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Liu, Cuicui

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Fabrication of Hybrid Nanostructures Based on the Block Copolymer Self-assemblies

LIU Cuicui

School of Physical and Mathematical Sciences

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Advisor: Associate Professor Chen Hongyu

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Abstract

This thesis summarized my postgraduate research work on the exploration of basic principles behind the micelles transformation during the BCP (block copolymer) self-assembly process, the fabrication of hybrid organic/inorganic nanostructures based on the BCP self-assembly, and the development of a general methodology for the preparation of metal nanoshell on the surface of hybrid nanostructures. These works were based on the study of self-assembly of amphiphilic BCP in mixed solvents. Specifically, self-assembling of BCPs in mixed solvent can form various morphologies in solution, including spheres, cylinders, discs, vesicles, *et al.* When inorganic nanoparticles were introduced into this system, hybrid organic/inorganic nanostructures with specific morphologies could be achieved. Besides, more complicated hybrid nanostructures containing metal core, polymer shell (the first layer) and metal nanoshell (the second layer) were synthesized using a general method.

In details, we studied the self-assembly of poly(styrene)-*block*-poly(acrylic acid) (PSPAA) in mixed solvent, and got unique toroidal micelles, which were topologically different from spheres, cylinders, and vesicles (**Chapter 2**). PSPAA has been employed to investigate the self-assembly of BCPs for decades. However, toroidal micelles synthesized from this polymer were rarely

observed. We ascribed our finding of toroidal micelles of PSPAA to three factors: 1. the "long-hair" PSPAA copolymer (relatively long PAA block) used in this system, which make the micelle transformation process very slow, allowing the intermediates to be trapped easily; 2. special experimental setup: mixed all ingredients together at a critical solvent ratio, giving enough time for polymers to assemble and evolving at higher temperature; 3. the addition of acid into the reaction, which decreased the charge repulsion among the PAA coronas, facilitating the aggregation of micelles and morphological evolution. Toroidal micelles were only one type of intermediates trapped during the whole micellar evolution process.

Based on **Chapter 2**, vesicle intermediates, bowl-like micelles, were trapped during the morphological transformation from toroidal micelles to vesicles (**Chapter 3**). The structural analysis of static shapes (surface to volume ratio (S/V) of the intermediates) revealed that S/V decreases from cylinder to lamella, to bowl-like micelle, and to vesicle, which did not give an obvious barrier in the transformation process. The real kinetic barrier lay in the movement of amphiphilic molecules and the merging of the polymer domains, which were required for folding lamella into vesicle. The key intermediates observed in our experiments were consistent with the location of main barrier in the transformation pathway.

When metal nanoparticles were introduced into the above BCP self-assembly

system, dumbbell-like nanostructures were easily synthesized (**Chapter 4**).

These hybrid dumbbell-like nanostructures possessed three characteristics: a) the structure was highly symmetric; b) the BCP domains generally located at the two opposite spherical caps of the gold nanoparticle (AuNP), and the other part of the AuNP is exposed to the outside environment; c) the BCP domains could be controlled to be spherical micelles, cylindrical micelles, and vesicular micelles.

In **chapter 5**, a general methodology for the preparation of metal nanoshell on the surface of AuNP@PSPAA (AuNPs encapsulated with amphiphilic block copolymer PSPAA) was developed. The core-shell AuNP@PSPAA was first synthesized via the self-assembly of PSPAA on the surface of AuNP. Various metal nanoshells including Ag, Au, Pt, Pd, Rh, and $\text{In}(\text{OH})_3$ have been synthesized on the surface of AuNP@PSPAA.

Finally, in **Chapter 6**, an effective and facile strategy to prepare transmission electron microscopy (TEM) samples for negatively charged colloids was developed by modifying the TEM grids with 3-aminopropyltriethoxysilane (APTES). The APTES-modified TEM grid could make the specimens evenly distribute on TEM grid through charge interaction between the negatively charged specimens and the positively charged amino-groups. Therefore, this method can effectively avoid aggregation and uneven distribution of samples, which usually occur during drying process in normal method..

