protein crystal. Despite of the highly selectivity of DNA networks, two limitations currently have prevented DNA-based gold arrays from solid state device integration: one is limited large scale fabrication and the other is instability during processing and drying. Unconventionally, Luo et. al. reported a DNA-based pathway for monolayer free-standing gold nanoparticle self-assembly. The 2-D structure was fabricated through assembling single-stranded DNA functionalized gold nanoparticles in a microhole-confined, drying-mediated process. The microhole-confined self-assembly improved the control over both internal order and overall morphologies of nanoparticle arrays. DNA in this method was used as a dry ligand without the requirement of specific
base-pairing functionality. It has overcome the instability issues associated with aqueous environments in traditional DNA based routes. Through controlling DNA length, both structure and functionality of the 2-D gold nanoparticle arrays could be manipulated. In addition to the above DNA strategy, nanocomposites based on functionalized polymers shows high stability and can be fabricated over macroscopic distance. However, there is a thermodynamic and kinetic gap which separates the phase behavior of thin film and bulk materials. This gap limits the transfer of highly ordered arrays from the bulk to thin films. Some research groups have been trying to use polymer thin film as the template to directly cast gold nanoparticles onto selective domains. Early in 1998, Sita et. al. deposited alkanethiol passivated gold nanoparticles on microphase-separated thin film poly(styrene-block-methacrylate) (PS-b-PMMA). The nanoparticles were selectively adsorbed onto the polystyrene phase during incubation. Müller-Buschbaum et. al. later improved the method by introducing the hydrodynamic flow of the gold nanoparticle suspension to the incubation system. The nanoparticle flow upon the polymer template resulted in a higher selectivity of immobilized gold nanoparticles to certain domains. Different from attaching gold nanoparticles onto selective domains, Jaeger et. al. demonstrated that by casting a ultra-thin gold film onto microphase separated PS-b-PMMA thin film, gold atoms could be driven by surface tension to aggregate preferentially to the PS domains. This trend was accelerated by thermal annealing to show highly-ordered gold hierarchical structures (Figure 2.25). Achermann et. al. incorporated gold ions into the cylindrical domains of block copolymer thin film and formed 2-D gold nanoparticle arrays through in situ
reducing gold salts. Recently, Xu et al. have developed a supermolecular approach to control spatial organization of gold nanoparticles within polymer thin film. Interestingly, during the processing, they found that, with certain gold nanoparticle payload, gold nanoparticles can be excluded onto the air-thin film interface to form 2-D hexagonal close-packed arrays. However, the mechanism has not been determined yet.

Figure 1.25 2-D gold nanoparticle arrays induced by polymer thin film template. a. TEM bright field image of ultrathin PS-b-PMMA copolymer thin film; b. aggregation of gold metal formed after being vapor-deposited onto thin film surface prior to thermal-annealing; c. highly-ordered 2-D gold arrays formed after annealing at 180 °C for 1 min; d. repeated deposition and annealing created denser arrays; d and e. same strategy was applied to induce 2-D silver arrays. (Ref. 114)
It’s notable that many efforts are still required in order to fabricate high-ordered 2-D gold nanoparticle arrays. To achieve this goal, Becker et. al. has developed a facile and novel approach based on peptide-derivatized block copolymer bioconjugates to directly

Figure 1.26 2-D tunable gold nanoparticle arrays induced by A3 peptide-derivatized polymer thin film template. a. Peptide-derivatative block copolymer can be fabricated into thin films with parallel and perpendicular cylindrical domains; gold nanoparticles can be selectively immobilized onto the peptide-rich domains; b and c. AFM images of hexagonal packed gold nanoparticle clusters and parallel nanowires. (Ref [117])
assemble 2-D hierarchical gold nanoparticle arrays on surface of the polymer thin film.\textsuperscript{131} By tethering A3 peptide at the end of PMMA, specific binding affinity to gold nanoparticle was implemented into cylindrical PMMA domains. By making using of cold zone annealing (CZA)\textsuperscript{129,132,133} for well-defined peptide-polymer thin film template and carefully manipulating the gold nanoparticle coating process, hexagonal gold nanoparticle clusters and parallel gold nanowires have been successfully demonstrated (Figure 1.26).

1.8 Three Dimensional (3-D) Gold Nanoparticle Superlattice

Gold nanoparticles can be viewed as artificial atoms exhibiting unique optical properties. As reviewed in the above sections, numerical studies have been done to fabricate 1-D gold chains and 2-D gold arrays in order to manipulate the plasmonic properties as well as to reveal the fundamental science behind. While the physical behaviors of 1-D and 2-D gold nanoparticle arrangement have been widely explored, studies on collective properties of 3-D gold nanoparticle assemblies are limited. To develop our fundamental understanding of the 3-D materials for novel applications, fabrication approaches for 3-D highly-ordered gold nanoparticle superlattice is playing a central role. Nowadays, several pathways have been investigated for this purpose, including blending of nanoparticles, DNA and polymer-based platform.

Inspired by blending organic colloidal particles of two different sizes for 3-D structure self-assembly, the similar principle has been applied to the formation of 3-D superlattices with inorganic nanoparticles in bimodal size distribution.\textsuperscript{134} In 2002, Weller \textit{et. al.}
employed magnetic CoPt$_3$ nanocrystals of two different sizes (4.5 and 2.6 nm) to build a trilayer superlattice. The structure was characterized by the building block of the AB$_5$ repeating unit. Murray et. al. naturally expanded the scope to incorporate the use of two different types of inorganic materials with distinct properties (PbSe and Fe$_2$O$_3$) for 3D superlattices. By varying the ratio of sizes between PbSe and Fe$_2$O$_3$ nanoparticles, superlattices with repeating units of AB$_2$ and AB$_{13}$ were obtained through a drying process which allowed the solvent to evaporate slowly enough for particle rearrangement. It was found that 3-D structures of the inorganic colloidal crystals constructed with bimodal hard spheres were strongly dependent on size ratio and the number ratio between large particle A and small particle B. By incorporating a variety of inorganic nanoparticles, there has been a rapid progress for the development of 3-D inorganic materials to reveal new structures and properties. In this background, Korgel et. al. introduced gold nanoparticles into 3-D structures for the first time. The formation of AB type superlattice was based on combination of two different nanoparticles: gold (5 nm) and iron (13 nm). Superlattices achieved above through blending different nanoparticles usually possess close-packed structures due to the attractive van der Waals or hard-sphere interactions during particle arrangement. The all attractive nature of nanoparticle interactions can limit majority of the particle arrangement to a close-packed way. Grzyhowski et. al., instead, employed a different strategy through using charged gold and silver nanoparticles with similar sizes to form large-scale, sphalerite (diamond-like) crystals. In this method, silver (d = 4.8 nm) and gold (d = 5.1 nm) nanoparticles were functionalized with $\omega$-functionalized alkane thiols: HS(CH$_2$)$_{10}$COOH (MUA) and
HS(CH$_2$)$_{11}$NMe$_3^+$Cl$^-$ (TMA), respectively. The charged nanoparticle cores were surrounded by a layer of counter ions which constructed the screening layer to direct the short-range interactions between nanoparticles. With the specific nanoparticle sizes, when the thickness of the screening layer became commensurate with the dimensions of the assembled nanoparticles, the structure reached the lowest energy and gave rise to non-close-packed 3-D structures. Formation of the nanocrystals was facilitated by smaller, charges nanoparticles to stabilize larger nanoparticles. In this point of view, better-quality crystals could be obtained from more polydisperse nanoparticle solutions (Figure 1.27). Murray et. al. further applied the electrostatic interactions to binary nanoparticle superlattice systems in order to create new packing. In this system, opposite electrical charges on nanoparticles could induce a certain affinity of one type of nanoparticle to another to facilitate maximization of the nanoparticle packing density. Electrical charges on sterically stabilized nanoparticles contributed to introducing 15 more different binary superlattices into the existing system. Gold-Copper (AuCu) superlattice has been successfully achieved.

Properties of gold nanoparticle 3-D superlattices fabricated using the blending method are usually limited by notable structural defects. Besides, diversity of superlattices is limited due to the fact that the identities of the particles being assembled often determine their packing that can be fabricated. The design of DNA platform can overcome the above barriers and outline a strategy to independently adjust the relevant superlattice parameters. Earlier work was primarily focused on testing the base-pairing recognition of DNA functionalized gold nanoparticles to form networks. Even
though the gold nanoparticle clusters were randomly aggregated, it suggested the possibility that DNA-modified gold nanoparticles were able to serve as basic building blocks to be rationally assembled through programmable base-pairing interactions towards highly-ordered superlattices. Mirkin et. al. and Gang et. al. independently discovered that DNA could be used to control the crystallization of gold nanoparticles,

Figure 1.27 3-D superlattice fabrication with inorganic nanoparticles in bimodal size distribution. a. Schematic illustration of TMA-functionalized silver nanoparticle and MUA-functionalized gold nanoparticle used for nanoparticle crystallization; b. AB unit cell formed by gold and silver nanoparticles; c. SEM image of the nanoparticle crystals. (Ref.124)
therefore allowing 3-D gold nanoparticle superlattices with face-centered cubic (FCC) and body-centered cubic (BCC).\textsuperscript{140, 141} In the work done by Mirkin \textit{et. al.}, through carefully manipulating the processing temperature, the author demonstrated that crystalline formed by the nanoparticle-oligonucleotide conjugates could be varied with different DNA components of which a single component system gave rise to FCC structure while a binary component system showed BCC structure (Figure 1.28).\textsuperscript{141} In addition, Mirkin \textit{et. al.} explored the effect of DNA linker length on the unit cell lattice parameters and the overall crystalline.\textsuperscript{142} They found that longer DNA connections resulted in a decrease in the overall crystalline and the order of the lattice, which was due to stronger conformational flexibility. Recently, Mirkin \textit{et. al.} summarized six rules that could be used to guide the design different gold nanoparticle superlattices.\textsuperscript{138} Those rules comprehensively explained the effect of particle size, ratio, hydrodynamic radius of DNA-nanoparticle, and rate of hybridization on the final structure of superlattices, thereby outlining a strategy to adjust nanoparticle structures through independent parameters.

Formation of 3-D gold nanoparticle superlattice with base-paring DNA in buffer solution faces the challenge of large scale fabrication and stability during drying and processing. Block copolymers, on the other hand, being able to self-assemble into well-defined arrays of 3-D nanostructures over macroscopic distances, have provided another platform for engineering gold nanoparticle superlattices.\textsuperscript{139} Specific interactions between mesophase-forming copolymers and nanoscopic particles can lead to highly organized hybrid materials under certain conditions. Morphology of such composite is
determined by characteristics of copolymers and nanoparticles. Balazs et. al. has developed a theory to predict the ordered phases where particles and polymers assembled

Figure 1.28  DNA-based 3-D superlattice fabrication. a. Schematic illustration of the assembly of DNA-gold nanoparticle conjugates into different structures; single component system presented f.c.c. structure while binary component system presented b.c.c. structure; b. SAXS pattern of single component crystalline; c. Integrated profile of (b) shows a f. c. c. structure; d. SAXS pattern of binary component crystalline; e. Integrated profile of (d) shows a b.c.c. structure. (Ref. 128b)
By taking into account effect of the entropic penalty of polymer chain end on nanoparticles, the author stated that large nanoparticles (particle diameter = 0.3 × natural size of polymer) preferred to staying in the middle of one phase while the small nanoparticles (particle diameter = 0.2 × natural size of polymer) preferred to staying in the interface between two phases (Figure 1.29 a and b). Later, Thomas et. al. experimentally verified the prediction. In their contribution, with certain surface modification, two types of nanoparticles, gold (3.5 nm) and SiO₂ (21.5 nm), were observed to localize in the interface dividing surface of poly(styrene-b-ethylene propylene) (PS-b-PEP) and the center of PEP polymer domain, respectively (Figure 1.29 c). Even though the enthalpic effect due to the nanoparticle surface modification cannot be neglected during particle arrangement, agreement between the experiment and simulation suggested a primary effect of entropic contribution on the formation of nanostructures. Following the work, Xu et. al. incorporated stimuli-responsive mechanism into the nanoparticle-BCP system without interfering with the nanoparticle assemblies. By attaching small molecules that favorably interacted with the nanoparticle ligands to the polymer side chains in a non-covalent manner, e. g. hydrogen bonding, nanoparticle-polymer interactions could be tailored by external stimuli and resulted in a change of nanoparticle arrangement. Hawker et. al. synthesized amphiphilic gold nanoparticles consisting of a gold core, an inner hydroxylated polyisoprene layer and an outer polystyrene shell. By carefully controlling the enthalpic interaction between the multilayered gold nanoparticles and the lamellae-forming poly(styrene-b-2-vinylpyridine) (PS-b-P2VP) matrix, the nanoparticles could be selectively arranged in the PS or P2VP
domains or at the interface. Recently, Xu et al. has applied the BCP strategy to assemble gold nanorods, which opens the door to controlling organization of anisotropic nanomaterials.

Figure 1.29 3-D superlattice fabricated by blending nanoparticles with block copolymer. a and b. Concentration profile of diblock copolymer-particle blend: \( \psi_A \), density distribution of A block; \( \psi_P \), density distribution of particle; \( \rho_P \), particle center for large particles (a) and small particles (b); TEM bright field image of PS-b-PEP blending with gold and SiO\(_2\) nanoparticles; gold nanoparticles are located in the interface of two blocks while SiO\(_2\) nanoparticles are located in the center of PEP block. (Ref. 132-133)
CHAPTER II
EXPERIMENTAL METHODS

2.1. Materials

The following chemicals were used as received: gold (III) chloride hydrate (HAuCl$_4$, Aldrich, 99.999%), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Aldrich, ≥99.5%), sodium azide (NaN$_3$, Aldrich, ReagentPlus$^\textregistered$, >99.5%), epichlorohydrin (Acros, 99%), $N,N$-dimethylformamide (DMF, Aldrich 99.9%), $N,N,N',N''$-pentamethyldiethylenetriamine (PMDETA, Aldrich, 99%), triethylamine (TEA, Aldrich, 99%), 2-bromoisobutyryl bromide (Aldrich, 98%), ammonium chloride (NH$_4$Cl, Aldrich, >99.5%), sodium hydroxide (NaOH, Aldrich, ≥97.0%), PEO-OH ($M_n = 10.0$ kg mol$^{-1}$, $M_w = 10.6$ kg mol$^{-1}$, PDI = 1.06, Polymer Source), trisodium citrate dihydrate (Aldrich, >99%), ascorbic acid (Aldrich, >99%), 3-amino-1-propanol (Aldrich, 99%), 1-Dodecanethiol (Fluka, 97%), hexadecyltrimethylammonium bromide (CTAB, Aldrich, >99%), tricaprylylmethylammonium chloride (Fluka, Aliquat-336), 1,3-dicyclohexylcarbodiimide (DCC, Aldrich, 99%), 4-(N,N-dimethylamino)pyridine (DMAP, Aldrich, 99%), deuterated chloroform (CDCl$_3$ Aldrich, 99.8 atom% D), sodium borohydride (98+%, Acros), sodium hydroxide (NaOH, Aldrich, >97.0%), 1-dodecanethiol (97%, Fluka), acetone (99.5%, Sigma), tricaprylylmethylammonium chloride (Fluka, Aliquat-336), 5-hexynoic acid (Aldrich, >99%), Fmoc-Phe-Wang resin.
(100-200 mesh, Novabiochem), amino acid (Novabiochem), 1-Methyl-2-Pyrrolidinone (NMP, 99%, Acros), piperidine (99%, Alfa Aesar), N,N-Diisopropylethylamine (DIPEA, peptide synthesis, Fisher Scientific), O-(Benzotriazol-1-yl)-N,N,N’,N’-tetramethyluronium (HBTU, 99.5%, CHEM-IMPEX INT’L INC), trifluoroacetic acid (TFA, Aldrich, 99%), methyl 2-bromo-2-methylpropanoate (Aldrich, 98%), silicon wafer (El-Cat Inc.), quartz (GM Associates, Inc.), hexane (95%, Aldrich), N,N-dimethylformamide (DMF, Aldrich 99.9%), methanol (MeOH, Fisher Scientific, reagent grade), toluene (Aldrich, HPLC grad, 99.8%), anisole (Aldrich, anhydrous, 99.7%), diethyl ether (Aldrich, >99%).

Methyl methacrylate (MMA, Aldrich, 99%) and styrene (Aldrich, 99%) were purified by stirring over freshly-ground calcium hydride for 12 hours and redistilled under vacuum before use. Cuprous bromide (CuBr, Aldrich, 98%) was freshly purified by stirring in acetic acid overnight, washed with acetone, and dried in vacuum. Dichloromethane (CH₂Cl₂, Fisher Scientific, reagent grade), and styrene (Aldrich, 99%) were purified by stirring over freshly-ground calcium hydride for 12 h and redistilled under vacuum before use.

2.2 Synthesis

2.2.1 Polymer and Organic Molecule Synthesis

The Polyethylene oxide precursor was purchased from Polymer Source (PEO-OH, $M_n = 10.0$ kg/mol, $M_w = 10.6$ kg/mol, PDI = 1.06). PEO-$b$-PS diblock copolymer was synthesized according to a previous method which is described below step by step.¹⁴⁸
*Poly(ethylene oxide)-Epoxy.* PEO-OH (\(M_n = 10.0 \text{ kg mol}^{-1}\), 2.5 g, 0.25 mmol) was dissolved in 15 mL epichlorohydrin with a 25 mL round-bottom flask. After completely dissolving, NaOH (4.00 g, 100 mmol) was added. The solution was left to stir for 36 h at room temperature. After filtering out insoluble solid, the supernatant was precipitated into cold ethyl ether for 3 times. Precipitate was collected and dried in vacuum for 24 h. The product was collected as a white powder (2.1 g, 84% yield). 

\(^1\text{H NMR (CDCl}_3, 300\text{ MHz, ppm, } \delta): 3.64 \text{ (br, } 908\text{H, } \text{CH}_2\text{CH}_2\text{O-}), \ 3.16 \text{ (m, } 1\text{H, } \text{CH(O)CH}_2\text{-}), \ 2.79 \text{ (m, } 1\text{H, } \text{-CH(O)CH}_2\text{-}), \ 2.61 \text{ (m, } 1\text{H, } \text{-CH(O)CH}_2\text{-}), \ 1.20 \text{ (s, } 9\text{H, } \text{(CH}_3\text{)}_3\text{C-O-}).\)

*Poly(ethylene oxide)-(N\(_3\))-OH.* PEO-Epoxy (\(M_n = 10.0 \text{ kg mol}^{-1}\), 2.0 g, 0.20 mmol) was dissolved in 15 mL DMF. NaN\(_3\) (0.17 g, 2.5 mmol) and ammonium chloride (0.14 g, 2.5 mmol) was added. The mixture was left to stir at 50 °C over night, diluted with 200 mL of DCM and washed with water for 3 times to remove inorganic salts. The organic layer was dried and excess solvent was removed by rotary evaporation. Concentrated solution was precipitated in cold ethyl ether to give out product as a white powder after drying in vacuum (1.4 g, 70% yield). 

\(^1\text{H NMR (CDCl}_3, 300\text{ MHz, ppm, } \delta): 3.64 \text{ (br, } 908\text{H, } \text{-CH}_2\text{CH}_2\text{O-}), \ 1.20 \text{ (s, } 9\text{H, } \text{(CH}_3\text{)}_3\text{C-O-}).\)

*Poly(ethylene oxide)-(N\(_3\))-Br.* PEO-(N\(_3\))-OH (\(M_n = 10.0 \text{ kg mol}^{-1}\), 1.00 g, 0.10 mmol) was completely dissolved in 25 mL anhydrous CH\(_2\)Cl\(_2\). After adding TEA (0.19 g, 1.9 mmol), the mixture was cooled to 0 °C with an ice bath and 2-bromoisobutyryl bromide (0.43 g, 1.9 mmol) was added dropwise within 20 min. The mixture was left to stir at room temperature for 24 h, diluted with 200 mL CH\(_2\)Cl\(_2\) and washed with water for 3 times to remove salts. Organic layer was collected, concentrated and precipitated in
excess cold ethyl ether. The product was collected as a white powder (0.8 g, 80% yield).

$^1$H NMR (CDCl$_3$, 300 MHz, ppm, δ): 5.12 (m, 1H, -CH$_2$CH(-CH$_2$N$_3$)OCO-), 3.64 (br, 908H, -CH$_2$CH$_2$O-), 1.93 (s, 6H, -(C=O)C(CH$_3$)$_2$Br), 1.20 (s, 9H, (CH$_3$)$_3$C-O-).

*Poly(ethylene oxide)-(N$_3$)-Polystyrene.* PEO-(N$_3$)-Br ($M_n = 10.0$ kg/mol, 0.6 g, 0.06 mmol) and styrene (3 mL) was dissolved in 5 mL of anhydrous toluene following by addition of CuBr (7 mg, 0.06 mmol). The mixture was degassed by 3 cycles of freeze-thaw operation. Keeping the mixture frozen, PMDETA (11mg, 0.06 mmol) was added under nitrogen. After three extra freeze-thaw operations, the light green solution was left to polymerize in 45 °C for 72 h. The reaction solution was passed through a silica column to remove Cu. Product was obtained through precipitating purified reaction solution in cold ethyl ether and hexane mixture (v:v = 1:1, 300 mL). The homo- and block copolymers were characterized by size exclusion chromatography (SEC, polystyrene standards) and $^1$H nuclear magnetic resonance ($^1$H NMR). The block copolymer was measured to have a number average molecular weight ($M_n$ overall) of 56 kg mol$^{-1}$, and a weight-average molecular weight ($M_w$ overall) of 60 kg mol$^{-1}$. The polydispersity (PDI) was measured to be 1.07 according to SEC chromatography. The molecular weight of PS block was calculated to be 46 kg mol$^{-1}$. Volume fractions of PEO and PS block are 17.9% and 82.1%, respectively. $^1$H NMR (CDCl$_3$, 300 MHz, ppm, δ): 6.30-7.40 (br, 2210H, phenyl rings), 3.64 (br, 909H, -CH$_2$CH$_2$O-), 1.67-2.15 (br, 442H, -CH$_2$CH(-Ar)-), 1.20-1.67 (br, 884H, -CH$_2$CH(-Ar)-), 1.20 (s, 9H, 85 (CH$_3$)$_3$C-O-), 0.93 (m, 6H, -(O=C=O)C(CH$_3$)$_2$CH$_2$-).

*S-1-dodecyl-S’-(r,r’-dimethyl-r”-acetic acid) Trithiocarbonate (TC).* synthesis was
referring to a previous method described as below. 1-dodecanethiol (20.2 g, 0.10 mol), tricaprylylmethylammonium chloride (1.0 g, 2.5 mmol) and acetone (58.0 g, 1.0 mol) were mixed and cooled to 0 °C under nitrogen atmosphere. Aqueous sodium hydroxide (50 wt%, 8.4 g, 0.105 mol) was slowly added into the mixture within 10 min under vigorous stirring. After stirring for another 20 min, carbon disulfide (7.6 g, 0.10 mol) dissolved in 10.0 g acetone was slowly added into the mixture within 30 min. During the time, color of the reaction solution gradually turned red. 17.8 g chloroform was added first followed by 50 wt% aqueous sodium hydroxide (40 g) was slowly added within 20 min. The mixture was left to stir overnight. 200 mL water was added to dilute the reaction solution followed by 80 mL of concentrated HCl to acidify the solution. After removing the acetone, the solid was collected, dissolved and stirred in 300 mL 2-propanol. Insoluble residue was removed via filtration. The 2-propanol solution was concentrated and the resulting solid was recrystallized in hexane. Product was yellow crystalline solid (10 g, 27.4%). \(^1\)H NMR: (CDCl\(_3\), 300 MHz, ppm, δ) 1.0 (t, 3H), 1.30-1.5 (m, 18H), 1.6-1.8 (m, 8H), 3.2 (t, 2H), 11.0 (s, 1H).

Poly(ethylene oxide)-SH. S-1-dodecyl-S’-(r,r’-dimethyl-r’’-acetic acid) trithiocarbonate-capped poly(ethylene oxide) (PEO-TC) and thiol-capped poly(ethylene oxide) (PEO-SH) were prepared as follows. \(\text{PEO-TC:} \) PEO-OH (2.0 g, 0.2 mmol) was dissolved in 10 mL DMF and reacted with TC (88.8 mg, 0.24 mmol) in the presence of DCC (41 mg, 0.2 mmol) and DMAP (3 mg, 0.024 mmol). The solid residue was filtered off, and the solvent was evaporated. The product was isolated through silica column chromatography (CHCl\(_3\)-MeOH = 7:3); \(^1\)H NMR (CDCl\(_3\), 300 MHz, ppm, δ): 4.24 (m,
2H, -CH₂O(CO)-, 3.64 (br, 908H, -CH₂CH₂O-), 3.27 (m, 2H, -SCH₂CH₂), 1.69 (s, 6H, -O(CO)C(CH₃)₂S-), 1.65 (m, 2H, -SCH₂CH₂CH₂-), 1.2–1.4(m, 18H, -SCH₂CH₂(CH₂)₆CH₃), 1.20 (s, 9H, (CH₃)₃C-O-). PEO-SH: PEO-TC (1.0 g, 0.1 mmol) was hydrolyzed by 3-amino-1-propanol (50 µL) in 5 mL DMF under nitrogen in order to prevent the thiol group from being oxidized. And the final product was precipitated in ether; ¹H NMR (CDCl₃, 300 MHz, ppm, δ): 3.64 (br, 908H, -CH₂CH₂O-), 1.20 (s, 9H, (CH₃)₃C-O-).

Alkyne-A3 peptide. A3 peptide (AYSSGAPPMPFP) was synthesized on an automated solid phase peptide synthesizer (CEM Liberty 1 Microwave Peptide Synthesizer) using standard Fmoc chemistry. The preloaded Fmoc-Phe-Wang resin substitution was 0.65 meq/g and the reaction scale was 0.1 mmol. The peptide was derivatized at the N-terminus with 5-Hexynoic acid using DIC (154 uL, 1 mmol) and HOBT (153 mg, 10 mmol) in DMF (5 mL). The reaction was performed at room temperature for 3 h. Then the resin was thoroughly washed and the alkyne functionalized A3 peptide was cleaved from the resin using standard conditions. The alkyne-A3 peptide was triturated, precipitated in cold diethyl ether and collected via centrifugation..

Polystyrene-Br. Purified styrene (10 g, 0.94 mol), methyl 2-bromo-2-methylpropanoate (22 mg, 0.11 mmol), CuBr (20 mg, 0.14 mmol) and anhydrous toluene (15 mL) were added into a 250 mL round-bottom flask equipped with a magnetic stirrer. The mixture was degassed by three freeze-thaw cycles. PMDETA (24mg, 0.14 mmol) was introduced into the mixture under protection of nitrogen and the
mixed solution became light green. Following three additional freeze-thaw cycles, the flask was immersed into a 110 °C oil bath. After 33 h, the reaction was quenched by liquid nitrogen. The reaction solution was passed through the column filled with silica gel to remove the Cu. The polymer was precipitated in cold MeOH, polystyrene and collected by vacuum filtration. 

$^1$H NMR (CDCl$_3$, 300 MHz, ppm, δ): 6.30-7.40 (br, 1980H, phenyl rings), 3.50 (s, 3H, CH$_3$O-), 1.67-2.15 (br, 396H, -CH$_2$CH(-Ar)-), 1.20-1.67 (br, 792H, -CH$_2$CH(-Ar)-), 0.93 (s, 6H, -(CH$_3$)$_2$(CH=O)C(C=O) -).

**Polystyrene-b-Poly(methyl methacrylate)-Br.** Polystyrene-Br (Mn = 42.1 kDa, 500 mg, 0.012 mmol), methyl methacrylate (892 mg, 8.92 mmol), CuBr (2.1 mg, 0.015 mmol) and anhydrous anisole (10 mL) were added to a 100 mL reaction flask equipped with a magnetic stirrer. The solution was degassed by three freeze-thaw cycles. PMDETA (2.5 mg, 0.015 mmol) was added to the mixture under nitrogen. Following three additional freeze-thaw cycles, the flask was immersed into an oil bath at 80 °C for 10 h and then quenched with liquid nitrogen. The reaction solution was passed through a column filled with silica gel. PS-b-PMMA-Br was precipitated by dropwise addition into cold diethyl ether and collected by vacuum filtration. The block copolymer (480 mg) was obtained after drying in vacuum for 24 h. 

$^1$H NMR (CDCl$_3$, 300 MHz, ppm, δ): 6.30-7.40 (br, 1980H, phenyl rings), 3.35-3.60 (br, 522H, CH$_3$O-), 1.67-2.15 (br, 396H, -CH$_2$CH(-Ar)-), 1.20-1.67 (br, 792H, -CH$_2$CH(-Ar)-), 0.60-1.00 (br, 522H, -(CH$_3$)-C(C=O) -).

**Polystyrene-b-Poly(methyl methacrylate)-N$_3$.** PS-b-PMMA-Br ($M_n = 42.1$ kDa, $M_n$ = 17.4 kDa, 450 mg, 0.008 mmol), sodium azide (10 mg, 0.16 mmol) and anhydrous
DMF (10 mL) were added into a 50 mL round-bottom flask. The mixture was stirred at 60 °C for 48 h. The solution was diluted with 250 mL CHCl₃ and washed with water 4 times to remove excess sodium azide. After removing the majority of solvent, the block copolymer was precipitated into diethyl ether and collected by vacuum filtration. The PS-b-PMMA-N3 was obtained after drying under vacuum for 24 h and the yield was 86.6% (390 mg). ¹H NMR (CDCl₃, 300 MHz, ppm, δ): 6.30-7.40 (br, 1980H, phenyl rings), 3.35-3.60 (br, 522H, CH₃O-), 1.67-2.15 (br, 396H, -CH₂CH(-Ar)-), 1.20-1.67 (br, 792H, -CH₂CH(-Ar)-), 0.60-1.00 (br, 522H, -(CH₂)C(C=O)-). FT-IR (cm⁻¹): 2100 (-N₃).

Polystyrene-b-Poly(methyl methacrylate)-A3. PS-b-PMMA-N₃ (350.0 mg, 0.006 mmol), alkyne-A3 peptide (39.4 mg, 0.03 mmol), PMDETA (4.0 mg, 2.0 × 10⁻² mmol) and anhydrous DMF (5 mL) were added to a 100 mL reaction flask with a magnetic stirrer. After polymer and peptide were dissolved, the mixture was degassed by three free-pump-thaw cycles. CuBr (3.0 mg, 0.02 mmol) was added under nitrogen and following three additional freeze-thaw cycles, the flask was stirred at 60 °C for 72 h. The solution was diluted with 250 mL of CHCl₃ and washed with saturated NaCl solution 4 times to remove metal salts and excess alkyne-A3 peptide. Anhydrous MgSO₄ was added to remove water from the collected polymer-peptide solution before vacuum filtration. After removing the majority of the solvent, the bioconjugate was precipitated into diethyl ether and collected by vacuum filtration. The PS-b-PMMA-A3 was obtained after drying under vacuum for 24 h and the yield was 30.8% (120 mg). ¹H NMR (CDCl₃, 300 MHz, ppm, δ): 6.30-7.40 (br, 1980H, phenyl rings), 3.35-3.60 (br, 522H, CH₃O-), 1.67-2.15 (br, 396H, -CH₂CH(-Ar)-), 1.20-1.67 (br, 792H, -CH₂CH(-Ar)-), 0.60-1.00 (br, 522H, -(CH₂)C(C=O)-).
Signal of peptide protons was too low to be detected. So conjugation of peptide and PS-b-PMMA was characterized using FT-IR. Disappearance of azid (N₃) absorption (2100 cm⁻¹) suggested successful conjugation.

2.2.2 Gold Nanoparticle Synthesis and Surface Modification

Gold nanoparticles were synthesized according to previous methods as described below.¹⁵⁰,¹⁵¹

_Gold nanoparticle (d = 10 ± 1.8 nm)._ An aqueous solution of HAuCl₄ (1 mmol L⁻¹, 50 mL) was heated to boiling for 30 min. Then a trisodium citrate solution (5 mL, 38.8 mmol L⁻¹) was quickly added into the solution. Upon initial color changed to red, the solution was cooled naturally to room temperature and stored and protected from light. Gold nanoparticles sizes were measured by TEM and analyzed with the digital imaging software “Image Tool”. 100 gold nanoparticles were counted for particle size and size distribution statistics. The concentration of the gold nanoparticles was calculated based on the average size (d = 10 nm) and density (19.3 g·cm⁻³) of gold nanoparticles.

_Preparation of Gold Seed._ A 20 mL aqueous solution containing 2.5×10⁻⁴ mol L⁻¹ HAuCl₄ and 1×10⁻⁴ mol L⁻¹ trisodium citrate was prepared. The solution was added with ice-cold, freshly prepared 0.1 mol L⁻¹ NaBH₄ solution (0.6 mL) while stirring. The pink solution was used as seeds within 2-5 h after preparation.

_Preparation of Incubation Solution._ A 200 mL solution ([HAuCl₄] = 2.5×10⁻⁴ mol L⁻¹, [CTAB] = 0.08 mol L⁻¹) was heated to boiling to a clear orange color. The solution was cooled to room temperature once the color turned to clear orange to be used as
incubation solution.

**Gold Nanoparticle (d = 20 ± 3.4 nm).** Freshly prepared 0.1 mol L\(^{-1}\) ascorbic acid solution (0.1 mol L\(^{-1}\), 0.05 mL) was added to 9 mL incubation solution. 1 mL seed solution was added to the mixture and stirred for 10 min. It was labeled as solution A. After 30 min, 1 mL solution A was added to a 9 mL incubation solution mixed with ascorbic acid solution (0.1 mol L\(^{-1}\), 0.05 mL) and stirred for 10 min. It was labeled as solution B. Particles prepared in this way were roughly spherical with a diameter of 20 ± 3.4 nm. Gold nanoparticles sizes were measured TEM and analyzed with the digital imaging software “Image Tool”. 100 gold nanoparticles were counted for particle size and size distribution statistics. The product was purified, stored and protected from light.

**Gold Nanoparticle (d = 48 ± 6.9 nm).** 0.05 mL freshly prepared 0.1 mol L\(^{-1}\) ascorbic acid solution was added to 9 mL incubation solution. 1 mL solution B was added to the mixture and stirred for 10 min. Particles prepared in this way were roughly spherical with a diameter of 48 ± 6.9 nm. Gold nanoparticles sizes were measured with the digital imaging software “Image Tool”. 100 gold nanoparticles were counted for particle size and size distribution statistics. The final product was purified, stored and protected from light.

**Gold Nanoparticle Surface Functionalization.** PEO-SH was dissolved in water and at a concentration of 5 mg mL\(^{-1}\). It was then added dropwise to gold nanoparticle (d = 10 ± 1.8 nm) solution based on a thiol/gold molar ratio of 1:200 under vigorous stirring. The mixture was stirred and incubated at room temperature for 3 days to reach
equilibrium. The reaction solution was purified 4 times via centrifugation at 13500 g for 20 min. Zeta potentials were measured at pH 5.5 in HAc/NaAc buffer before (-34.9 ± 4.3 mV) and after the reaction (-10.2 ± 2.4 mV) to confirm the successful ligand derivatization. For gold nanoparticles (d = 20 ± 3.4 nm and 48 ± 7.9 nm), PEO-SH was dissolved in water at a concentration of 5 mg mL⁻¹ and added dropwise to the gold nanoparticle solution at a thiol/gold molar ratio of 1:200. The mixture was kept in an ultrasonic bath for 30 min at 60 °C first before stirring. Then it was allowed to vigorously stir for 3 days for equilibrium. The reaction solution was purified 4 times by centrifugation at 13500 g for 20 min. Zeta potential of gold nanoparticles before and after surface functionalization was measured at pH 7.0 in 1×PBS buffer. Zeta potential of gold nanoparticle (d = 20 ± 3.4 nm) decreased from 33.7 ± 3.6 mV to 6.4 ± 3.7 mV after functionalization with PEO ligands. Similar change was observed for gold nanoparticle (d = 48 ± 7.9 nm), of which the zeta potential decreased from 34.7 ± 2.2 mV to 5.1 ± 4.6 mV after PEO functionalization. pH is essential for zeta potential measurement. In this experiment, two types of nanoparticle precursors were utilized: sodium citrate gold nanoparticle and CTAB gold nanoparticles, which were stabilized in 0.1 mol L⁻¹ HAc/NaAc buffer (pH 5.5) and 1×PBS buffer (pH 7.0), respectively. Zeta potentials of citrate-stabilized gold nanoparticles and the corresponding PEO-functionalized nanoparticles were measured at pH 5.5 in HAc/NaAc buffer while CTAB-stabilized gold nanoparticle and the PEO-correspondents were measured in 1×PBS buffer. To measure zeta potential of the gold nanoparticles, 750 µL of sample was transferred from a 1 mL disposable syringe to a zeta cell with air bubbles dislodged.
Sample concentration was 1.0 µg mL\(^{-1}\) in buffer solution. Measurement was conducted at 25 °C and an applied voltage of 150 V. An equilibration time of 2 min was employed prior to starting measurement. 3 individual measurements were done for each sample. A viscosity of 0.891 centiPoise (cP), a dielectric constant of 78.6 and Henry function of 1.5 were used for calculation.