Chapter 1 Introduction

1.1 Self-assembly of Small Molecular Amphiphiles

Self-assembly of molecules is a typical procedure in nature, where a chaotic system of pre-existing molecules re-organizes to form an ordered structure or pattern because of the specific interactions among the molecules themselves. This process is ubiquitous in nature and also in daily life.¹⁻⁴ For example, the cell membrane consists of lipid bilayer, which was produced by the self-assembly of amphipathic phospholipids;⁵ soap bubbles, which emerge as soap is used, are made from the self-assembly of surfactants. In both cases, the phospholipids and surfactants are all classified as amphiphilic molecules, which consist of hydrophobic tail and hydrophilic head.

With the purpose of understanding the self-assembly of amphiphiles and exploring the application of such structures, a lot of work has been done to gain knowledge of the structures, properties, and theories of the self-assemblies.⁶⁻⁹ The self-assembly of low molecular weight amphiphilic molecules has been studied for several decades, and a great variety of morphologies including spheres, cylinders, lamella, and vesicles have been found.¹⁰⁻¹³ It is thought that the shape of the final aggregates was primarily a result of the function between the hydrophobic tail and hydrophilic head, which can influence the curvature of

the interface between the hydrophobic part and hydrophilic part. Generally, "packing parameter", p , is applied to explain the variety of morphologies.⁹

Packing parameter is defined in the below Equation:

$$p=v/al$$

where v is the volume of the hydrophobic part, a is the contact area of the hydrophilic head, and l is the length of the hydrophobic chain,⁹ as shown in Figure 1-1. Normally, when $p \leq 1/3$, spherical micelles are the favoured morphology; when $1/3 \leq p \leq 1/2$, cylindrical micelles; when $1/2 \leq p \leq 1$, lamellar or vesicular micelles.⁹

Figure 1-1. Schematic showing the shape of amphiphiles in terms of packing parameter.

The similar morphologies to those aggregates of low molecular weight amphiphiles were also observed when amphiphilic BCPs were employed in the self-assembly study. Compared to small molecular weight amphiphiles, amphiphilic BCPs exhibit relatively higher stability and slow molecular dynamics during the self-assembly. Therefore, amphiphilic BCPs offer a nice

platform to study micelle transformation.^{1,14-17} Considerable attention has been devoted to investigate BCPs self-assemblies and also to explore the potential applications of such assemblies in various fields, such as biomaterials, nanoreactors, drug delivery vehicles, *etc* for many decades.^{1,15,18-20}

Thanks to the great advances in the synthesis of BCPs, various polymers with different structural designs, such as linear BCPs, star-like copolymers, graft copolymers, dendritic copolymers, have been synthesized successfully by using living radical polymerization and anionic polymerization.²¹ Besides, the molecular weight, the block length, and the chemical properties can also be well controlled in polymerization. These copolymers are able to self-assemble into various aggregates, relying on the property of the copolymers and the experimental conditions. Among these copolymers, amphiphilic linear BCPs, containing both hydrophilic block and hydrophobic block, were attracted the most attentions in many decades due to the unique properties endowed by their immiscible blocks.

Self-assembly of BCPs in solution can produce various morphologies, covering spherical, cylindrical, lamellar, vesicular, and large compound micelles. The pioneer works on self-assembly of BCPs in solution can be traced back to 1995.^{14,22,23} Since that time, a mountain of experimental and theoretical works has been done. This introduction will focus on the basic principles for the self-assembly of BCPs in the mixed solvents, the synthesis of BCPs micelles

with embedded metal nanoparticles, and the potential application of these micelles.

1.2 Accessible Morphologies via Self-assembly of Block Copolymers

Various morphologies from the self-assembly of BCPs have been produced in the solution. For example, the morphologies include simple spherical micelles, cylindrical micelles, netlike micelles, vesicular/lamellar micelles, large compound micelles (LCMs), large compound vesicles (LCVs), tubular micelles, onion-like micelles, hexagonally packed hollow hoops, hamburger-like micelles, phase-segmented micelles, helical micelles, *etc.*^{14,23,24} The amphiphilic BCPs utilized into the production of these aggregates were plentiful, including poly(styrene)-*block*-poly(acrylic acid) (PS-*b*-PAA), poly(styrene)-*block*-poly(ethylene oxide) (PS-*b*-PEO), poly(styrene)-*block*-poly(vinylpyridine) (PS-*b*-PVP), poly(4-vinylpyridine)-*block*-poly(styrene)-*block*-poly(4-vinylpyridine) (P4VP-*b*-PS-*b*-P4VP), poly(acrylic acid)-*block*-poly(methyl acrylate)-*block*-poly(styrene) (PAA-*b*-PMA-*b*-PS), poly(butadiene)-*block*-poly(ethylene oxide) (PBD-*b*-PEO), poly(lactic acid)-*block*-poly(ethylene oxide) (PLA-*b*-PEO), *etc.*^{20,25-30} In the following part, we only choose some representative examples to discuss.

1.2.1 General Preparation Method

The most commonly used method for preparation of BCP micelles is called "co-solvent" method. Generally, this method applies to these amphiphilic BCPs, whose hydrophobic segments show a high T_g (glass transition temperature), such as PS-*b*-PAA (PSPAA) with $T_{g,PS} \approx 100\text{ }^{\circ}\text{C}$.^{24,31,32} Generally, the self-assembly of these BCPs occurs in mixed solvents: a good solvent mixed with a selective solvent. Take amphiphilic BCPs, PS-*b*-PAA, as an example, the detailed experimental process was as follows: a good solvent (DMF, THF, or dioxane), which can dissolve both the hydrophobic and hydrophilic blocks, was first utilized to dissolve PS-*b*-PAA copolymer. The copolymer chains are unimolecularly dispersed and exist as the random coils conformation under this condition. Then, a selective solvent like H₂O, which can only dissolve the hydrophilic PAA block, was slowly added into the BCPs solution. The introduction of H₂O decreased the solubility of the hydrophobic PS part in the solution. At CWC (critical point of water concentration), the PS chains tended to aggregate to form small spherical micelles (micellization) to minimize the interfacial energy between the PS part and the outside solvent.^{33,34} If a huge amount of water was introduced into the system in one shot, the CMC (critical micellization concentration) of BCPs became very low, and almost all of the polymer chains involved in the micellization quickly.^{25,33} The final morphologies of the self-assembly were depended on the component of the

BCPs, the ratio of good solvent to poor solvent, the properties of solvent, the reaction temperature, and the introduction of additives. Finally, the good solvent can be removed by centrifugation (dilute the resulting solution with water and centrifuge this solution at high speed)³⁵⁻³⁷ or by dialysis of this solution against water.^{24,31,32}

The second method is called "solvent-free" method (direct hydration approach).²⁷⁻²⁹ This method generally applies to these amphiphilic BCPs, whose hydrophobic segments have a relatively lower T_g , such as PBD-*b*-PEO with $T_{g,PBD} \approx -90\text{ }^{\circ}\text{C}$.^{27,28} Typically, the BCPs were first dissolved in a good solvent, followed by drying and annealing of the BCPs/solvent blends at a higher temperature. Then, the dried sample was hydrated with water.

The other methods include layer-by-layer assembly,^{38,39} direct synthesis method,^{40,41} microfluidic method,^{42,43} and so on. The diversity of the developed methods for the preparation of BCPs' aggregates allows various morphologies to be achieved and thus provides abundant potential applications in the future.

1.2.2 Various Morphologies from the Self-assembly

Spherical micelles are the simplest assembly among the other morphologies. They consist of spherical hydrophobic central part encircled by the hydrophilic corona, as shown in inserted scheme of Figure 1-2(a).^{23,44,45} This type of micelles can be formed once the BCPs reach their CMC in the mixed solvent.

Therefore, they are the first aggregates during the BCPs self-assembly process, and also, it can be considered that they are the starting states for other more complex morphologies. One characteristic for the spherical micelles is that the size of spherical micelles cannot unlimited increase, since the radius of core is limited by the physical length of coiled hydrophobic blocks. Another characteristic is that the hydrophilic coronas endow the spherical micelles with well solubility in water. Thus, the hydrophobic core can be used as a nanocontainer or nanocarrier for loading genes,^{46,47} the fluorescence probe,⁴⁷ as well as the hydrophobic drug.^{21,48} Because of their unique properties, considerable works have been done to investigate the use of such spherical micelles in drug delivery system and biological imaging field. Several derivatives such as Janus micelles (Figure 1-2b),⁴⁹⁻⁵² multi-compartment micelles (Figure 1-2c),^{53,54} and zwitterionic micelles⁵⁵ have been developed based on this core-shell spherical micelles.