2. 3 Fabrication

_Micelle Formation._ PEO-b-PS (10 mg) was dissolved in 10 mL pre-filtered anhydrous DMF to obtain a 0.1 wt% stock solution. It was stirred at room temperature for 24 h to ensure polymer completely dissolved. Ultrapure water (18 MΩ cm\(^{-1}\)) was added dropwise into the solution. Each drop added was ~0.1 wt % water of the total solution weight, and at least a 15 min interval was maintained between two consecutive drops. The final water content was 4.76%. Then solution was sealed and left to stir overnight before dialysis.

_Thin Film Preparation._ Amorphous quartz coverslips were rinsed in toluene followed by 1 h of ultraviolet-ozone (UVO) treatment, which completely removed surface contamination. Block copolymer films were spin-coated (4500 rpm, 30 sec) from toluene solution (2.0 wt%) onto cleaned substrates. Prior to the annealing process, residual solvent was extracted by drying samples at 60 °C for 24 h under vacuum. Oven annealed samples were kept in a vacuum oven at 210 °C for 48 h to achieve a morphology that did not evolve further with time. Cold Zone Annealing - Sharp (CZA-S) and Cold Zone Annealing – SoftShear (CZA-SS) were performed under a temperature gradient (\(\nabla T\))
of 45 °C/mm at varying annealing velocities from 5 μm/s to 100 μm/s. The maximum temperature (T\text{MAX}) of the temperature gradient was set at 210 °C. Although 210 °C is above the peptide degradation temperature, the short exposure time due to continuous annealing process does not harm the peptide groups.

2.4 Equipment

Size Exclusion Chromatography (SEC). SEC analysis was performed with a Waters 150-C Plus instrument equipped with three HR-Styragel columns (100 Å mixed bed 50/500/10³/10⁴ Å, mixed bed 10³/10⁴/10⁶ Å) and a double detector system (differential refractometer, Waters 410; laser light scattering detector, Wyatt Technology, DAWN EOS, \(\lambda = 670\) nm) with THF as solvent (flow rate = 1.0 mL min⁻¹, \(T = 30°\) C). Regular SEC calibrations were conducted with PS standards (Polymer Laboratories).

\(^1H\) Nuclear Magnetic Resonance (\(^1H\) NMR). \(^1H\) NMR spectra was conducted in CDCl₃ with a Varian Mercury 300 spectrometer. The spectra were referenced to the residual proton impurities in CDCl₃ at 7.25 ppm. Typical acquisition parameters were a relaxation delay of 1 s, an observed pulse of 7.1 µs corresponding to a 90° flip angle and a scan number of 128. Data was analyzed using Varian software VNMRJ VERSION 2.2 REVISION C.

Fourier Transform Infrared (FTIR). Polymer was dissolved in THF and the solution was evenly casted onto KBr disk to form thin film. FTIR spectra were measured with a Digilab Excalibur FTS 3000 Series FTIR spectrometer (DIGILAB, Randolph, MA) through the polymer films on KBr disks. Data analysis was done by Win-IR Pro
Software.

**UV-Visible Spectroscopy (UV-Vis):** UV-Vis spectra for gold-micelle hybrid nanoparticles and gold nanoparticle thin films were recorded on a Hewlett Packard 8453 UV-Vis Spectrophotometer System. Gold-micelle solution was put into cells with external dimension of 45 x 12.5 x 12.5 (H x W x D [mm]) and internal dimension of 44.5 x 9.5 (H x W [mm]). Height of the testing solution was between 1/2 to 2/3 of the internal height of the cell. Spectrum was performed in the range of 400-700 nm with each data point recorded for every 2 nm. Gold nanoparticle thin film loaded on transparent quartz substrate was fixed in front of the pinhole and the signal was accumulated for 30 sec for each measurement. Background was subtracted by performing the same measurement with a quartz substrate coated with polymer thin film. Spectrum was performed with the range of 400-700 nm with each data point recorded for every 2 nm. Data was analyzed through UV-Visible ChemStation Software.

**UV-Visible Spectroscopy (UV-Vis):** UV-Vis spectrum for gold dimers were recorded on a SYNERGY Mx Mult-Mold Microplate Reader (BioTek® Instrument, Inc) using a 96 well plate. 200 µL gold nanoparticle solution was tested for each sample. Spectrum was performed from 400-700 nm with each data point recorded for every 1 nm. Reader control is via BioTeck’s Gen5™ Data Analysis Software.

**Dynamic Light Scattering (DLS):** Laser Light scattering experiments were conducted using a Brookhaven Instrument coupled to a BI-200SM goniometer, a BI-9000AT correlator, and an EMI-9863 photomultiplier tube for photon counting. A Meller Griot 35 mW He-Ne laser was used as the light source (632.8 nm). A cylindrical glass
scattering cell with a diameter of 12 mm was placed at the center of a thermostat bath (0.01 °C) with decahydronaphthalene used for refractive index matching. The solutions were filtered into the scattering cells through syringe filters of 0.45 or 1.0 μm pore size. Both the scattering intensity and hydrodynamic diameter were measured at a scattering angle of 90° and a temperature of 25 °C. The measurement was repeated for 3 times and each time the collection time is 30 min. The average number of counts was 157.2 kcp. For data analysis, exponential decay is essential, which is related to the motion of particles, specifically to the diffusion coefficient. When the sample is monodisperse, decay is simply a single exponential. The general relationship for the photoelectron count time correlation function is

\[ g^{(2)}(\tau) = 1 + \beta |g^{(1)}(\tau)|^2 \]

where

\( g^{(2)}(\tau) \): normalized second-order correlation function; \( \beta \): optical constant of the instrument;

\( g^{(1)}(\tau) \): normalized first-order correlation function.

For spherical particles,

\[ g^{(1)}(\tau) = \exp(-\Gamma \tau) \]

\[ \Gamma = D_T q^2 \]

\[ q = 4\pi n_0 \sin(\theta/2) / \lambda_0 \]

Where

\( \theta \): scattering angle;

\( \lambda_0 \): wavelength of the laser;
\( n_0 \): refractive index of the solvent;

\( q \): magnitude of scattering vector.

When the sample is in a low concentration region, concentration dependence of the translational diffusion coefficient \( D_T \) can be expressed as:

\[
D_T = D_0 (1 + k_d c)
\]

where

\( D_0 \): translational diffusion coefficient at infinite dilution;

\( k_d \): diffusion second virial coefficient;

\( c \): concentration.

The hydrodynamic diameter is given by the Stokes-Einstein equation:

\[
d_h = k_B T / (3\pi\eta D_0)
\]

Where

\( k_B \): Boltzmann constant,

\( T \): absolute temperature,

\( \eta \): solvent viscosity.

For particles with a size distribution, in the cumulant method, autocorrelation function can be analyzed using an approximate equation:

\[
g^{(1)}(\tau) = \exp[-\Gamma\tau + (\mu_2/2)\tau^2 - (\mu_3/3!\tau^3 + ...]
\]

where

\( \Gamma \): average decay rate,

\( \mu_2 / \Gamma^2 \): second order polydispersity index.

For PS-b-PEO spherical micelles, data analysis was through Cumulant method. The
intensity autocorrelation functions were single-exponential decays with baselines that were unity within the precision of the measurements. DLS diffusion coefficients were evaluated by fitting the normalized correlation function.

*Transmission Electron Microscopy (TEM):* Bright field images of TEM were recorded in a JEOL-1230 microscope with an accelerating voltage of 120 kV. 3 μL of the testing solutions were deposited onto copper grids. After ca. 3 min, the excess solution was wicked away by a piece of filter paper. The sample was then allowed to dry under ambient conditions. TEM images were recorded on a digital CCD camera and processed with the accessory digital imaging system. Micelle and gold nanoparticles sizes were measured with the digital imaging software “Image Tool”. Numbers of gold nanoparticles on each micelle were counted directly through TEM images. And for each condition, more than 500 nanoparticles were counted for particle counting statistics based one more than 30 TEM microphotography. Blank micelles and micelle-gold aggregates were not taken into account. For gold dimer characterization, the concentration of the gold nanoparticles prepared was calculated based on the total amount of HAuCl₄, average size (d = 10 nm, 20 nm and 48 nm by TEM) and density (19.3 g·cm⁻³) of gold nanoparticles. Concentrating gold nanoparticles solution was performed by centrifugation at 21000 g for 20 min to assure discarded supernatant was clear.

*Electrospray ionization mass spectrometry (ESI-MS):* All experiments were preformed with a Bruker HCT Ultra II quadrupole ion-trap (QIT) mass spectrometer (Bruker Daltonics, Billerica, MA). Samples were prepared by adding methanol (20% v/v) to water. Sample solutions were introduced by direct infusion into the instrument at
a rate of 360 μL hr⁻¹ using a syringe pump. A potential of +4.0 kV (negative ion mode) was applied to the entrance of the sampling capillary, which is orthogonal to the grounded spraying needle. Nitrogen was used as the nebulizing gas (10 psi), which flows coaxially with the spray and aids in the formation of charged droplets, and as the drying gas (8 L min⁻¹, 300 °C), which heats the sampling capillary to help in the droplet desolvation process. Ions were allowed to accumulate up to a maximum time of 200 ms before mass-selective ejection through ramping up of the RF potential of the ring electrode for m/z measurement. All quoted m/z values are monoisotopic. Data was analyzed using Compass Data Analysis Version 4.0.

Zeta (ζ) Potential: ζ-potential measurements were performed on a Nano Zetasizer90 instrument with a 633 nm He-Ne laser from Malvern Instrument UK, Inc. The instrument calculates the ζ-potential by determining the electrophoretic mobility. Samples were incubated at 25 °C for 2 min and an acquisition of 20 counts was taken for each measurement. The average value of 3 independent measurements was taken for each solution. Data was automatically analyzed by the Nano Zetasizer90 software through equations as below:

\[
UE = \frac{2ez \cdot f(ka)}{3h}
\]

where

\(UE\) : electrophoretic mobility
\(e\) : dielectric constant;
\(z\) : zeta potential;
\(h\) : viscosity;
For gold nanoparticles, a viscosity of 0.891 centiPoise (cP), a dielectric constant of 78.6 and Henry function of 1.5 were used for calculation.

Surface Enhanced Raman Scattering (SERS): Raman spectra were acquired with a Horiba Jobin Yvon Labram HR-800 Raman spectrometer with a wavelength of 514.5 nm. The fresh prepared gold dimer solution was spun-casting onto silicon wafer before testing. An acquisition time of 30 s was used for each spectrum. Calculation of SERS enhancement was given by the following equations:

The contrast for the particles is given by:

\[
\text{Contrast} = \frac{I_c - I_w}{I_w} = \frac{I_c}{I_w} - 1
\]

where

- \(I_c\): the integrated intensity of the Raman peak (peak area) from silicon with the particles;
- \(I_w\): the integrated intensity from silicon without particles.

To estimate the enhancement factor for the signal, the ratio of the volumes contributing to the signal when particles are present, \(V_{SERS}\), and to the signal without particles, \(V_{Raman}\), must be taken into account.

\[
\text{Enhancement Factor} = \text{Contrast} \left(\frac{V_{Raman}}{V_{SERS}}\right)
\]

\[
\frac{V_{Raman}}{V_{SERS}} = \frac{\pi (r1)^2 (d1)}{\pi (r2)^2 (d2)}
\]

where

- \(r1\): laser spot size.
- \(d1\): penetration depth (0.68 tr) of 514.5 nm laser light into silicon wafer.
- \(r2\): average radius of dimer.
d2: penetration depth (20 nm) of 514.5 nm laser light when particles are present.

**Cold Zone Annealing (CZA):** The basic structural design of our modified zone annealing apparatus is based on the design concept described previously by Lovinger et al. Low molecular weight PDMS oil (“Thermal C10” Julabo USA Inc.) is circulated at -5 °C using a chiller system (Julabo F12-ED Refrigerated/Heating Circulator) to cool the cold blocks. The hot zone is generated by a low resistance (0.025 Ω cm⁻¹) nickel-chrome wire covered with a 3 mm (outer diameter) ceramic insulation that is powered with a high current source (Digital DC Power Supply, Model 1692, B&K Precision Corp.). The cold blocks are separated from the nickel – chromium wire at a distance of 1 mm. The height of the wire is fine tuned so as to achieve desired $\nabla T_{\text{max}}$ of 45 °C/mm. The temperature gradients thus produced are characterized using a thermal IR imaging camera having an accuracy of 0.1 °C (Testo 875 Thermal Imager Kit). AFM was used for topological analysis of thin films after CZA.

**Quartz Crystal Microbalance (QCM):** QCM measurement was performed on QE401-F1343 (Biolin Scientific / Q-Sense, Västra Frölunda, SWEDEN) with the sensors of QSX303 SiO₂ (Biolin Scientific / Q-Sense, Västra Frölunda, SWEDEN) spin-coated with polymer thin films from 2 w% PS-b-PMMA and PS-b-PMMA-A3 toluene solutions ($d_{\text{thickness}}$ (PS-b-MMA-A3) = 150 nm, $d_{\text{thickness}}$ (PS-b-MMA) = 150 nm). Gold nanoparticle water suspension (~30 nmol L⁻¹, pH = 5.5) was used as the incubation solution. Q-Sense software was utilized for data analysis.

**Atomic Force Microscopy (AFM):** AFM images were recorded with Dimension V (Veeco Instrument Inc., Santa Barbara, CA). The controller is Nanoscope V (Veeco
Instrument Inc., Santa Barbara, CA). TM 200 Tapping Mode Probes (Tip Radius < 15 nm, f: 145 – 230 kHz, k: 25-95 N/m, SensaProbes, Santa Clara CA) were used under tapping mode. AFM image was analyzed using Veeco’s NanoScope® software.

Grazing Incidence Small-Angle X-ray Scattering (GISAXS): measurements were performed at the D1 beamline of the Cornell High Energy Synchrotron Source (CHESS) and at the X9 beamline of the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory. At CHESS D1, 9.8 keV X-ray photons with a 1.5% bandwidth from a multilayer monochromator impinged on thin film samples coated on silicon wafers at an angle of 0.12°, at the first waveguide resonance of the film. GISAXS images were collected with a CCD camera (MedOptics) at 1493 mm from the sample. The CCD was protected from the intense scattering in the incident plane with a rod-like beamstop. Sample alignment and measurement of the reflectivity curve close to the film critical angle was performed with an ion chamber. Background of subtracted by the dark scan, with the shutter closed to remove any noise introduced by feign light and air. During the experiment, 10 GISAXS measurements were taken for each sample. Data conversion to q-space was accomplished using silver behenate powder as a standard. The intensity of the samples (I) were plotted with respect to q, where $q = \frac{4\pi}{\lambda} \sin(\theta/2)$, $\lambda$ is the wavelength of the incident X-ray beam and $\theta$ is the scattering angle. Spacing between adjacent domains was calculated through $d = \frac{2\pi}{q}$. GISAXS data analysis was done by IsGISAXS Version 2.6 data analysis software.
CHAPTER III
HIGH-FIDELITY FABRICATION OF GOLD-POLYMER JANUS NANOPARTICLES USING A SOLUTION

3.1. Motivation

The reason that inorganic-organic hybrid Janus nanoparticles have attracted intensive attention is mainly due to their great potential for biomedical applications. On one hand, inorganic nanoparticles, e.g. Au, Co, Fe$_3$O$_4$, are sensitive to optical or magnetic stimulus and can be used as probing agent.\textsuperscript{113, 152, 153} On the other hand, organic particles, e.g. micelles, liposome, can perform as nanocarriers.\textsuperscript{154} Colloidal micelles, formed through self-assembly of amphiphilic block copolymers, possess various morphologies which can be tuned by varying block copolymer composition, molecular weight, solvent properties, block copolymer concentration, etc.\textsuperscript{151, 155-157} Compared to assemblies from small molecular surfactants, micelles generally show higher stability, which is important for medical and biological applications. Recently, inorganic nanoparticles, especially gold nanoparticles, have been rendered as superb candidate of bioimaging agent for drug delivery and gene therapy due to their outstanding optical performance as well as non-toxicity.\textsuperscript{158} So the construction of gold-micelle Janus nanoparticles leads to a promising pathway for a basic drug-delivery system. However, because of the symmetric shape and isometric composition of spherical gold and micelle colloidal
particles, it’s difficult to control the stoichiometry to precisely link one gold nanoparticle to one micelle sphere. To provide an effective solution, we developed a facile and novel method to synthesize well-defined populations of asymmetric, hybrid gold-micelle Janus nanoparticles possessing gold nanoparticles growing from within the corona of micelles.

3.2. Results and Discussion

Figure 3.1  Schematic illustration of the process to generate gold–micelle asymmetric Janus nanoparticles with micelle template possessing PEO corona and PS core. PEO-b-PS was synthesized and fabricated into micelles. HAuCl₄ was reduced into gold nanoparticles within the PEO corona in the presence of reducing agent (HEPES). Number of gold nanoparticles per micelle was manipulated through adjusting the concentrations of micelles and HEPES.
Figure 3.1 summarizes the procedures we utilized to obtain gold–micelle Janus nanoparticles. In our method, the amphiphilic block copolymer PEO-b-PS was synthesized to form micelles. The PEO corona can locally accumulate and sequesters AuCl₄⁻ ions. The formation of inorganic–organic hybrid nanoparticles occurs via in situ reduction of HAuCl₄ into gold nanoparticles within the PEO corona. Through careful adjustment of the reducing agent as well as micelle template concentrations, we are able to obtain gold–micelle Janus nanoparticles as a nearly monodisperse product.

Figure 3.2 Synthetic route for PEO-b-PS. PEO-OH precursor was first conjugated with epichlorohydrin to form PEO-Epoxy, which further went through ring-opening reaction to give hydroxyl group to be functionalized with 2-bromoisobutyryl bromide. PEO-(N₃)-Br was used as the macromolecular initiator for PS chain growth to form PEO-b-PS.
The PEO-b-PS polymer was synthesized according to an established procedure, as described in Figure 3.2. The molecular characterization of the PEO precursor and block copolymer were determined by size exclusion chromatography (SEC) in Figure 3.3 and the data are summarized in Table 3.1. ¹H NMR spectrum (Figure 3.4) was used to confirm the molecular weight of PS block with the following equation:

\[ M_n^{PS} = M_n^{PEO} \times \left( \frac{S_B}{S_A} \right) \times \left( \frac{104 \times 4}{44 \times 5} \right) \]

where

S_A: Integration of the peak at 3.64 ppm (-OCH₂CH₂-);

S_B: Integration of broad peaks between 7.40 and 6.30 ppm (protons on phenyl rings of PS block)

Figure 3.3  SEC spectrum of PEO precursor (black) and PEO₂₂₇₋b-PS₄₄₂ (blue). Two peaks are clearly separated from each other indicating complete initiation.
Total molecular weight of PEO-b-PS block copolymer was calculated to be 56 000 Da, with volume fraction of PEO ~16.9% and PS ~83.1%.