Figure 1-2. TEM/STEM images and corresponding schematics of spherical micelles and their derivatives, (a) spherical micelles, (b) Janus micelles, and (c) multi-compartment micelles. (Reprinted with permission from ref.45, 52 and 54, respectively. Copyright 1999 from American Chemical Society; 2008 from the Royal Society of Chemistry; and 2012 from American Chemical Society.)

Cylindrical micelles (rod) consist of a hydrophobic cylindrical centre encircled by the hydrophilic corona, as illustrated in inserted scheme of Figure 1-3(a).⁵⁶⁻⁶⁰ Generally, the formation process of the cylindrical micelles was as follows: After micellization, the small spherical micelles collide with each other to form large spherical micelles. Since the radius of core is limited by the physical length of coiled hydrophobic blocks, the size of spherical micelles cannot unlimitedly increase. At some critical value, the large spherical micelles would change to cylindrical micelles to decrease the interfacial tension. Due to the similar packing mode of BCPs, the diameter of the cylinders is generally

similar to that of the initial spheres, while the length is variable from several nanometers to several micrometers. Because cylindrical micelles can act as the template for loading inorganic nanoparticles like semiconductors and magnetic particles, considerable works have been done to adjust the length of the cylindrical micelles. For example, Eisenberg et al. found that the rod length of PS-*b*-PAA assembly can be well controlled by simply tuning the added quantity of selective solvent (water): long cylinders at 13.0 % water (Figure 1-3a), and short cylinders at 25.9 % (Figure 1-3b).⁵⁶ Recently, Winnik and co-workers developed a new "living self-assembly" method to get the well controllable length of cylindrical micelles from polyferrocenyldimethylsilane-containing BCPs.⁶¹ Besides these simple cylindrical micelles, more complex cylindrical micelles have also been synthesized, including toroidal/multi-toroidal micelles (Figure 1-3c and 3d),³⁶ segmented cylindrical micelles (Figure 1-3e),^{62,63} network-like micelles,³³ helical/double-helical micelles (Figure 1-3f and 3g).⁶⁴⁻⁶⁷

Figure 1-3. TEM images and corresponding schematics of cylindrical micelles and complex cylindrical micelles, (a) long cylinders, (b) short cylinders, (c) toroidal micelles, (d) multi-toroidal micelles, (e) segmented cylindrical micelles, (f) helical micelles, and (g) double-helical micelles. (Reprinted with permission from ref.56, 36, 62, and 64, respectively. Copyright 1999 from American Chemical Society; 2011 from Wiley-VCH Verlag GmbH & Co; 2007 from American Association for the Advancement of Science; 2008 from the Royal Society of Chemistry.)

Vesicular micelles (lamellar micelles) are fluid-filled sacs that have a bilayer wall assembled from amphiphilic molecules or copolymers, as shown in Figure 1-4(a).^{1,7,15,23,68-70} The most notable examples are the various membrane-enclosed sacs in living cells. Studying the formation of vesicles and their behavior is of critical importance for understanding biomembranes.

Moreover, vesicles are ideal artificial nanoscale compartments, which are being explored as nanoreactors and drug delivery vehicles.^{19,20,71,72} Vesicles can directly emerge from the phase-segregation of agglomerated amphiphiles^{16,73-75} or transform from the pre-existing micelles, relying on the amphiphile's property and the initial condition.^{7,16,41,56} In the latter scenario, it is well known that the micellar evolution goes from spheres to cylinders, to lamellae, and finally to vesicles. Generally, vesicles are more frequently to be observed than lamellar micelles because of their relatively lower *surface-to-volume* ratio (more thermodynamically stable).³¹ Besides these simple vesicles, some other complicated vesicles were also synthesized, such as tubular micelles (elongated vesicles),⁷⁶ large-compound vesicles,³¹ onion-like vesicles (multi-lamellar vesicles),⁷⁷ as Figure 1-4(b), (c) and (d) showed. Most of the reported vesicles were closed bilayers, which consisted of amphiphilic diblock copolymers. When amphiphilic triblock copolymers or multi-block copolymers were used for the synthesis of vesicles, monolayered or multi-layered vesicles were generally formed.⁷⁸ Take amphiphilic triblock copolymers ABC (A, C: hydrophilic block; B: hydrophobic block) for example, they can form monolayered vesicles composed of hydrophobic core layer (B blocks) along with hydrophobic A blocks and C blocks located on the inner and outer side, respectively.⁷⁹