Figure 3.4 $^1$H-NMR of PEO precursor and PEO$_{227}$-b-PS$_{442}$. The in-chain functional group N$_3$ can be used for extend the functionality. (a) PEO-Epoxy: (CDCl$_3$, 300 MHz, ppm, δ): 3.64 (br, 908H, -CH$_2$CH$_2$O-), 3.16 (m, 1H, -CH(O)CH$_2$), 2.79 (m, 2H, -CH(O)CH$_2$), 2.61 (m, 1H, -CH(O)CH$_2$), 1.20 (s, 9H, (CH$_3$)$_3$C-O-). (b) PEO-(N$_3$)-OH: $^1$H NMR (CDCl$_3$, 300 MHz, ppm, δ): 3.64 (br, 908H, -CH$_2$CH$_2$O-), 1.20 (s, 9H, (CH$_3$)$_3$C-O-). (c) PEO-(N$_3$)-Br: $^1$H NMR (CDCl$_3$, 300 MHz, ppm, δ): 5.12 (m, 1H, -CH$_2$CH(CH$_2$N$_3$)OCO-), 3.64 (br, 909H, -CH$_2$CH$_2$O-), 1.93 (s, 6H, (-COC(CH$_3$)$_2$Br), 1.20 (s, 9H, (CH$_3$)$_3$C-O-). (d) $^1$H NMR (CDCl$_3$, 300 MHz, ppm, δ): 6.30-7.40 (br, 2211H, -CH$_2$CH(-Ar)-), 3.64 (br, 908H, -CH$_2$CH$_2$O-), 1.67-2.15 (br, 442H, -CH$_2$CH(-Ar)-), 1.20-1.67 (br, 884H, -CH$_2$CH(-Ar)-), 1.20 (s, 9H, (CH$_3$)$_3$C-O-), 0.93 (m, 6H, -O-CO-C((CH$_3$)$_2$)CH$_2$-).
Table 3.1 Molecular characterization of block copolymer

<table>
<thead>
<tr>
<th>Polymer</th>
<th>(M_n/\text{Da})</th>
<th>(M_w/\text{Da})</th>
<th>(M_w/M_n)</th>
<th>(N_{\text{PEO}})</th>
<th>(N_{\text{PS}})</th>
<th>(\phi_{\text{PEO}})</th>
<th>(\phi_{\text{PS}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEO</td>
<td>10 000</td>
<td>10 600</td>
<td>1.06</td>
<td>227</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PEO-b-PS</td>
<td>56 000</td>
<td>60 000</td>
<td>1.07</td>
<td>227</td>
<td>442</td>
<td>16.9%</td>
<td>83.1%</td>
</tr>
</tbody>
</table>

\(a\) Molecular weight and molecular weight distribution were measured by SEC.

\(b\) Degree of polymerization, \(N\), was calculated based on the equation:

\[
N = \frac{\text{total } M_w \text{ of polymer}}{M_w \text{ of monomer unit}} = \frac{M_w}{M_0}.
\]

\(c\) Volume fraction was calculated based on the equation:

\[
\Phi = \frac{\text{volume of polymer block}}{\text{total volume of copolymer}} = \frac{V(A)}{V(\text{total})}
\]

PS-b-PEO is an amphiphilic copolymer, which self-assembles into monodisperse core–shell micelles in aqueous solution under the appropriate conditions. Details of the thermodynamics have been discussed and reviewed previously.\(^{155-157}\) To prepare spherical micelles, PEO\(_{227}\)-b-PS\(_{442}\) was first dissolved in pre-filtered anhydrous DMF via stirring at room temperature for at least 24 h to obtain a 0.1 wt% stock solution. Water was chosen as the selective solvent for the PEO block. Ultrapure water (18 MU cm\(^{-1}\)) was added drop wise to 10 mL of a stock solution (0.1 wt%) to reach a predetermined water and DMF ratio (1 : 20). Water was added slowly (rate \(~40 \mu\text{L h}^{-1}\)) in order for the solution to reach the thermodynamic equilibrium. After the solution reached equilibrium, it was quickly quenched in a large amount of water and dialyzed for 2 days in a 500–1000 Da molecular weight cutoff (MWCO) tubing (Spectrum Laboratories, Inc., Rancho Domingo, CA) before lyophilization. Figure 3.5a shows a representative bright
field TEM image of spherical micelles of PEO$_{227}$-b-PS$_{442}$ from DMF/water solution with a water concentration of $\sim$4.76%. The average diameter of the spherical micelles was measured to be $\sim$30 nm with a relatively narrow size distribution. Figure 3.5 b shows

Figure 3.5 Characterization of micelle size and size distribution. a. Bright field TEM image of spherical PEO-b-PS micelles showing an average size $\sim$30 nm; b. hydrodynamic radius, $D_h$, measured by dynamic light scattering (intensity average) shows a nearly monodisperse micelle population with the average size measuring 40 nm; inset is the correlation function.
the hydrodynamic diameter, $D_h$, and distribution of the micelles was measured utilizing dynamic light scattering (DLS). It indicates that the average diameter is about 40 nm, which is larger than the TEM results due to the collapse of corona after drying and the scattering from the highly hydrated shell chains in solution.

Figure 3.6  ESI-MS spectrum of the master solution (0.7 mmol L$^{-1}$ HAuCl$_4$ in water).

All gold-containing ions are labeled.
According to our strategy, micelle coronas are functioning as template, in which the gold salts can be accumulating preferentially due to the dative bonding effect of the PEO chains. The locally concentrated PEO chains are able to form pseudo-crown ether cavities, which can bind gold ions and further aid in the formation of gold nanoparticles.

![ESI-MS spectrum](image)

Figure 3.7  ESI-MS spectrum after the addition of micelles to the master solution. All gold-containing ions are labeled.
in the presence of reducing agent. This effect has been reported previously that PEO with molecular weights larger than 1.45 kDa could accumulate gold salts within the polymer chains similar to a crown ether solubilization effect observed for other cations.\textsuperscript{17, 159, 160} With certain reducing agent, the complexes can be reduced to gold nanoparticles in water under mild conditions. However, there’s no experimental evidence directly demonstrating the gold salts-accumulating effect. Here, using electrospray ionization mass spectrometry (ESI-MS), we have confirmed the preferential accumulation of gold

![ESI-MS spectrum of the supernatant after centrifuging to remove micelles. All gold-containing ions are labeled.](image)

Figure 3.8  ESI-MS spectrum of the supernatant after centrifuging to remove micelles. All gold-containing ions are labeled.
salts in the micelle solution through measuring intensity change of gold ions under different conditions. We first prepared the master aqueous solution with gold ion concentration of 0.735 mmol L\(^{-1}\). A separate aliquot was made in which micelles ([polymer] = 1.0 \times 10^{-6} \text{ and } [HAuCl}_4 = 0.735 \text{ mmol L}^{-1}\) were added. The third solution

![ESI-MS spectrum](image)

Figure 3.9 ESI-MS spectrum of the supernatant after reducing HAuCl\(_4\) to gold nanoparticles and centrifuging to remove gold nanoparticles and micelles. All gold-containing ions are labeled.
was made with the same polymer and HAuCl₄ concentration but in 0.3 mol L⁻¹ HEPES buffer. After an equilibrium time period, each of the above two solutions was split into two aliquots, one of which was centrifuged to keep only the supernatant. Each of the solutions was then assessed using ESI-MS to measure the intensity of free gold ions which represented the gold concentration in each solution. The intensity of free gold ions was reduced drastically from 352 523 (arbitrary units) in the master solution to 58 984 in solution with micelles to 4930 in the reduced supernatant in a step-wise fashion. The individual spectrum are presented in Figure 3.6 – 3.9 and summarized in Table 3.2.

Table 3.2  ESI-MS characterization of free gold intensity in different solutions

<table>
<thead>
<tr>
<th>Solution Intensity</th>
<th>HAuCl₄(master)</th>
<th>HAuCl₄+Micelle</th>
<th>Supernatant</th>
<th>Reduced to Au</th>
</tr>
</thead>
<tbody>
<tr>
<td>m/z 266.9</td>
<td>352523</td>
<td>110601</td>
<td>58984</td>
<td>4930</td>
</tr>
<tr>
<td>m/z 338.8</td>
<td>25440</td>
<td>2454</td>
<td>1651</td>
<td>1131</td>
</tr>
<tr>
<td>m/z 480.9</td>
<td>115411</td>
<td>83794</td>
<td>38553</td>
<td>1253</td>
</tr>
<tr>
<td>m/z 489.9</td>
<td>57589</td>
<td>25244</td>
<td>12120</td>
<td>11014</td>
</tr>
</tbody>
</table>

m/z 266.8, [AuCl + Cl]⁻;

m/z 388.7, [AuCl₃ + Cl]⁻;

m/z 480.8, [Au₂Cl(O₂)H₂O]⁻;

m/z 489.9, [Au₂(O₂)₃]⁻
When the block copolymer is processed into micelles with PEO corona and PS core, they can function as the template for the *in situ* growth of gold nanoparticles. At constant gold salt concentration, it has been reported that reducing agent is playing an important role in regulating gold nanoparticle size and number. When reducing ability of solution is weak, nucleation rate is slower compared to particle growth rate. Gold nanoparticles can present larger size but smaller population, vice versa. To facilitate Janus particle formation, we kept concentration of gold salts constant and varied reducing agent (HEPES) and micelle concentrations. Dialyzed micelles were resuspended and diluted into stock solution with HEPES buffer at several different predetermined concentrations. Then a stock solution (2.45 mmol L\(^{-1}\)) of chloroauric acid (HAuCl\(_4\)) was added (300 µL) to 700 µL of each prepared micelle HEPES solution to yield a final HAuCl\(_4\) concentration of 0.735 mmol L\(^{-1}\). From the master mixture, aliquots were divided into 4 groups according to different HEPES concentrations, 0.1 mol L\(^{-1}\), 0.3 mol L\(^{-1}\), 0.5 mol L\(^{-1}\) and 0.7 mol L\(^{-1}\), respectively. Within each HEPES concentration, 4 different polymer concentrations were subdivided. The final range of diblock copolymer concentration, which was ranging from \(2.0 \times 10^{-7}\) mol L\(^{-1}\) to \(1.4 \times 10^{-6}\) mol L\(^{-1}\), was identical for the 0.1 mol L\(^{-1}\) and 0.3 mol L\(^{-1}\) HEPES groups. The polymer concentration was lower for the higher HEPES concentration samples (0.5 mol L\(^{-1}\) and 0.7 mol L\(^{-1}\)), which was ranging from \(8.0 \times 10^{-8}\) mol L\(^{-1}\) to \(5.6 \times 10^{-7}\) mol L\(^{-1}\). We have found that when the HEPES concentration is too high (>0.7 mol L\(^{-1}\)), it can raise instability issues of the micelles. The micelles disassemble as the HEPES concentration increased beyond this threshold. However, if the concentration of micelles remains
below the critical value for each set of experiments within a specific HEPES concentration, the micelles gradually lose the ability to sequester sufficient amounts of gold salts for controlling the resulting morphologies of gold nanoparticles. The composition information for each reaction system is shown in Table 3.3.

<table>
<thead>
<tr>
<th>Composition</th>
<th>[Polymer]/mol L⁻¹</th>
<th>Composition</th>
<th>[Polymer]/mol L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPES 0.1 mol L⁻¹</td>
<td></td>
<td>HEPES 0.3 mol L⁻¹</td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>1.4 × 10⁻⁶</td>
<td>2a</td>
<td>1.4 × 10⁻⁶</td>
</tr>
<tr>
<td>1b</td>
<td>1.0 × 10⁻⁶</td>
<td>2b</td>
<td>1.0 × 10⁻⁶</td>
</tr>
<tr>
<td>1c</td>
<td>6.0 × 10⁻⁷</td>
<td>2c</td>
<td>6.0 × 10⁻⁷</td>
</tr>
<tr>
<td>1d</td>
<td>2.0 × 10⁻⁷</td>
<td>2d</td>
<td>2.0 × 10⁻⁷</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Composition</th>
<th>[Polymer]/mol L⁻¹</th>
<th>Composition</th>
<th>[Polymer]/mol L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPES 0.5 mol L⁻¹</td>
<td></td>
<td>HEPES 0.7 mol L⁻¹</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>5.6 × 10⁻⁷</td>
<td>4a</td>
<td>5.6 × 10⁻⁷</td>
</tr>
<tr>
<td>3b</td>
<td>4.0 × 10⁻⁷</td>
<td>4b</td>
<td>4.0 × 10⁻⁷</td>
</tr>
<tr>
<td>3c</td>
<td>2.4 × 10⁻⁷</td>
<td>4c</td>
<td>2.4 × 10⁻⁷</td>
</tr>
<tr>
<td>3d</td>
<td>8.0 × 10⁻⁸</td>
<td>4d</td>
<td>8.0 × 10⁻⁸</td>
</tr>
</tbody>
</table>

Figure 3.10 – 3.13 show representative TEM bright field images of gold-micelle hybrid nanoparticles under each reaction condition and the corresponding statistical histograms representing distributions of numbers of gold nanoparticles per micelle under different the fabrication conditions according to Table 3.3. For each reaction condition,
more than 500 nanoparticles were counted for the statistical plot. Gold nanoparticle aggregates and blank micelles were not counted for the histograms. In Figure 3.10, HEPES concentration is kept constant of 0.1 mol L$^{-1}$ while the polymer concentration is

![Representative TEM images under reaction conditions where HEPES concentration was constant at 0.1 mol L$^{-1}$ while polymer concentration was gradually reduced from 1a to 1d, was were $1.4\times10^{-6}$ mol L$^{-1}$, $1.0\times10^{-6}$ mol L$^{-1}$, $6.0\times10^{-7}$ mol L$^{-1}$ and $2.0\times10^{-7}$ mol L$^{-1}$, respectively. The histogram shows the overall fraction of micelles with different loading (1-4) of gold nanoparticles. As polymer concentration decreases, the overall portion of Janus nanoparticle is decreasing and the capability to control gold nanoparticles is also impaired.](image-url)

Figure 3.10
reduced gradually from $1.4 \times 10^{-6}$ mol L$^{-1}$ to $2.0 \times 10^{-7}$ mol L$^{-1}$. Janus nanoparticles have been observed at higher polymer concentration ($1.4 \times 10^{-6}$ mol L$^{-1}$ in 1a and $1.0 \times 10^{-6}$ mol L$^{-1}$ in 1b) and lower polymer concentrations ($1.0 \times 10^{-6}$ mol L$^{-1}$ in 2a, $6.0 \times 10^{-7}$ mol L$^{-1}$ in 2c, and $2.0 \times 10^{-7}$ mol L$^{-1}$ in 2d), respectively.

The histogram shows the overall fraction of micelles with different loading (1-4) of gold nanoparticles. The overall portion of Janus particles is reduced as polymer concentration decreases. Control over gold nanoparticle growth is improved compared with Figure 3.10 when polymer concentration is low.

Figure 3.11 Representative TEM images under reaction conditions where HEPES concentration was constant at 0.3 mol L$^{-1}$ while polymer concentration was gradually reduced from 2a to 2d, which was $1.4 \times 10^{-6}$ mol L$^{-1}$, $1.0 \times 10^{-6}$ mol L$^{-1}$, $6.0 \times 10^{-7}$ mol L$^{-1}$ and $2.0 \times 10^{-7}$ mol L$^{-1}$, respectively. The histogram shows the overall fraction of micelles with different loading (1-4) of gold nanoparticles. The overall portion of Janus particles is reduced as polymer concentration decreases. Control over gold nanoparticle growth is improved compared with Figure 3.10 when polymer concentration is low.
L⁻¹ in 1b). When polymer concentration is reduced (6.0 × 10⁻⁷ mol L⁻¹ in 1c and 2.0 × 10⁻⁷ mol L⁻¹ in 1d), average number of gold nanoparticle per micelle is enhanced. Meanwhile, reduced polymer concentration led to aggregation of gold nanoparticles, which is attributed to the fact that PEO volume fraction is not sufficient enough to maintain regularity on gold nanoparticle formation under those reaction conditions. The general trend of hybrid nanoparticle morphology change is depicted in the statistical histogram. As polymer concentration decreases, the overall portion of Janus nanoparticle is decreasing. The same general trend is observed for 0.3 mol L⁻¹ HEPES solution in Figure 3.11. When polymer concentration is high (1.4×10⁻⁶ mol L⁻¹, 2a), nearly all the hybrid nanoparticles present Janus morphology (~90%). On the contrary, lower polymer concentration yield hybrid nanoparticles with varied numbers (1 – 4) of gold nanoparticles on each micelle. In the statistical plot of group 2a, percentage of nanoparticle composition decreases sharply from ~90% to ~ 0% as the gold nanoparticle/micelle ratio increases from 1 to 4. Differently, the decrease slows down when comes to group 2d where the Janus nanoparticle percentage is only ~40% ([Polymer] = 2.0 × 10⁻⁷ mol L⁻¹). Decrease in the Janus nanoparticle fraction is attributed reduced micelle population, assuming gold nanoparticle population was identical. Interestingly, higher HEPES concentration has a better control of gold nanoparticle formation. In group 1d and 2d, even though polymer concentration is identical, higher HEPES concentration prevents massive formation of gold aggregates. As HEPES concentration reaches 0.5 mol L⁻¹, the highest Janus nanoparticles overall fraction drops to ~65% (Figure 3.12). As the polymer concentration is reduced, Janus nanoparticle production
is depressed and the ratio of gold nanoparticle/micelle is shifting to a higher value.

TEM bright field image shows that, from Figure 3.12 (3a) to Figure 3.12 (3d), Janus

![Representative TEM images under reaction conditions where HEPES concentration was constant at 0.5 mol L$^{-1}$ while polymer concentration was gradually reduced from 3a to 3d, which was 5.6×10$^{-7}$ mol L$^{-1}$, 4.0×10$^{-7}$ mol L$^{-1}$, 2.4×10$^{-8}$ mol L$^{-1}$ and 8.0×10$^{-8}$ mol L$^{-1}$, respectively. The histogram shows the overall fraction of micelles with different loading (1-6) of gold nanoparticles. The overall portion of Janus nanoparticle is further reduced and the distribution peak of gold nanoparticle/micelle ratio is shifting to a higher value as polymer concentration decreases.](image)

Figure 3.12

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nanoparticle morphology is gradually decreasing. Instead, population of micelles which carry more than one gold nanoparticle is increasing. Figure 3.13 shows that when the

![Representative TEM images](image)

Figure 3.13  Representative TEM images under reaction conditions where HEPES concentration was constant at 0.7 mol L\(^{-1}\) while polymer concentration was gradually reduced from 4a to 4d, which was 5.6×10\(^{-7}\) mol L\(^{-1}\), 4.0×10\(^{-7}\) mol L\(^{-1}\), 2.4×10\(^{-8}\) mol L\(^{-1}\) and 8.0×10\(^{-8}\) mol L\(^{-1}\), respectively. The histogram shows the overall fraction of micelles with different loading (1-8) of gold nanoparticles. The overall portion of Janus nanoparticle is further reduced and the distribution peak of gold nanoparticle/micelle ratio is shifting to an even higher value as polymer concentration decreases.
concentration of HEPES is further enhanced to 0.7 mol L\(^{-1}\), Janus nanoparticle population drops to ~45% in group 4a when the polymer concentration is the highest (5.6 \(\times\) 10\(^{-7}\) mol L\(^{-1}\)). In group 4b, where polymer concentration is 4.0 \(\times\) 10\(^{-7}\) mol L\(^{-1}\), overall fraction of the hybrid nanoparticle with gold nanoparticle/micelle ratio of 1:1, 2:1 and 3:1 is similar. As the polymer concentration is reduced to 8.0 \(\times\) 10\(^{-8}\) mol L\(^{-1}\), Janus nanoparticle population was further depressed. Interestingly, the maximum occupation takes place where each micelle carries 5 gold nanoparticles. Under this particular reaction condition, the gold nanoparticle/micelle ratio is extended to 8 based on observation, which never occurs under other reaction conditions.

To facilitate comparison, we combined the above four statistical histograms together, as shown in Figure 3.14. In Figure 3.14, the highest yield of gold-micelle Janus nanoparticles occurs when micelles are concentrated. Assuming that gold nanoparticle population was constant, higher micelle concentration can reduce the gold nanoparticle/micelle ratio for each hybrid particle. We also observed that maximum overall percentage of Janus nanoparticles was present in 2a in Table 3.3 over the 16 recipes. In Figure 3.14a and b ([HEPES] = 0.1 and 0.3 mol L\(^{-1}\)), for all the eight recipes, the overall portion of hybrid particles is decreasing as the gold nanoparticle/micelle ratio increases. Moreover, the decreasing rate becomes faster when polymer concentration goes higher. This observation indirectly verifies the hypothesis that when large number of micelles exists, production of hybrids of which each micelle entity carries more than one gold nanoparticles is depressed. A transition occurs in Figure 3.14c ([HEPES] = 0.5 mol L\(^{-1}\)). Within the plot, distribution of 3c shows that the peak of the number of gold
nanoparticles per micelle is shifting to a higher level and a significant drop of gold–micelle Janus hybrids yield has been observed. The trend becomes much clear.

Figure 3.14 Statistical histograms depicting the distribution of gold nanoparticles/micelle ratio for all 16 reaction conditions listed in Table 2. Within each window, HEPES concentration is constant while polymer concentration is reduced from a to d. (a) 0.1 mol L\(^{-1}\) HEPES; (b) 0.3 mol L\(^{-1}\) HEPES; (c) 0.5 mol L\(^{-1}\) HEPES; and (d) 0.7 mol L\(^{-1}\) HEPES.
as the HEPES concentration is further increased. In Figure 3.14d ([HEPES] = 0.7 mol L\(^{-1}\)), population of Janus nanoparticle is minimized. In the distribution of 4c and 4d, majority of the hybrids shows 3 to 5 gold nanoparticles on each micelle.

**Figure 3.15**  a. Statistical histogram of gold–micelle Janus nanoparticles and multi-particle clusters under different reaction conditions. The overall portion is changing with HEPES and micelles concentrations; b. bright field TEM images of Janus nanoparticles; c. TEM bright field images of the raspberry-like gold–micelle hybrid, of which each micelle has multiple gold nanoparticles.
To better demonstrate the effect of reaction conditions on the Janus particle fraction, we have isolated the percentages of Janus nanoparticles for all the 16 recipes as a function of HEPES and block copolymer concentrations generated from Figure 3.15. Figure 3.15a shows the general transition tendency of the Janus nanoparticle heterogeneity fraction according to variations in HEPES and block copolymer concentrations. In this plot, the z-axis represents the overall portion of gold–micelle Janus morphologies in the hybrid nanoparticle population. The x- and y-axes indicate the extent to which the hybrid morphologies are simultaneously affected by HEPES and polymer concentrations, respectively. For each set of columns with the same HEPES value, the overall percentage of Janus nanoparticles is decreasing as micelle concentration becomes more and more dilute. Concentrated micelle solutions (1.4×10⁻⁶ mol L⁻¹) with low HEPES concentrations (0.1 mol L⁻¹ and 0.3 mol L⁻¹) are able to host the growth of high-fidelity gold–micelle Janus particles. The optimal conditions (~90%) for Janus particle formation are 0.3 mol L⁻¹ HEPES and 1.4×10⁻⁶ mol L⁻¹ polymer (Table 3.3, 2a).

The instability of micelles in concentrated HEPES solution has limited the parallel comparison of the effect of micelle concentrations at the same reducing agent concentration. However, in the overlapping regime, specifically in the dilute micelle regime (2.4×10⁻⁷ mol L⁻¹), the blue columns indicate that with concentrated HEPES (0.5 mol L⁻¹ and 0.7 mol L⁻¹), the Janus nanoparticles fraction is significantly reduced to less than 20%. This number is much lower than that (~40%) when polymer concentration is 2.0×10⁻⁷ mol L⁻¹ and HEPES concentration is 0.1 mol L⁻¹ and 0.3 mol L⁻¹. From the comparison we can infer that higher HEPES concentration is able to produce more gold
nanoparticles. It can be attributed to the fact that higher reducing agent concentration leads to stronger reducing ability, thereby allowing available gold to be divided to more gold nanoparticles. Figure 3.15b shows a bright field TEM image of the gold–micelle hybrid with highest Janus nanoparticles occupation (Table 3.3, 2a). The bifunctional nanoparticles show distinct Janus morphology, with the micelles diameter averaging 30 nm and gold approximately 10 nm. The gold nanoparticles appear black and micelles are light colored in the image because of the higher electron density of gold nanoparticles, which prevents transmission of the electrons. Figure 4.15c shows bright field TEM images of the gold–micelles hybrid synthesized with low micelle but high HEPES concentration (Table 3.3, 4d). Each micelle is coupled with multiple gold nanoparticles with diameters around 20 nm which present raspberry-like morphology.

In order to investigate the effect of polymer and HEPES concentrations on the nanocrystals within the hybrid nanoparticles, UV-Vis absorbance was measured for each product of the 16 reaction conditions. The gold nanocrystal absorbance spectrum is dominated by a characteristic surface plasmon resonance (SPR) peak, of which the details have been discussed and reviewed. The nanocrystal SPR peak appears as a broad absorbance band centered near a wavelength of 520 – 530 nm when the size of gold cluster exceeds 1.5 nm. The SPR maximum wavelength $\lambda_{\text{max}}$ deviates depending on particle size, shape, dielectric constant and temperature. In Figure 3.16, each curve shows the shift of $\lambda_{\text{max}}$ of gold nanocrystals by varying the polymer concentration while keep HEPES concentration constant. Each point represents the average of three independent UV-Vis measurements. For all the four sets of curves, increasing the
polymer concentration will result in blue-shifting of $\lambda_{\text{max}}$. In the reaction condition when $[\text{HEPES}] = 0.1 \text{ mol L}^{-1}$, $\lambda_{\text{max}}$ is blue-shifting of as polymer concentration increases.

Figure 3.16 a. Shift of the average plasmon peak wavelength of gold–micelle hybrids when changing micelle and HEPES concentrations. Points with different colors lying on the solid and dashed lines have the corresponding full UV-Vis spectra in (b); b. Eight full UV-Vis spectra curves according to the peak wavelength lying in solid and dashed lines in (a), which clearly show the blue-shift of the dashed lines from the corresponding solid lines.
can be attributed to reduced gold nanoparticle size and enhanced Janus population. As indicated in Figure 3.10, lower polymer concentration produces gold nanoparticle with larger sizes which can lead to higher wavelength plasmon absorption. The situation gets improved when polymer concentration is enhanced, where more PEO chains are involved in controlling gold nanoparticle synthesis. With the same polymer concentration, control over the particle synthesis has been improved when HEPES concentration is increased to 0.3 mol L\(^{-1}\). Comparing with two curves, \(\lambda_{\text{max}}\) is smaller than that of 0.1 mol L\(^{-1}\) HEPES solution. This is because higher reducing agent concentration can produce gold nanoparticles with smaller size, narrower size distribution and larger population. For curves when HEPES concentration is 0.5 mol L\(^{-1}\) and 0.7 mol L\(^{-1}\), effect of gold nanoparticle size on absorption becomes minor. Different from a reduction in the gold nanoparticle size, the blue shifting is caused by an increasing spacing between adjacent gold nanoparticles. According to Figures 3.12 and 3.13, as polymer concentration increases, average number of gold nanoparticle on each micelle decreases and releases the spacing among neighboring particles. Besides the effect of polymers, HEPES concentration also has a strong impact on plasmon of gold nanocrystals. It has been reported that the amount of reducing agent is able to influence the relative rates of the two independent processes: nucleation and growth of the metal particles. As the HEPES concentration is increasing, reducing ability of the solution is enhanced and the available gold can be divided into more and more nuclei to make a difference in nanoparticle numbers and diameters. In dilute HEPES solution (0.3 mol L\(^{-1}\)), existing gold is divided into fewer nanoparticles. Higher micelle concentration facilitates
generation of Janus nanoparticles. When the HEPES concentration is high (0.7 mol L\(^{-1}\)), there will be a larger number of nanoparticles and the corresponding gold nanoparticle size will be smaller. In this case, when the polymer concentration is low (8.0 \(\times\) 10\(^{-8}\) mol L\(^{-1}\)), instead of forming Janus nanoparticles, each micelle can induce several gold nanoparticles representing raspberry-like morphology. The absorption peak is red-shifting to a higher wavelength, which is due to the reduced nanoparticle interspacing. In the window separated by both dashed and solid lines, with the same micelle content, \(\lambda_{\text{max}}\) is sharply blue-shifting towards 530 nm as the HEPES concentration is increased, which also suggests that the size of gold nanocrystals is getting smaller and more uniform. Full UV-visible spectrophotometry curves with the \(\lambda_{\text{max}}\) on the solid and dashed lines in Figure 3.16a are correspondingly present in Figure 3.16b. The set of dashed lines are entirely blue shifted from the solid ones with the same color.

3.3 Worm-like Periodical Gold Nanoparticle Chains

The PEO-b-PS (\(M_n^{PEO} = 10\) kDa, \(M_n^{PS} = 46\) kDa) block copolymer used in the above experiment possesses longer hydrophobic PS chain and shorter hydrophilic PEO chain. Due to the high fraction and large relative size of the insoluble block, micelles formed with this polymer fall into crew-cut categories. As reported earlier, multiple morphologies of crew-cut micelles, e.g. spheres, rods, vesicles, lamellae and large compounds, have been prepared.\(^{155}\) Utilizing the poly(styrene-b-acrylic acid) (PS-b-PAA), Eisenberg et. al. found that keeping the PS block length constant, decreasing PAA block length results in a morphological transition ranging from sphere, rods, vesicle
and lamellae. When the length of PAA block is extremely short, micelles present highly polydisperse spheres.\textsuperscript{157} The morphology change of crew-cut micelles is attributed to force balance of the self-assembly system. Typically, there are three parameters that control the process: stretch of the hydrophobic blocks in the core, surface tension between the core and the outside solvent, and the repulsion among the corona chains. Those three factors are influenced by changing water content, nature of common solvent, initial copolymer concentration and copolymer composition and so on.

Figure 3.17 Morphological change of PEO-b-PS micelles with different water content. a. TEM bright field image of micelle spheres (polymer: 0.1 wt\% in DMF, H\textsubscript{2}O: 4.76 wt\%); b TEM bright field image of micelle peal-like chains (polymer: 0.1 wt\% in DMF, H\textsubscript{2}O: 17.0 wt\%).
With the above PEO-b-PS block copolymer, we observed that when water content was low (polymer: 0.1 wt% in DMF, H$_2$O: 4.76 wt%), block copolymers form monodisperse spherical micelles. Interestingly, when the water content was increased to > 17.0 wt%, we found that morphology of the micelles changed from spheres to pearl-like chains with different lengths (Figure 3.17). The pearl-like morphology stayed unchanged when water content was continuously enhanced. No micelles of rod or vesicle morphologies have been observed, even under extremely slow water addition speed (10 µL/30 min; every water droplet was dropped onto the inner wall of vial first and slowly flew into the solution under viscous stirring). In particular, the change of water content has an effect on the surface tension between the PS core and the solvent. Addition of water increases the Flory-Huggins $\chi$ parameter between PS block and the solvent, which drives the PS block to segregate in order to reduce the surface energy. At the early stage of the micelle formation, PS block aggregation number is low and the chain is stretched, which allows the formation of spherical micelles. Also at this stage, the PS core is highly swollen with common solvent and the aggregation number of PS blocks increases as the addition of water increases. Consequently, core dimension will increase. However, the core dimension increase is not indefinitely. Minimizing the surface energy between PS core and solvent can be offended by increasing of the corona repulsion and PS chain stretching. In order to minimize the overall energy of the self-assembly system, morphology of the micelles will change to release the repulsion effect and the stretched chains. Contrarily, upon the increase of water content, the common solvent in the micelle core is gradually lost, allowing the PS chains to be
kinetically frozen into certain state once the temperature is below $T_g$. In an extreme instance, if the PS chain molecular weight is high enough ($T_g$ is high), morphology of

Figure 3.18  TEM bright field images of pearl-like micelle-gold hybrid superstructures fabricated under different reaction conditions. 1-D worm-like gold nanowire was produced under soft reducing environment. a. $[\text{HAuCl}_4] = 0.735 \text{ mmol L}^{-1}$, $[\text{polymer}] = 2.0 \times 10^{-7} \text{ mol L}^{-1}$, $[\text{NaBH}_4] = 0.01 \text{ mol L}^{-1}$; b. $[\text{HAuCl}_4] = 0.735 \text{ mmol L}^{-1}$, $[\text{polymer}] = 2.0 \times 10^{-7} \text{ mol L}^{-1}$, $[\text{HEPES}] = 0.5 \text{ mol L}^{-1}$; c. $[\text{HAuCl}_4] = 0.735 \text{ mmol L}^{-1}$, $[\text{polymer}] = 2.0 \times 10^{-7} \text{ mol L}^{-1}$, $[\text{HEPES}] = 0.3 \text{ mol L}^{-1}$.
micelles can be frozen into spheres. At some critical value of polymer composition and the water content, when the common solvent in the micelle core is still enough to allow high mobility of PS chain, a morphological transition from sphere to rod and vesicle can be observed. The morphology we have observed using this specific PEO-b-PS occurred when the spherical micelles began to transform into rods. Pearl-like chain is one of the middle states that has been observed before.\textsuperscript{161}

Using the pearl-like micelle chains to incubate gold nanoparticles, we found that under the same condition incubating gold-micelle Janus nanoparticles, a 1-D “worm-like” gold nanoparticle morphology has been observed. Figure 3.18 shows the TEM bright field image of the superstructures incubated under different reaction condition. When using strong reducing agent (NaBH\textsubscript{4}), small gold nanoparticle seeds have been observed all over the micelles (Figure 4.18a). A soft reducing agent but higher concentration ([HEPES] = 0.5 mol L\textsuperscript{-1}) allows larger gold nanoparticles compared with the seeds (Figure 3.18b). Among those nanoparticles, the ones growing in between two neighboring micelle spheres are much larger than those on the body surface. A reduction of the HEPES concentration ([HEPES] = 0.3 mol L\textsuperscript{-1}) resulted in diminishing of the gold nanoparticles growing on the micelle body surface, leaving them located in the junction of spheres to form periodical structures (Figure 3.18c). This observation is due to the heterogeneous dispersion of PEO along the chain (Figure 3.19). PEO chain density between two neighboring spheres is higher than that on the sphere body. Since PEO chains absorb and sequester gold ions, when the micelle chains are put into HAuCl\textsubscript{4} water solution, junction regions along the chain can absorb more gold ions to induce
locally concentrated areas. Strong reducing agent accelerates nucleation rate and rapid growth of the crystal, resulting in large amount of gold seeds. With soft reducing agent with allows slower nucleation rate, gold nanoparticle formation first occurs at the place where concentration of gold ions is higher. The study of the kinetics behavior and nanoparticle growth of the gold-micelle periodical chains is still at the early stage. We have included our preliminary results here mainly as an interesting observation.

Figure 3.19 1-D gold nanowire fabrication based on pearl-like micelles. a. Pearl-like micelle chains; circled is the junction area between two adjacent spheres; b. schematic illustration of the concentrated PEO chains in the junction area; c. gold nanoparticles grew from the junction area to form 1-D worm-like structure.
3.4 Conclusion

In this part, we have demonstrated a new aqueous route to synthesize hybrid nanostructures with reduced symmetry that is scalable. Core-shell micelles function as a template to incubate gold nanoparticles via the relative volume fraction of PEO. Morphologies of gold-micelle nanoparticles can be changed from Janus structure to raspberry-like structure by adjusting the concentrations of micelle and HEPES buffer which functions as the reducing agent. The anisotropic structures were evident in TEM images, which statistically show the transition of overall portion of Janus particles with the change of reaction conditions. The formation of Janus nanoparticles is more favored when micelle content is high while HEPES concentration is low. On the contrary, in dilute micelle but concentrated HEPES solution, each micelle sphere is able to grow several gold nanocrystals, which present raspberry-like structure. Controlling nucleation and growth of inorganic structure with organic matrix is one of the fundamental processes for manipulation mineral deposition into microstructures. Patterning gold nanoparticles using polymer template is cost-effective relative to other methods and has potentially wide ranging applications. Due to the simplicity of our process, this method has great potential for large scale production. Under certain conditions, the individual spherical micelles can transform into pearl-like chains, creating higher PEO concentration in the junction region between two neighboring spheres. It further led to gold nanoparticle growth in the junction areas to form worm-like periodical gold nanoparticle chains. Even though mechanism of the phenomenon is still under investigation, we envision that our approach can be extended to different geometries and materials with the diversity of
core shell structures that are reported in the literature. This approach is a fast, more precise and economically feasible strategy that will afford the rational design and synthesis of complex gold nanoparticle superstructures. This strategy will also broaden existing “bottom-up” fabrication strategies.
CHAPTER IV
A FACILE SOLUTION TEMPLATE APPROACH TO GOLD NANOPARTICLE
DIMERS

4.1 Motivation

Surface plasmons are well known for the ability to confine light and generate intense local electromagnetic field, which can be applied to novel techniques such as SERS. To benefit from the emerging technology that allows single molecular detection, substrates that can afford intense and sustainable SERS signals with high reproducibility, e.g. gold dimers, are significantly desirable. As described in Chapter I, methods from the “top-down” and “bottom-up” principles have been widely investigated for gold dimer engineering. However, existing fabrication methods suffer from high cost (photolithography) and/or defective optical properties (molecular linkage). Cost-effective methods to precisely control and assemble the functional structure are scarce. In the previous chapter, we described a facile pathway to grow gold nanoparticle from micelles nanospheres in order to form Janus hybrid nanoparticles in situ. Inspired by the principle, we hope through replacing the PS cores of the micelles with the pre-synthesized gold nanoparticles, a metallic template can be obtained to fabricate gold dimers (Figure 4.1). So objective of the current work is to develop a facile strategy for gold dimer synthesis via a one step surface nucleation growth pathway: PEO
functionalized gold spherical nanoparticles are utilized as the template to grow additional gold nanoparticles within the PEO corona in an aqueous solution. We are looking for the optimized reaction condition for well-defined gold dimers which can show strong potential for SERS measurement.