Figure 1-4. TEM images and corresponding schematics of vesicular micelles and complicated vesicles, (a) vesicles, (b) tubular micelles (elongated vesicles), (c) large-compound vesicles, and (d) onion-like vesicles (multi-lamellar vesicles). (Reprinted with permission from ref.70, 76, 31, and 77, respectively. Copyright 2004 from Wiley-VCH Verlag GmbH & Co; 1998 from American Chemical Society; 1996 from American Chemical Society; 2000 from Wiley-VCH Verlag GmbH & Co.)

Figure 1-5. TEM images and corresponding schematics, (a) hexagonally packed hollow hoops (HHHs), and (b) Large-compound micelles (LCMs). (Reprinted with permission from ref.80, and 14, respectively. Copyright 1997 from American Physical Society; 1995 from American Association for the Advancement of Science)

The spherical, cylindrical, and vesicular micelles are the most common micelles observed in the experiments. In addition, there were other more complicated micelles reported in the literature. For example, hexagonally packed hollow hoops (HHHs) (Figure 1-5a) were obtained from the self-assembly of PSPAA amphiphilic BCPs.⁸⁰ Unlike the above normal core-shell structures, in this type of structure, the hydrophilic PAA chains formed the "cores" of the hoop, whereas the hydrophobic PS chains encircled these "cores", forming the coronas. Large-compound micelles (LCMs) consist

of multiple inverse micelles (Figure 1-5b).¹⁴ The surface of such LCMs is surrounded by a layer of hydrophilic blocks, allowing their stability in the solution. Take PS-*b*-PAA LCMs for example, the PAA cores are located in the continuous hydrophobic PS phase, whose surface is surrounded by the hydrophilic PAA layer (Inserted scheme in Figure 1-5b).¹⁴

1.2.3 Mechanism for the Morphological Transformation

It is well known that the morphology of the BCP aggregates is influenced by three factors: the stretching of the hydrophobic blocks in the core, the interfacial tension between the core and the outside solvent, and the interaction among the hydrophilic coronas.^{14,24,31} The self-assembly of BCPs is a phase separation process intrinsically. When a selective solvent is added into the solution of BCPs/good solvent, the two immiscible blocks will separate from each other to minimize the interfacial energy between the two blocks. Most of the mechanistic studies in the literature focused on the thermodynamic driving forces, for example, by analyzing the packing parameters.^{7,9,81} The specific morphology of the self-assemblies is primarily determined by the ratio of the hydrophobic block to hydrophilic block, which can influence the curvature of the interface between the hydrophobic part and hydrophilic part.⁹ Generally, "packing parameter", p , is used to explain the variety of morphologies.^{7,9,81} Packing parameter is defined in the below Equation:

$$p=v/al$$

where v is the volume of the hydrophobic part, a is the contact area of the hydrophilic head, and l is the length of the hydrophobic chain, as shown in Figure 1-6. As a general rule,⁹ when $p \leq 1/3$, spherical micelles are the favoured morphology; when $1/3 \leq p \leq 1/2$, cylindrical micelles; when $1/2 \leq p \leq 1$, lamellar or vesicular micelles.

Figure 1-6. Various self-assembled structures formed by amphiphilic block copolymers in a block-selective solvent. The type of structure formed is due to the inherent curvature of the molecule, which can be estimated through calculation of its dimensionless packing parameter, p . (Reprinted with permission from ref. 9. Copyright 2009 from Wiley-VCH Verlag GmbH & Co.)

According to this rule, the final shape of BCP aggregates can be dominated by changing these factors that influenced the final values of p , for example, the

property of copolymers, the ratio of good solvent to selective solvent, the type of solvents, and the quantity of added ions.