Figure 4.1 Micelles with PS core and PEO corona are able to induce gold nanoparticle growth within the PEO corona in aqueous solution. Theoretically, if the PS core is replaced with a gold nanoparticle, a metallic template can be attained to fabricate gold dimers under certain reaction condition.
4.2 Results and Discussion

Figure 4.2 briefly illustrates the gold dimer fabrication strategy through a surface nucleation pathway to grow the second gold nanoparticle from the nanoparticle template tethered with PEO chains. Mineralization of PEO chains to induce the gold nanoparticle

![Diagram of gold nanoparticle synthesis](image)

Figure 4.2 Synthesis of gold nanoparticle dimers using template gold nanoparticles surface functionalized with PEO ligands. Mono-thiol capped PEO was synthesized to functionalize gold nanoparticles. Template nanoparticles were incubated with HAuCl₄ and HEPES solution to yield gold dimers.
formation in certain reducing environment has been reported by several research groups.\textsuperscript{159, 160} It has provided opportunities to incubate gold nanoparticles from PEO functionalized substrates possessing different morphologies and structures. In the previous work, we have shown that gold nanoparticles can be induced within PEO corona tethered on spherical PS core. Using the same principle, we can change the PS core into a spherical gold nanoparticle as the substrate to support the PEO corona. Serving as the precursor, PEO functionalized gold nanoparticles can be utilized to induce the formation of gold dimers through carefully manipulating reaction conditions.

In current study, gold nanoparticle with a narrow size distribution (d = 10 ± 1.8 nm) were synthesized according to a traditional citrate strategy.\textsuperscript{150} Particles synthesized with this method exhibit a mono-disperse size which makes the particles superb candidate as the template. Mono-thiol capped PEO (PEO-SH, $M_n = 10$ kDa) was synthesized to functionalize gold nanoparticles to form the precursors according to established procedures.\textsuperscript{162, 163} \textsuperscript{1}H-NMR in Figure 4.3 suggested the successful synthesis of mono-capped PEO-SH. Zeta potentials were performed at pH 5 in HAc/NaAc buffer to detect PEO tethering. Binding of electrically neutral PEO to negatively charged citrate-gold nanoparticles resulted a zeta potential increase from $-34.9 \pm 4.3$ mV (before reaction) to $-10.2 \pm 2.4$ mV (after reaction), which confirms the successful ligand derivatization. To induce gold nanoparticle growth with PEO chains, it’s critical to choose proper reducing agent. An arbitrary comparison has been made in Figure 5.4. After purification, gold nanoparticle precursors were collected and suspended in a HAuCl$_4$ aqueous solution for an equilibrium period (30 min). The incubation solution
was divided into two aliquots. Addition of reducing agent NaBH₄ (0.01 mol L⁻¹) to one of the aliquots yielded the less well-defined star-like gold nanostructures (Figure 5.4a),

Figure 4.3 ¹H-NMR (CDCl₃, 500 MHz, ppm, δ) spectrum of S-1-dodecyl-S’-(r,r’-dimethyl-r’’-acetic acid) trithiocarbonate (TC) capped PEO (PEO-TC, a) and thiol capped PEO (PEO-SH, b) PEO-SH was synthesis through cleaving the alkyl chain from PEO-TC. (a) PEO-TC: 4.24 (m, 2H, -CH₂O(CO)-), 3.64 (br, 910H, -CH₂CH₂O-), 3.27 (m, 2H, -SCH₂CH₂-), 1.69 (s, 6H, -O(CO)C(CH₃)₂S-), 1.65 (m, 2H, -SCH₂CH₂CH₂-), 1.2–1.4(m, 18H, -SCH₂CH₂(CH₂)₆CH₃), 1.20 (s, 9H, (CH₃)₃C-O-). (b) PEO-SH: ¹H NMR (CDCl₃, 500 MHz, ppm, δ): 3.64 (br, 910H, -CH₂CH₂O-), 1.20 (s, 9H, (CH₃)₃C-O-).
while HEPES (0.5 mol L\textsuperscript{-1}) gave out gold dimers (Figure 5.4b). The dramatic structural difference is attributed to different reducing abilities. NaBH\textsubscript{4} is a strong reducing agent commonly used in inducing nanoparticle seeds. The reducing ability of HEPES is much weaker compared with NaBH\textsubscript{4}, which allows slower nucleation rate to form larger nanoparticles. From our previous research outcomes (Chapter III) we concluded that reduction of HAuCl\textsubscript{4} with HEPES in PEO aqueous solution might be a promising procedure to induce spherical gold nanoparticle formation. Nevertheless, reaction conditions need to be optimized in order to quantitatively form well-defined gold dimers.

Figure 4.4 Different reducing agent can produce different gold nanostructures using PEO-functionalized gold nanoparticle template. a. PEO functionalized gold nanoparticles incubated with NaBH\textsubscript{4} (0.01 mol L\textsuperscript{-1}); b. PEO functionalized gold nanoparticles incubated with HEPES (0.5 mol L\textsuperscript{-1}).
The fact is that not only the identity of reducing agents but their concentrations can affect the reducing ability of reaction solution and consequently make a change on gold nanostructure. In this way, different concentrations of reducing agent are able to bring changes in nanoparticle numbers, sizes and size distribution through adjusting the independent rates of nucleation formation and particle growth. Besides, within certain reducing environment and constant gold salt dose, volume fraction of PEO can also affect

Figure 4.5  Dilute HEPES (0.1 mol L\(^{-1}\)) solution with low PEO-functionalized gold nanoparticle concentration (~15 nmol L\(^{-1}\)) shows little control over gold nanoparticle formation.  a. PEO-functionalized gold nanoparticle templates; b. Gold nanostructure produced under in the solution of 15 nmol L\(^{-1}\) PEO-functionalized gold nanoparticles, 0.7 mmol L\(^{-1}\) HAuCl\(_4\) and 0.1 mol L\(^{-1}\) HEPES; insufficient PEO content with dilute HEPES concentration produced massive gold nanoparticle aggregates.
the nanoparticle formation, given the extreme when the amount of PEO is insufficient to prevent aggregation. A test reaction was performed using a solution consisting of 15 nmol L\(^{-1}\) PEO-gold nanoparticles, 0.7 mmol L\(^{-1}\) HAuCl\(_4\) and 0.1 mol L\(^{-1}\) HEPES. Different from the nanoparticle templates which are uniform in size and well-dispersed, large population of gold nanoparticle aggregates with wide range of nanoparticle sizes have been observed under this reaction condition, as shown in Figure 4.5. Limited control on newly grown nanoparticle may result from low HEPES concentration and small amount of template particles content (PEO volume fraction) synergistically. Situation is similar in gold-micelle fabrication with low HEPES and micelle concentrations. Without sufficient regularity on particle growth, the system produced gold aggregates (Chapter III).

To optimize reaction condition, we have systematically studied the effect of HEPES and gold nanoparticle template on the structure of final products (Figure 4.6). First, we enhanced the concentration of PEO functionalized gold nanoparticle to \(\approx 30\) nmol L\(^{-1}\). With the same HEPES concentration (0.1 mol L\(^{-1}\)) and HAuCl\(_4\) concentration (0.7 mol L\(^{-1}\)), aggregation has been significantly reduced. Figure 4.6a shows the aggregates constituted of nanoparticles of different sizes. Keeping PEO functionalized gold nanoparticle concentration (\(\approx 30\) nmol L\(^{-1}\)) and HAuCl\(_4\) concentration (0.7 mol L\(^{-1}\)) constant, three more different concentrations (0.3 mol L\(^{-1}\), 0.5 mol L\(^{-1}\) and 0.7 mol L\(^{-1}\), respectively) have been employed to test the nature of HEPES in the current system. Accordingly, Figures 4.6b – d show representative TEM bright field images of the products prepared under each reaction condition. Compared to gold nanoparticle
templates which are nearly monodisperse in size, newly grown gold nanoparticles exhibit larger particle size as well as broader size distribution, which distinguishes them from the

Figure 4.6  Optimizing reaction condition for gold dimer fabrication through varying HEPES concentration and template gold nanoparticle content. TEM bright field images show gold nanoparticle structures under different reaction conditions: a – d. [HAuCl₄] = 0.7 mmol L⁻¹, [PEO-AuNPs] ≈ 30 nmol L⁻¹, [HEPES] is 0.1 mol L⁻¹, 0.3 mol L⁻¹, 0.5 mol L⁻¹ and 0.7 mol L⁻¹ respectively; d – f: [HAuCl₄] = 0.7 mmol L⁻¹, [HEPES] = 0.7 mol L⁻¹, [PEO-AuNPs] is ~30 nmol L⁻¹, ~45 nmol L⁻¹ and ~60 nmol L⁻¹ respectively.
template nanoparticles. As HEPES concentration is increasing, structural transition from gold nanoparticle aggregates to well-defined dimeric species can be clearly observed. Meanwhile, enhanced HEPES concentration has resulted in a narrower particle size distribution, which can be attributed to a faster nucleation rate followed by a slower particle growth rate. Controlled-growth of gold nanoparticles from PEO functionalized gold nanoparticle templates to generate gold dimers takes place as HEPES concentration reaches to above 0.3 mol L⁻¹ (Figure 4.6b). When [HEPES] = 0.7 mol L⁻¹, well-defined gold dimers have been incubated (Figure 4.6d). From Figure 5.6d to 5.6f, PEO functionalized gold nanoparticle concentration (overall PEO volume fraction) was increased from ~30 nmol L⁻¹ to ~60 nmol L⁻¹ while amounts of HAuCl₄ and HEPES were maintained at 0.7 mmol L⁻¹ and 0.7 mol L⁻¹, respectively. Keeping HAuCl₄ supply constant, we hoped the increase in initial PEO volume fraction was able to better shape newly grown particle size and size distribution. However, compared to HEPES, effect of PEO concentration on structure of the final products is limited and few structural differences have been observed with the enhanced PEO volume fraction (Figure 4.6d – 4.6f). One subtle change according to the observation is that, when concentration of PEO functionalized gold nanoparticle is increased to 60 nmol L⁻¹, size differences between two adjacent gold nanoparticles fade. One possible explanation for this phenomenon is that, under constant gold salt supply, increasing the PEO volume fraction can subtract the amount of gold ions been absorbed within each template particle and result in smaller but narrow-disperse nanoparticles.

Size differences between gold template nanoparticles and newly grown nanoparticles
can be utilized as an important contrast to calculate the yield of gold dimers. Well-defined gold dimers with notable internal nanoparticle size differences have been prepared under the optimized reaction condition ([HAuCl₄] = 0.7 mmol L⁻¹, [PEO-AuNPs] ≈ 45 nmol L⁻¹ and [HEPES] = 0.7 mol L⁻¹). Comparison has been made between TEM

Figure 4.7  PEO functionalized gold nanospheres (45 nmol L⁻¹) prior to (a) and after (b) incubation with HEPES (0.7 mol L⁻¹) and HAuCl₄ (0.7 mmol L⁻¹) to form gold dimers. Gold dimers with each composed of two nanoparticles with different sizes, were circled out. The population of dimers vs spheres was ~30%.
images of PEO functionalized gold nanoparticle templates and gold dimers induced with the templates (Figure 4.7a and 4.7b). Figure 4.7a shows the image of pure PEO functionalized gold nanoparticle templates. Each of the particles is presenting nanospherical shape with uniform diameter (d = 10 ± 1.8 nm) and no traces to aggregate. After incubation, some of the nanoparticle templates have been coupled individually with a second nanoparticle to show gold dimer superstructures (Figure 4.7b). Gold dimers with internal particle size differences have been circled out and the overall population was calculated to be ~ 30% according to TEM bright field images.

The inset in Figure 4.8a shows a magnified image of one of the typical gold dimers fabricated under the above reaction condition. It constitutes of two individual gold nanoparticles with different diameters, the bigger one is ~20 nm and the smaller one is ~10 nm. Size of the smaller particle is consistent with that of the PEO functionalized gold nanoparticle template (Figure 4.5a), which suggests that the particle with larger size is newly incubated from the surface of the small particle (PEO functionalized gold nanoparticle template). A “gap” formed in between the two particles with a width narrower than 1 nm is clearly observed, which is usually treated as the active SERS site. More TEM bright field images are shown in Figure 4.9 from different reaction batches. UV-visible absorption spectroscopy was used to characterize the surface plasmon resonance absorption of the nanoparticles in solution. As shown in Figure 4.8b, freshly prepared gold nanoparticle suspension (d = 10 ± 1.8 nm) produces a characteristic plasmon resonance peak (λ_{max}) at 524 nm, which red shifts to 526 nm after functionalizing with PEO. Plasmon absorption peak and width are dependent on particle
size, shape and surrounding medium. The slight red shift of $\lambda_{\text{max}}$ can be attributed to the enhanced refractive index due to PEO chain binding on gold nanoparticles. Generation of dimers based on the PEO functionalized nanoparticle template results in the $\lambda_{\text{max}}$ red-shifting to 534 nm as well as a broadening of the peak-width. This result can be attributed to the interaction of nanoparticles within each gold dimer according to Mie theory, which states that when the distance between the individual particles within a gold dimer becomes shorter than the sum of their radii, the SPR band will shift to a higher wavelength accompanied with a broadening of the width. SERS measurement (Figure 4.8c) was performed on silicon wafer which was directly spun-casted with gold dimers on the surface. It has shown a contrast of 1.05 compared to the signal from silicon wafer without gold dimers. The contrast$^{164}$ for the particles is given by:

$$\text{Contrast} = \frac{I_c - I_w}{I_w} = \frac{I_c}{I_w} - 1$$

where

- $I_c$: the integrated intensity of the Raman peak (peak area) from silicon with the particles;
- $I_w$: the integrated intensity from silicon without particles.

To estimate the enhancement factor for the signal, the ratio of the volumes contributing to the signal when particles are present, $V_{\text{SERS}}$, and to the signal without particles, $V_{\text{Raman}}$, must be taken into account.

$$\text{Enhancement Factor} = \text{Contrast} \left( \frac{V_{\text{Raman}}}{V_{\text{SERS}}} \right)$$

$$\frac{V_{\text{Raman}}}{V_{\text{SERS}}} = \frac{\pi (r1)^2 (d1)}{\pi (r2)^2 (d2)} = \frac{\pi (1 \, \mu m)^2 (0.68 \, \mu m)}{\pi (0.020 \, \mu m)^2 (0.020 \, \mu m)}$$

where

- $r1$: laser spot size;
d1: penetration depth (0.68 μm) of 514.5 nm laser light into silicon wafer;\textsuperscript{159}

r2: average radius of gold dimer;

d2: penetration depth (20 nm) of 514.5 nm laser light when particles are present.

Figure 4.8  a. TEM bright field image of gold dimers (inset is the magnified image depicting the asymmetric structure with a gap width ~1 nm); b. UV-Vis absorption spectrum of template gold nanoparticles prior to PEO functionalization (black line), after PEO functionalization (red line), and formation of gold dimers (blue line); c. SERS spectrum with gold dimers (red line) and without gold dimers (black line).
To calculate enhancement factor, estimation of effective radius of SERS area is required according to the above equation. If the radius of the dimer (~20 nm) was used to calculate $V_{\text{SERS}}$, a first approximation estimate of the enhancement factor is $\sim 10^5$. Instead, if only the radius of the gap (~1 nm) was considered, the enhancement factor can be calculated to be $\sim 10^8$. It suggests the strong potential for intense SERS of gold dimers synthesized using the current solution template method. For more intense SERS signals, laser polarization exactly parallel to the longitudinal axis is required.

![TEM bright field images of gold dimers incubated in the solution of HEPES (0.7 mol L$^{-1}$) and HAuCl$_4$ (0.7 mmol L$^{-1}$). Gold dimers, each composed of two nanoparticles with different sizes, were circled out. Inset is the magnified image showing the particle size differences. The average population of dimers vs spheres is ~30%.

Figure 4.9 TEM bright field images of gold dimers incubated in the solution of HEPES (0.7 mol L$^{-1}$) and HAuCl$_4$ (0.7 mmol L$^{-1}$). Gold dimers, each composed of two nanoparticles with different sizes, were circled out. Inset is the magnified image showing the particle size differences. The average population of dimers vs spheres is ~30%.}
In our previous work to fabricate gold-micelle hybrid nanoparticles, HEPES concentration not only affect the induced gold nanoparticle size and size distribution but also the average number of gold nanoparticles grown from each spherical PEO cluster. On one hand, when HEPES concentration is higher, nanoparticle size is smaller and distribution is narrower. On the other hand, higher HEPES concentration leads to a stronger reducing environment, thereby the available gold can be divided into more nanoparticles on each PEO clusters to make a raspberry-like structure for the final products. In the current system, increased HEPES concentration has shown positive effect on shaping nanoparticle growth. Dilute HEPES solution induces gold nanoparticle aggregation while concentrate HEPES solution produces well-defined gold dimers. However, different from the previous results (Chapter III), images shown in Figure 4.6 doesn’t follow the same trend to present superstructures other than gold dimers, e.g. trimer or tetramer, with enhanced HEPES concentration. One explanation for the phenomenon is attributed to limited number of PEO chains tethered on each nanoparticle template. Considering a spherical micelle with the radius of the core \( R_{\text{core}} \) formed by a copolymer in which the degree of polymerization of core-forming PS block is \( N_{\text{PS}} \), total chain number of each micelle can be calculated from the equation below:

\[
N_{\text{agg}} = f \frac{(4/3)\pi R_{\text{core}}^3}{V_s N_{\text{PS}}}
\]

where

\( V_s \): Volume per polystyrene repeat unit (0.167 nm\(^3\));

\( f \): volume fraction of PS blocks.
For the micelles with $R_{\text{core}} \sim 15$ nm, $N_{PS} \sim 442$, $f \sim 83\%$, the total chain number is estimated to be $\sim 161$. If the diameter of the core is reduced to 5 nm, according to the calculation,

![Micelle Diagram](image1)

![PEO-AuNP Diagram](image2)

Figure 4.10  Comparison between gold-micelle superstructure and gold-PEO-gold superstructure incubated in solution of NaBH$_4$ and HAuCl$_4$. TEM bright field images show distinct structural difference, which suggests that the two templates are not identical.
number of the block copolymer chain in each micelle is estimated to be less than 10. Taking into account the process that thiol-capped PEO chains are gradually binding onto the 10 nanometer gold nanoparticle surface, each polymer chain can create an exclusive volume after immobilization and prevents the second chain coming into the same volume. Different from micellization, the step by step binding mechanism can set a maximum loading of polymer chains on each particle with predetermined particle size, which can further affect the mount of gold ions attracted and the number of potential nucleation sites. Comparison was made between PEO-b-PS micelles and PEO functionalized gold nanoparticle through incubation micelles and PEO functionalized gold nanoparticles in NaBH$_4$ (0.01 mol L$^{-1}$) and HAuCl$_4$ (0.07 mmol L$^{-1}$) solution separately. After incubation, spherical nanoparticle seeds formed within the micelle corona while irregular-shaped nanocrystals were induced from the gold nanoparticle surface (Figure 4.10). Given the same PEO molecular weight, the observation suggests that PEO chain number and density on gold nanoparticle templates can be different from that of the micelles, which can result in the structure differences of final products. To test the hypothesis, non-entangled Gaussian chain model was taken into account to estimate the average number of PEO chains on each gold nanoparticle according to an established method with slight modification.$^{165}$ Figure 4.11 shows a schematic illustration of single PEO chain tethered on the gold nanoparticle surface. The chain can rotate in a cylindrical fashion about a fixed point. In the present model, the fixed point is defined by the gold-thiolate bond. Assuming the PEO chain can be modelled as a random coil with a radius gyration ($R_g$) to be calculated using the following equation:
\[ R_g = a \cdot \sqrt{N / 6} \]

where

\( a \): statistical segment length (7.0 Å for PEO\(^{160}\));

\( N \): number of repeating unit.