Besides the use of the packing parameter to study the mechanism, analysis of the surface-to-volume ratio (S/V) of the specific micelle types³⁶ was another method to study the thermodynamic driving forces. Typically, larger S/V means a larger area of the unfavorable polymer-solvent interface and less amount of the favorable internal interactions (van der Waals and hydrophobic) within the polymer domains. By setting all micelles to have a same diameter/thickness, it is easy to calculate that the S/V decreases in this sequence: sphere, cylinder, lamella, and vesicle.³⁶ Hence, vesicle would be favored if there is no competitive driving force.

However, in fact, a lot of other morphologies beyond the vesicle were obtained in the experiments. That is because in these experiments, many types of interaction potentials favor the decrease in the S/V while some others favor the increase in the S/V . For example, in the system of self-assembly of PSPAA, these interactions are the hydrophobic and van der Waals interactions among PS blocks. In general, these interactions in the core are maximized if the S/V is minimized. There are also some other interaction potentials in the literature, such as H-bonding,⁸² Columbic^{83,84} or dipolar interactions,⁸² π - π stacking,⁸⁵ additional packing interactions resulted from the order or crystallinity within the core,^{86,87} etc. But, the counteractive force is the steric and charge repulsion

among the coronas, which is weaker at high curvature. Hence, the repulsion potentials favor the formation of spheres which have relatively high S/V . At equilibrium, the structure of a surfactant micelle is a result of the balance between the interaction potentials and the repulsion potentials. Any condition that leads to dominant interaction potentials favors the formation of vesicles. Reducing the repulsions is one way to achieve that: for example, use of PS-*b*-PAA with shorter PAA chains,³² protonation of the PAA chains by acid,²³ inclusion of homo-polystyrene in the PSPAA cores,²⁴ increasing ionic strength for more effective shielding,³¹ mixing of cationic and anionic surfactants.⁸³ Alternatively, increasing the interactions in the core can also lead to dominant interaction potentials: for example, increasing water content in the solvent (stronger interfacial tension),^{34,56} use of polymer blocks with more hydrophobic nature, and use of polymer that packs well in the core.

1.3 Embedding Preformed Nanoparticles into Micelles

Amphiphilic BCPs can self-assemble into various micelles in mixed solvents, such as spherical, cylindrical, lamellar, and vesicular micelles.^{1,7,8,23,24,27,88} These controllable self-assembly morphologies provide excellent templates for constructing organic/inorganic hybrid nanomaterials, such as metal, magnetic, or semiconducting nanoparticles embedded in the polymer domains.^{6,36,89-93} The produced hybrid nanomaterials generally possess not only the properties of both

organic/inorganic parts but also some new characteristics resulted from the size distribution of inorganic NPs.

Two main approaches have been used to incorporate nanoparticles (NPs) into the BCP aggregates. One is the *in situ* synthesis of NPs on the BCP aggregates, which means that metal ions are first linked to or adsorbed into the aggregates, and then transformed to metal NPs by chemical reductions.^{91,93} One typical example is using polystyrene-*block*-polyvinylpyridine (PS-*b*-P4VP) as the template to incorporate the inorganic precursors selectively into P4VP part followed by a chemical reduction step to obtain metallic nanoparticles.⁹³ However, one drawback of this methodology is that it cannot be employed for the as-prepared BCP aggregates that were incapable of linking metal ions. The other method is the *ex situ* synthesis of NPs on the BCP aggregates, in which the metal NPs stabilized by the organic ligands on the surface are first prepared, followed by co-assembly of the amphiphilic BCPs with them in the mixed solvents.⁹⁴⁻⁹⁹ This methodology does not need the post chemical reduction. Besides, after surface modification of the as-prepared NPs, they can be linked to or embedded in the selective segments of the BCP aggregates, which means the controllable located position of the NPs in the BCP aggregates.

The *ex situ* synthesis of BCP micelles with embedded NPs was more convenient than the *in situ* preparation method. Thus, this method has attracted significant attentions for decades, and various morphologies of BCP micelles

with embedded NPs have been fabricated. In the following part, we will summarize the reported different BCP micelles with embedded NPs prepared from this "*ex situ*" method.