When \( N = 227 \), \( R_g = 4.2 \) nm.

Figure 4.11 Side and top projection of a single PEO chain immobilized onto a gold nanoparticle surface through the gold-thiolate bonding. The top view shows projection of a rotating chain onto a surface.
The circular projection shown in Figure 4.11 implies that the PEO chain creates a void space with the radius equal to $R_g$. The footprint of each of the PEO chain on the surface of the nanoparticle can be estimated to be approximately equal to $\pi r^2$, where $r = R_g$. Therefore the area covered by the respective PEO is given by $\pi (R_g)^2$. The surface

Figure 4.12  Footprint of PEO void space on gold nanoparticle surface. Each circle represents the surface area occupied by a single PEO chain on gold nanoparticle.
area occupies by each PEO chain ($M_n = 10$ kDa) is $56 \text{ nm}^2$. Assuming gold nanoparticle surface was fully covered by PEO chains and each chain occupied a spherical footprint, there is void space among spherical circles, as shown in Figure 4.12. Three orthorhombic unit cells are shown, with the surface area of the orthorhombic cell is $3\sqrt{3}d^2$. The area of the two circles in that unit cell is $3\pi d^2/2$. The ratio of these numbers is 0.907. So the number of PEO chains on each gold nanoparticle surface can be calculated as follows:

$$n = 0.907S_{\text{AuNP}} / S_{\text{PEO}}$$

For the gold nanoparticles with an average diameter $\sim 10$ nm,

$$n \approx 6$$

Figure 4.13  Control experiment I: PEO modified gold nanoparticle ($d = 20 \pm 3.4$ nm) before (a) and after (b) surface nucleation. More than one gold nanoparticle was incubated from each particle template.
To verify the effect of PEO chain loading of each nanoparticle template on structure of the final products, control experiments have been done using enlarged gold nanoparticle templates to afford enhanced PEO chain payload for each of them. Diameter of gold nanoparticle template was nearly doubled ($d = 20 \pm 3.4$ nm, Figure 4.13a). Accordingly, average PEO chain number on each nanoparticle was increased to $\sim 25$ based on above calculation. In order to keep overall PEO volume fraction constant and leave average chain number on each nanoparticle the only difference between the two

Figure 4.14 Control experiment II: PEO functionalized gold nanoparticle ($d = 48 \pm 6.9$ nm) before (a) and after (b) surface nucleation. The product presents raspberry-like superstructure with the core surrounded with multiple newly grown gold nanoparticles.
systems, concentration of gold nanoparticle template (d = 20 ± 3.4 nm) was reduced to ~8 nmol L⁻¹ in the incubation solution ([HAuCl₄] = 0.7 mol L⁻¹ and [HEPES] = 0.7 mmol L⁻¹). After reaction, more than one gold nanoparticle was induced from each nanoparticle template (Figure 4.13b). The inset in Figure 4.13b shows a magnified image of gold nanoparticle superstructure with a core surrounded with 3 nanoparticles on the surface. Each particle has a diameter ~ 20 nm.

To enhance the size contrast between template nanoparticles and newly grown nanoparticles, freshly prepared gold nanoparticles with even larger diameters (d = 48 ± 6.9 nm) were functionalized with PEO chains. Under the same assumption, average number of PEO chains on each of the enlarged particle was estimated to be ~140 to achieve an overall coverage. TEM bright field images of the gold nanoparticles tethered with PEO are shown in Figure 4.14a. Particles are evenly dispersed and no aggregation has been observed. With the same reaction procedure, PEO functionalized gold nanoparticles suspensions (~ 2.5 nmol L⁻¹) were incubated with HAuCl₄ (0.7 mmol L⁻¹) and HEPES (0.7 mol L⁻¹). Based on the above PEO chain number estimation, concentration of PEO functionalized gold nanoparticles (d = 48 ± 7.9 nm) was reduced by a factor of 18 in order to keep the PEO volume fraction roughly the same as that in the above two incubating solutions, leaving ratio of PEO chain number to gold particle template number the only difference. Figure 4.14b shows the TEM images of nanoparticle superstructures after surface nucleation. PEO functionalized gold precursors have been observed to be surrounded with multiple newly grown gold nanoparticles with distinguishably smaller sizes. The inset in Figure 4.14b shows a
magnified TEM bright field image of the gold superstructure, clearly depicting the raspberry-like structure with multiple gold nanoparticles (d ≈ 20 nm) grown from the original nanotemplate (d ≈ 48 nm). With the same overall PEO volume fraction and HEPES concentration, number of newly formed gold nanoparticle from each gold nanoparticle template is increasing as size of the latter becomes larger. The control experiments have provided the direct evidence that PEO chain loading on each particle has been playing a primary role in determine superstructures of the final products. It has also explained the phenomenon that formation of gold dimers took place when the size of gold nanoparticle templates was small (d = 10 ± 1.8 nm).

4. 3 Conclusions

In conclusion, PEO functionalized gold nanoparticles (d = 10 ± 1.8 nm) have been used to template well-defined gold dimer formation in situ under proper reaction condition. The synthesized gold dimers are able to support strong SERS signals. Gold dimers with reduced symmetry have been identified and particle size differences within each pair have been utilized as the contrast to distinguish newly grown particle from the particle template. Average PEO chain loading on each gold nanoparticle template is playing a central role in determining the structure of final product. Through increasing template nanoparticle size to enhance PEO chain payload, structure of the final product can be manipulated from gold dimer to raspberry-like structure. The cost-effective fabrication method we have provided here requires least surface modification, one-pot reaction process and mild reaction condition. It contributes to broadening existing
“bottom-up” fabrication strategies for gold dimer nanostructures that can be applied for SERS measurement. This method can also be applied in fabricating gold-related heterometallic superstructures through carefully choosing nanoparticle templates that can be functionalized by PEO chains. With more specific chemical modification, a wide range of nanoparticle organizations can be envisioned, which are useful in testing electromagnetic theories in various electronic or optoelectronic applications.
CHAPTER V

NANOMANUFACTURING OF GOLD NANOPARTICLE ARRAYS USING PEPTIDE-DERIVATED BLOCK COPOLYMER TEMPLATES

5.1 Motivation

Collective surface plasmons (SPs) generated by two-dimensional (2-D) gold nanostructures are attractive due to their potentials to allow us channeling light within sub-wavelength scale (plasmonics) and conducting single molecular detection (plasmonic sensing). However, methods for fabricating programmable, highly ordered arrays of gold nanoparticles with nanoscale precision are limited. Electron beam (E-Beam) lithography and focused ion beam (FIB) lithography are two typical fabrication techniques from the “top-down”. However, E-Beam and FIB methods are expensive and time-consuming, especially at scales below 100 nm and developing patterns over large areas remains challenging. Moreover, these methods will not work at length scales below 20 nm. Alternatively, methods from the “bottom-up”, e. g. programmable DNA template, polymer-nanoparticle blend and polymer thin film template allow complex nanostructures to be fabricated with smaller size and lower cost. Nevertheless, each method has certain shortcomings. Even though well-defined gold nanoparticle superlattice can be incubated with this method, two issues limit their use in solid-state device integration: limited scalability and the structural
instability during processing and drying. Nanocomposites based on interaction between gold nanoparticles and functionalized polymers have shown higher stability and can be fabricated over macroscopic distance. Nevertheless, there is a thermodynamic and kinetic gap that separates the respective phase behavior of the thin film and bulk materials due to surface interactions and finite size effects. The gap further limits the transfer of highly-ordered arrays from bulk to thin film. Investigators have selectively deposited surface functionalized gold nanoparticles that were tuned to have wetting preference to one block, onto polymer thin films with microphase separation. While the approach overcomes the thermodynamic issues, it introduced additional challenges regarding the optimal nanoparticle surface modification.

In this work, we are highly motivated to design a facile and cost-effective approach for 2-D gold nanoparticle arrays. The strategy we have come up with is to directly manipulate 2-D hierarchical gold nanoparticle arrays on the surface of peptide functionalized polymer thin films with well-defined nanopatterns. Selectively implementing gold-binding affinity to a peptide tethered to the chain end of one polymer phase limits the challenges associated with gold nanoparticle surface modification. In addition, the aqueous solution-based processing method yielded hexagonally close packed gold nanoparticle clusters and parallel gold nanowires-based on selectively deposit gold nanoparticles on to block copolymer thin film.
5.2 Results and Discussion

Figure 5.1 briefly illustrates the strategy for highly-ordered 2-D hierarchical gold nanostructure fabrication based on poly(styrene-block-methyl methacrylate)-A3 peptide.

PS-b-PMMA-A3 peptide was synthesized and fabricated into thin films with highly-ordered hexagonal and linear surface patterns. The thin film templates were used to direct gold nanoparticles self-assembly on PMMA domains to form 2-D hierarchical hexagonal gold nanoparticle clusters and parallel gold nanowires. Our surface directed approach is highly versatile and with further refinement, can be adapted to 3 dimensional (3-D) systems.
(PS-b-PMMA-A3) thin film. PS-b-PMMA is a typical BCP system with strong segregation that can self-assemble into periodical structures such as cylinders, lamellae, gyroid, etc. A3 peptide (AYSSGAPPMPPF), identified through phase display, has shown strong binding affinity to gold nanoparticles with the strength of adsorption calculated to be -63 kcal/mol for {111} surface and -9 kcal/mol for {100} surface. 

Figure 5.2 Synthetic route for A3 peptide-deviratized block copolymer bioconjugates.

PS-b-PMMA was synthesized through a sequential polymerization followed by the “click” chemistry to tether A3 peptide at the end of PMMA.
Tethering A3 peptide at PMMA chain end has embedded preferential wetting ability to gold nanoparticles into PMMA domains of the polymer thin film. Through carefully controlling fabrication process, the hybrid molecule PS-b-PMMA-A3 can be engineered into thin films with either perpendicular or parallel PMMA cylinders to guide free-standing gold nanoparticle arrangement on the surface.

Figure 5.3 ESI-MS spectrum of alkyne functionalized A3 peptide. Monoisotopic mass-to-charge ratios (m/z) are valued: 1337.5 (Alkyne-A3 peptide +Na⁺); 1353.5 (Alkyne-A3 peptide +Na⁺+K⁺-H⁺); 1359.5 (Alkyne-A3 peptide +2Na⁺-H⁺).
PS-b-PMMA-A3 ($M_n = 59.5$ kDa, $\Phi_{PS} = 70.7\%$, $\Phi_{PMMA} = 29.3\%$) was synthesized through sequential atom-transfer radical-polymerization (ATRP) from PS to PMMA, followed by “click” chemistry to conjugate A3 peptide at the end of the PMMA block (Figure 5.2). Molecular weight of A3 peptide with an alkyne end group was determined.

![Figure 5.4 SEC spectrum (THF, RI detector, PS standard) of PS-Br and PS-b-PMMA-Br. PS-Br was polymerized and used as the macromolecular initiator to grow PMMA block. PS-Br: $M_n = 42.1$ kDa, $M_w = 43.9$ kDa, PDI = 1.02. PS-b-PMMA-Br: $M_n = 59.5$ kDa, $M_w = 69.0$ kDa, PDI = 1.16.](image)
with electrospray ionization mass spectrometry (ESI-MS) in Figure 5.3. All three components (1337.5: alkyne-A3 peptide + Na+, 1353.5: alkyne-A3 peptide+ Na+K-H+, and 1359.5: alkyne-A3 peptide + 2Na-H+) suggests the successful synthesis.

Figure 5.5 ¹H NMR (CDCl3, 300 MHz, ppm, δ) spectrum of PS-Br (a) and PS-b-PMMA-Br (b). PS-Br was polymerized and used as the macromolecular initiator to grow PMMA block. PS-Br: 6.30-7.40 (br, 1980H, phenyl rings), 3.50 (s, 3H, CH₃O⁻), 1.67-2.15 (br, 396H, -CH₂CH(-Ar)-), 1.20-1.67 (br, 792H, -CH₂CH(-Ar)-), 0.93 (s, 6H, -(C=O)C(CH₃)₂). PS-b-PMMA-Br: 6.30-7.40 (br, 1980H, phenyl rings), 3.35-3.60 (br, 522H, CH₃O⁻), 1.67-2.15 (br, 396H, -CH₂CH(-Ar)-), 1.20-1.67 (br, 792H, -CH₂CH(-Ar)-), 0.60-1.00 (br, 522H, -(CH₃⁻)C(O) -).
The molecular characterization of the PS precursor and PS-b-PMMA block copolymer was determined by size exclusion chromatography (SEC) shown in Figure 5.4 Figure 5.6 FT-IR spectrum of PS-b-PMMA-Br, PS-b-PMMA-N₃ and PS-b-PMMA-A3. Typical absorption of azide functional group is at 2100 cm⁻¹. In this reaction, the azide signal showed up when PS-b-PMMA-Br was converted to PS-b-PMMA-N₃ and disappeared after “click” reaction to tether A3 peptide at the PMMA end.
$^1$H NMR spectrum (Figure 5.5) was used to confirm the molecular weight of PMMA block with the following equation:

$$M_{m}^{PMMA} = M_{m}^{PS} \times \left( \frac{S_B}{S_A} \times \left( \frac{104 \times 3}{100 \times 5} \right) \right)$$

where

$S_A$: Integration of the peak at 3.5 ppm (-OCH$_3$);

$S_B$: Integration of broad peaks between 7.40 and 6.30 ppm (protons on phenyl rings of PS block)

Total molecular weight of PS-b-PMMA block copolymer was calculated to be 59 500 Da, with volume fraction of PS ~70.7%, and PMMA ~29.3%. Conjugation of alkyne-A3 peptide with PS-b-PMMA-N3 was characterized through Fourier transform infrared (FTIR) spectrum (Figure 5.6). Modification of azide group (-N$_3$) at PMMA chain end gave rise to the absorption peak at 2100 cm$^{-1}$, which disappeared after click reaction.

Quartz crystal microbalance (QCM) was used to assess the availability of the A3 peptide on the surface on the BCP thin film and the non-specific adsorption of the gold nanoparticles to the BCP surface. The QCM measurement indicated that the gold nanoparticles absorbed with significant selectivity to the PS-b-PMMA-A3 relative to PS-b-PMMA, and that the gold nanoparticles were bound to an extent that they remained immobilized upon rinsing. Frequency changes were measured by flowing a gold nanoparticle solution (30 nmol L$^{-1}$, pH 5.5) through sensor chips spin-coated (2.0 wt% in toluene, 4000 rpm, 30 sec) with PS-b-PMMA-A3 and PS-b-PMMA (d$_{thickness}$ (PS-b-MMA-A3) $\approx$ 150 nm, d$_{thickness}$ (PS-b-MMA) $\approx$ 150 nm) respectively (Figure 5.7a).

When the gold nanoparticle solution was introduced to the PS-b-PMMA thin film, the
QCM frequency dropped slightly indicating non-specific adsorption, remained constant during incubation and recovered to the original values as the gold nanoparticle solution was washed out. This subtle frequency drop can be attributed to a solution density.

![Graph showing QCM measurement](image)

**Figure 5.7** Comparison of wetting ability to gold nanoparticles between A3-functionalized BCP and non-A3 functionalized BCP. a. QCM measurement of PS-b-PMMA (black) and PS-b-PMMA-A3 (red) in gold nanoparticle water solution (30 nmol L⁻¹, pH 5.5); sharp frequency drop of PS-b-PMMA-A3 suggests stronger wetting ability to gold nanoparticle compared to PS-b-PMMA; b. AFM image of pre-annealed PS-b-PMMA thin film after gold nanoparticle solution (30 nmol L⁻¹, pH 5.5, 6 h) incubation; few gold nanoparticles were immobilized; c. AFM image of pre-annealed PS-b-PMMA-A3 thin film after gold nanoparticle solution (30 nmol L⁻¹, pH 5.5, 6 h) incubation; gold nanoparticles were immobilized and formed clusters.
difference between water and the gold nanoparticle suspension. The recovery of the oscillation frequency shows that very few gold nanoparticles bind non-specifically to the thin film without A3 peptide. However, a sharper, irreversible frequency drop was observed for polymer end-functionalized with A3 peptide, indicating both the availability and selective immobilization of gold nanoparticles to the PMMA domains. To confirm the wetting differences of gold nanoparticles between thin film with and without the attached A3 peptide, control experiments incubating both pre-thermal annealed (180 °C, 48 h) PS-PMMA-A3 and PS-b-PMMA thin films were performed in concentrated gold nanoparticle solutions (30 nmol L⁻¹, 6 h). Few nanoparticles have been observed on PS-b-PMMA thin film as shown in the AFM phase image in Figure 5.7b. In contrast, gold nanoparticle clusters covered the majority of the PS-PMMA-A3 thin film surfaces after incubation (Figure 5.7c). The control experiments provide direct evidence that gold nanoparticles have a selective and strong affinity to the A3 functionalized BCP compared to the peptide-free BCP.

Creating well-defined gold nanoparticle hierarchical structures involves two essential steps. The first step requires the generation of a highly ordered BCP template with perpendicular oriented PMMA cylindrical domains with surface-exposed A3. The second step is the selective adsorption of gold nanoparticles onto the A3 functionalized PMMA domains. Conventional thermal annealing (Temperature ‘T’ = 180 °C, 48 hr) of PS-b-PMMA-A3 in a vacuum oven leads to a ‘finger print’ horizontal morphology shown in Figure 5.8a. After static thermal annealing for 48 h, polymer chains attain their equilibrium morphology. Static-thermal-annealed PS-PMMA-A3 films exhibit PMMA
cylindrical domains that show horizontal orientation with the lattice spacing of \( \sim 48 \text{ nm} \). The observed spacing between two adjacent cylinders shown in Figure 5.8a was schematically depicted in Figure 5.8b, which is smaller than the distance between two neighboring cylinders in 3-D space. In order to fabricate highly-oriented thin film which allows to template gold nanoparticles, PS-PMMA-A3 was coated on quartz substrates and subsequently annealed through cold zone annealing with a sharp temperature gradient.

Figure 5.8  Comparison between observed domain spacing and theoretical domain spacing  a. AFM image of PS-b-PMMA thin film after static thermal annealing; observed cylinder-to-cylinder spacing is 48 nm, which is smaller than the real cylinder-to-cylinder spacing in 3-D space; b. schematic illustration of the observed cylindrical domain spacing in 3-D space.
termed as CZA-S. CZA-S annealing of PS-b-PMMA films at temperature much below the BCP order-disorder transition temperature was previously shown to rapidly orient cylinders perpendicular to the substrate.\textsuperscript{132} CZA-S annealing of PS-PMMA-A3 thin films ($T_{\text{max}} = 210^\circ \text{C}$, Temperature Gradient ‘$\nabla T’ \sim 45^\circ \text{C/mm}$, Velocity ‘$v’ = 5 \mu \text{m/s}$) led

![Comparison between observed domain spacing and theoretical domain spacing](image)

**Figure 5.9** Comparison between observed domain spacing and theoretical domain spacing  

a. AFM image of PS-b-PMMA-A3 thin film after CZA-S; observed cylinder-to-cylinder spacing between PMMA phase is 55 nm; b. schematic illustration of the observed cylinder-to-cylinder spacing in 3-D space, which is identical to the theoretical cylinder-to-cylinder spacing.
to similar results i.e. vertically oriented PMMA cylinders with A3 linkages as can be seen in Figure 5.9a. CZA-S annealed PS-PMMA-A3 films exhibit hexagonal close-packed vertically orientated PMMA cylinders with a cylinder-to-cylinder spacing of \( \approx 55 \) nm (Figure 5.9b). According to the equation:

\[
l_o = \frac{\sqrt{3}}{2} l
\]

where

\( l \): theoretical cylinder-to-cylinder spacing;

\( l_o \): observed cylinder-to-cylinder spacing.

\( l_o \) is calculated to be \( \sim 48 \) nm, which is consistent with the measurement in Figure 5.8a. Phase and height images of a 5 \( \mu \text{m} \times 5 \mu \text{m} \) hexagonally close packed thin film template are shown in Figure 5.10. The 3-D height profile indicates that heights of PMMA cylinders are in the range less than 2 nm.

In the second step, gold nanoparticles, as an inorganic target with an average diameter \( \sim 12 \) nm, were prepared according to an established citrate-based procedure without additional surface modification.\textsuperscript{150} Three competing phenomena direct the gold nanoparticle self-assembly on the thin film surface: 1) A3 peptide availability at the free surface of the vertically oriented PMMA-A3 section is given by a probability distribution, depending on chain end conformation ending at the free surface as well as the surface wetting properties of the A3 peptides; 2) selective immobilization of gold nanoparticles to the PMMA-A3 exposed domain of the thin film, 3) gold nanoparticle aggregation which can simultaneously affect resolution of the final hierarchical structure. According to Figure 5.7c, 6 h incubation of thin film with 30 nmol L\textsuperscript{-1} gold nanoparticle incubation
solution led to gold nanoparticle clusters of which the sizes are large enough to covering both PS and PMMA domains. In order to depress interactions of nanoparticles to reduce aggregation, peptide-thin film template was immersed in a dilute gold nanoparticle solution (~3 nmol L⁻¹) under vigorous stirring.

Figure 5.10  AFM image of PS-b-PMMA-A3 thin film with a 5 µm × 5 µm scan area after CZA-S.  a. Thin film presents highly-orient cylinders perpendicular to the substrate; b. the corresponding 3-D height image shows that height of PMMA cylinders is under 2 nm.

AFM phase images depicting the formation of gold nanoparticle hierarchical patterns on the templates were recorded for different incubation periods (Figure 5.11).  Figure
5.11a shows the template prior to gold nanoparticle deposition. After 8 h incubation, a small population of gold nanoparticles was sporadically dispersed on the thin film surface (Figure 5.11b), which steadily increased with prolonged incubation time (Figure 5.11c).

![Image](image.png)

Figure 5.11 Time-dependent self-assembly of hexagonally close packed gold nanoparticle clusters on thin film template with perpendicular oriented cylinders. The cylindrical array was incubated in dilute gold nanoparticle water solution (3 nmol L\(^{-1}\), pH 5.5) for different periods of time: a. 0 h; b. 8 h; c. 16 h, d. 24 h and e. 32 h. Coverage of gold nanoparticles on thin film increased with incubation time.
The physico-chemically attached gold nanoparticles to the peptide patterned surface presented a hierarchical hexagonal gold pattern after 24 h (Figure 5.11d). While free gold nanoparticles were deposited on the PMMA domain of the thin film, they interacted with nanoparticles immobilized on the film surface to form aggregates large enough to cover the PS domains. As the incubation time was increased to 36 h, gold nanoparticle aggregation spreads all over the template, as shown in Figure 5.11e.

Figure 5.12  AFM image of hexagonally close packed gold nanoparticle clusters. Inset is the magnified image showing hexagonal repeating units with a domain spacing ~57 nm.
Incubated under the identical condition as shown in Figure 5.11d, Figure 5.12 shows a high resolution AFM phase image displaying the in situ self-assembly of hexagonally close packed gold nanoparticle hierarchical structure. The inset image is an enlarged area showing the repeating units of hexagonal configuration. The center-to-center distance of two adjacent gold nanoparticle cluster domains is measured to be ~57 nm,

Figure 5.13  a. AFM phase image of hexagonally close packed gold nanoparticle clusters on PS-b-PMMA-A3 thin film template; b. the corresponding 3-D height image shows an average height of the gold cluster domain of ~10 nm.

which is consistent with that between the neighboring PMMA domains of PS-b-PMMA-A3 thin film template underneath. The observation suggests that
hexagonally close packed A3 peptide domains in the thin film are able to induce gold nanoparticle arrangement on the surface. However, aggregation of gold nanoparticles can result in different gold cluster domain sizes and deviation of gold-dot location, thereby leading to defects. Figure 5.13 shows the high resolution phase (a) and height (b) of the template after gold nanoparticle deposition. The 3-D height image in Figure 5.13b indicates that height of the gold nanoparticle layer is ~10 nm, which is consistent with the average gold nanoparticle diameter (~12 nm). It suggests that there is a single layer of gold nanoparticles on the thin film. However aggregates were not eliminated. Grazing incidence small angle X-ray scattering (GISAXS) was performed to dimensionally probe the gold nanoparticle superstructure in conjunction with the block copolymer morphology. The GISAXS measurements of these films are shown in Figure 5.14. The X-ray incident angle was chosen to be at the first waveguide resonance, which is just above the critical angle of the BCP film. This ensured that the X-ray’s homogeneously illuminated the entire film. Figure 5.14a shows the typical GISAXS scattering pattern that is seen for these films after gold deposition. The inset in Figure 5.14a shows the GISAXS scattering pattern of the BCP film prior to gold deposition. It shows the presence of vertical streaks which are characteristic of vertically oriented cylinders. This is in accordance to previous results on CZA-S annealing of BCP thin films. The first order scattering peak position in Figure 5.14a coincides with the domain spacing of the block copolymer lattice, which is the primary lattice. The higher order scattering, as seen in Figure 5.14a, is characteristic of the gold nanoparticle lattice. Figure 5.14b shows the integrated intensity of Figure 5.14a. The gold nanoparticle
scattering peak positions are indicated in the figure. The first order peak position of the nanoparticle lattice has the value \( q \approx 0.523 \text{ nm}^{-1} \) corresponding to a lattice spacing \( \approx 12 \text{ nm} \).

Figure 5.14  a. GISAXS scattering pattern for film after gold nanoparticle deposition; (inset is the GISAXS scattering pattern of the BCP film prior to gold nanoparticle deposition); b. Integrated intensity of GISAXS scattering pattern of thin film template after gold nanoparticle deposition shows a primary lattice spacing of 48 nm and the secondary lattice being the gold nanoparticle lattice with a lattice spacing of 12 nm.
The nanoparticles are roughly 12 nm in size as was discussed earlier. Therefore, the lattice spacing is equal to the gold nanoparticle size, and this is only possible when the gold nanoparticles are touching each other. Additionally, the second order scattering peak for gold lattice has the value $q \sim 0.91 \text{ nm}^{-1}$. The relative peak position for gold lattice is $1: \sqrt{3}$, which indicates the gold nanoparticles are arranged in hexagonally close packed lattice with a domain spacing of 12 nm. These quantitative observations confirm the presence of gold nanoparticle super-lattice, the primary lattice being the block copolymer lattice with a lattice spacing of 48 nm and the secondary lattice being the gold nanoparticle lattice with a lattice spacing of 12 nm. The observation of gold lattice formation provides evidence as to the interaction of immobilized gold nanoparticles on the PS-PMMA-A3 surface compared to the free gold nanoparticles suspended in solution. Given a dilute solution and adequate incubation time, gold nanoparticles can self-anneal themselves to attain “lower energy” or affinity binding positions on the BCP surface, such as with the A3 peptide. Given the density of the A3 peptide on the top surface is not 100% due to chain end conformations ending up in film interior as well, the high density of hexagonally close packed gold nanoparticles observed is in fact remarkably high. Plasmon absorption of gold nanoparticles immobilized on thin film was compared with those dispersed in solution through UV-Visible spectroscopy measurement in Figure 5.15. The well dispersed gold nanoparticles water solution exhibiting a deep-red color presented in a sharp absorption peak at 525 nm (Figure 5.15a). Inset of the plot is the TEM bright field image of individual gold nanoparticles. The plasmon peak red shifted to 555 nm together with a peak width broadening after gold nanoparticles were
immobilized onto thin film (Figure 5.15b). The above signal change is attributed to plasmon coupling resulted from the formation of gold nanoparticle cluster within each domain, which leads to a higher wavelength absorption.

![Figure 5.15](image.png)

**Figure 5.15** Comparison between plasmon absorption spectrum of gold nanoparticles in solution and on template  

a. UV-Vis absorption of gold nanoparticle in water suspension with the peak absorption wavelength at 525 nm (inset is the TEM bright field image of the gold nanoparticles); b. UV-Vis absorption of hexagonally closed packed gold nanoparticle clusters arranged on template with the peak absorption wavelength at 555 nm. Plasmon absorption peak shifted to a higher wavelength accompanied with a width broadening after deposition due to the formation of nanoparticle clusters.
One of the key advantages of using a thin film as a template lies in its morphological tunability which can be used to guide different gold nanoparticle patterns. To illustrate the idea, we have modified the thin film processing procedure to generate parallel PMMA cylinders for 2-D gold-lines assembly. Thin films with highly oriented parallel cylinders along the substrate were obtained through soft-shear CZA annealing (termed ‘CZA-SS’ annealing) the BCP thin film. Like CZA-S annealing, CZA-SS annealing is a continuous process that is compatible with roll-to-roll processing. Excitingly, CZA-SS

Figure 5.16 Comparison between observed cylinder-to-cylinder domain spacing and theoretical domain spacing  a. AFM image of PS-b-PMMA-A3/PS-b-PMMA thin film after CZA-SS with the observed cylinder-to-cylinder spacing of 37 nm; b. schematic illustration of the observed cylinder-to-cylinder spacing (37 nm) and the theoretical cylinder-to-cylinder spacing (43 nm) in 3-D space.
is an extremely rapid process where pristine unidirectional BCP line patterns can be fabricated at the rate of 12 mm/min. In order to demonstrate that this novel nanomanufacturing procedure is robust as well at the lower lattice spacing and with heterogeneous BCP systems, we prepared a 1:1 (wt./wt.) blends of PS-b-PMMA-A3 with

Figure 5.17  Time-dependent AFM images shows the self-assembly of gold nanowires on thin film template with horizontal oriented cylinders. Template was incubated in dilute gold nanoparticle water solution (3 nmol L⁻¹, pH 5.5) for different periods of incubation time: a. 0 h; b. 16 h; c. 32 h; d. 48 h and e. 64 h. Coverage of gold nanoparticles on template increased with incubation time.
non-A3 functionalized PS-b-PMMA with a number-average molecular mass of 35 kDa. With the addition of smaller molecular mass PS-b-PMMA, the observed BCP lattice spacing decreased to 37 nm, compared with 48 nm in PS-b-PMMA-A3 thin film without blending (Figure 5.16a). Based on the observed spacing between adjacent cylinders, the cylinder-to-cylinder distance in real 3-D space is calculated to be ~43 nm (Figure 5.16b).

Figure 5.18 Experimental and theoretical illustration of the template control on minimum gold nanoparticle line-to-line distance. a. Parallel gold nanowires after an incubation time of 40 h; b. an enlarged area showing the linear configuration of the gold nanoparticle arrangement with the minimum line-to-line distance ~80 nm; c. schematic illustration of the ideal minimum cylinder-to-cylinder distance (74 nm) on the template surface to induce gold nanowires.
Blending of non-A3 functionalized polymer also resulted in a reduction of peptide chain concentration in each PMMA domain, which further weakened the preferential wetting ability of thin film to gold nanoparticles. In order to compensate for this decrease in peptide concentration, we have doubled the incubation time to study gold nanoparticle self-assembly. In Figure 5.17a, a small amount of gold nanoparticles have been immobilized and notably aligned on the thin film template. Immobilized nanoparticle population was increasing over time and gradually reached a full coverage after 64 h of incubation time (Figure 5.17b – d). However, the weakened affinity and selectivity of thin film template to gold nanoparticles and elongated incubation time in turn facilitates the formation of nanoparticle aggregation. During incubation, these two competing issues appear to be similar in rate and binding amount. Furthermore, the enhanced coverage of gold nanoparticles on the template is accompanied by growing aggregation grains, which renders fabrication of well-defined gold-lines with high resolution difficult. Figure 5.18a shows a 2-D gold-lines surface pattern after 40 h incubation, where straight and parallel gold nanoparticle lines can be observed partially covering the thin film and propagating across the overall a 2 µm × 2 µm scanning area. A magnified image is shown in Figure 5.18b, where gold nanoparticle has been aligned anisotropically and displayed linear configuration. In this area, the distance between two adjacent gold lines was measured to be ~ 80 nm, nearly two times the BCP lattice spacing shown in Figure 5.16a, with an explanation as illustrated in Figure 5.18c. The observed ~37 nm spacing comes from cylinders from two adjacent layers (blue and yellow). In the same layer, the shortest distance between two cylinders is ~ 74 nm, two times of that from the
observation. When the layer is exposed to gold nanoparticle solution, instead of 37 nm, the closest gold line-to-line distance should be 74 nm, which is close to the ~80 nm spacing we have observed. In the ideal circumstance, when cylinders in the same layer are exposed to gold nanoparticle solution, the closest gold line-to-line distance should be 74 nm instead of 37 nm, which is close to the ~80 nm spacing we have observed in Figure 5.18b. Even though the parallel cylindrical thin film is able to template the line-to-line spacing.

![Figure 5.19](image)

**Figure 5.19** Comparison between plasmon absorption spectrum of gold nanoparticles in solution and on template  

a. UV-Vis absorption of gold nanoparticles in water suspension with the absorption peak at 525 nm (inset is the TEM bright field image of the gold nanoparticles); b. UV-Vis absorption of parallel gold nanowires arranged on template with the absorption peak at 555 nm. Plasmon absorption peak shifted to a higher wavelength accompanied with a width broadening after deposition due to the reduced interparticle spacing.
distance, distribution of nanoparticles within each line varies significantly, from single gold nanoparticle to cluster. This phenomenon can be attributed to unevenly dispersed peptide chains in each PMMA column and/or the interaction between immobilized gold nanoparticles to free ones in solution. The area where more gold nanoparticle are located is more likely to form nanoparticle aggregates as incubation time goes on. Further incubation of the thin film increases coverage of gold nanoparticle and aggregation simultaneously, as shown in Figure 5.17. Although complete nanoparticle coverage has not been achieved, the above free-standing gold nanoparticle thin film is able to afford SPs. Compared with that of well-dispersed gold nanoparticles in water solution (Figure 5.19 a), gold nanoparticles shows a broad absorption spectrum of which

![Polymer and Peptide](image)

Figure 5.20 Future strategy: increasing peptide chain number at the end of PMMA block to enhance the binding affinity and selectivity of thin film to gold nanoparticles.
the peak centered ~ 550 nm (Figure 5.19b). The plasmon peak red-shifting and broadening after gold nanoparticle deposition is attributed to formation of closely packed nanoparticle clusters as shown in Figure 5.18b. Taking advantage of CZA-SS technique, we have successfully demonstrated robustness of the peptide-polymer BCPs to induce gold nanoparticle self-assembly and arrangement. Moreover, results on the above two gold nanoparticle superstructures (gold nanoparticle clusters and gold nanowires) support the fact that peptide concentration has played an important role in balancing the competing issues regarding gold nanoparticle deposition. Enhanced peptide concentration can strengthen the preferential wetting ability of thin film to gold nanoparticles, correspondingly, reduce the possibility to form nanoparticle aggregates if one carefully maintains the incubation process. These offer a promising pathway to solve the paradox and reduce defects of the hierarchical structures in the future. A strategy for the molecular design is shown in Figure 5.20.

5.3 Conclusions

In summary, we have demonstrated a scalable continuous processing approach to precisely assemble gold nanoparticles into well-defined 2-D arrangements over macroscopic distances. A peptide-derivatized block copolymer, PS-b-PMMA-A3, was synthesized in order to couple the binding affinity of A3 peptide to gold nanoparticle while templating the microphase separation of the BCP. A CZA technique dynamically fabricated the hybrid BCP into hexagonally well packed thin films that selectively bind gold nanoparticles through interactions with the attached A3 peptide at the PMMA chain.
end. Using a thin film template, 2-D hexagonally close packed hierarchical structures were demonstrated by controlling gold nanoparticle incubation conditions to maximize selective immobilization of nanoparticles to the template but minimize nanoparticle aggregation. Under the same principle, 2-D gold nanowire configuration has been successfully fabricated by changing CZA processing procedures to softshear mode. The ability to separate formation of organic template from gold nanoparticle decoration overcomes stability issues arising from traditional aqueous assembly provide new opportunities to fabricate 2-D well packed gold nanoparticle superstructures. Our method may further expand the use of bio-functionalized block copolymers in solid-state devices where the plasmonic modes could be varied with interchangeable templates.
REFERENCES


60. Nie, S. Science 1997, 275, 1102.