1.3.1 Spherical Micelles with Embedded NPs

The first and representative example for the fabrication of simple spherical micelles inserted with metal NPs was reported by Taton et al.^{94,95,99,100} The detailed preparation process was shown in Figure 1-7. Typically, amphiphilic diblock copolymers, PS-*b*-PAA, were first dissolved in a good solvent (DMF), followed by addition of purified citrate-stabilized AuNPs. After that, a small amount of hydrophobic ligand, 1-dodecanethiol, was introduced into the mixture to hydrophobically modify AuNPs, facilitating the AuNPs embedded into the hydrophobic domain of the aggregates. To induce the micellization of PS-*b*-PAA, a selective solvent, water, was added into the system. Lastly, 2,2'-(ethylenedioxy)bis(ethylamine) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDC) were added into the solution to crosslink the PAA block permanently. TEM images (Figure 1-7a to 7c) showed that the resulted morphologies were core/shell nanostructures with AuNPs as the core and PSPAA as the shell.

Figure 1-7. The top scheme showing the preparation of core/shell AuNPs embedded into PS(PMMA)-*b*-PAA micelles; TEM images of AuNP@PSPAA with different size of AuNPs: (a) 4.1 nm, (b) 12.3 nm, and (c) 31.0 nm. (Reprinted with permission from ref.95 and 94. Copyright 2005 from Wiley-VCH Verlag GmbH & Co; 2005 from American Chemical Society.)

Based on this system, Chen et al. developed a more facile and more general method to encapsulate metal NPs with amphiphilic PS-*b*-PAA copolymers.^{37,96,97} Figure 1-8 shows the schematic of controlling the position of AuNPs in PSPAA micelles and corresponding TEM images.⁹⁷ This position control was achieved by a binding competition between hydrophobic ligand (L_A) and hydrophilic ligand (L_B) on the surface of AuNPs, which led to selective attachment of PS-*b*-PAA on the hydrophobic side of the AuNPs. The preparation method used in this study was quite easy and efficient: a mixture of AuNPs, PS-*b*-PAA, L_A or L_A and L_B, DMF and water was firstly heated at 110

°C for 2 h, and then cooled down to room temperature (RT). By using this method or similar method with a little modification, AuNPs with different size can be encapsulated with these micelles; and PS-*b*-PAA micelles can also encapsulate some other NPs including AgNPs, Ag nanocubes, Au@Ag NPs, Fe₂O₃ NPs, Silica NPs, *etc.*^{37,101-103}

Figure 1-8. Schematic diagram showing the formation of homocentric (a and b) and eccentric (a, c, and d) AuNP@polymer. TEM images of purified AuNP@polymer when $[L_A]:[L_B]$) 1:0 (e); 1:22 (f); 1:132 (g), corresponding to b, c, and d, respectively. All scale bars are 50 nm. (Reprinted with permission from ref. 97. Copyright 2008 from American Chemical Society.)

1.3.2 Cylindrical Micelles with Embedded NPs

Cylindrical micelles can also be employed to encapsulate preformed NPs. Precisely control the location of the incorporated NPs into the specific region of cylindrical micelles is very important for fabricating 1D nanostructures. And a lot of studies have been focused on this project due to the potential applications of such nanostructures in making novel optoelectronic and microelectronic instruments.¹⁰⁴

Recently, Eisenberg and co-workers reported that the preformed NPs were controlled to incorporate only into the central portion of the cylinders, as shown in Figure 1-9(a).^{89,105} The preparation method was as follows: first, the NPs were stabilized with amphiphilic diblock copolymers, which have a similar composition to that of the BCPs utilized in the following micelle-forming step; then, the micelles were prepared by using the conventional method except the existence of BCP-stabilized metal NPs in the mixed solvents. This methodology forced the metal NPs to locate in the vicinity of the micelle's centre, which could avoid the large energy produced by the asymmetric stretching of BCP blocks on the metal NPs that did not locate near the centre.⁸⁹

Chen et al.³⁶ reported the controlled localization of hydrophobically functionalized AuNPs into the hydrophobic domains of the PSPAA toroids (closed cylinder) (Figure 1-9b). The preparation method was facile. First, citrate-AuNPs were modified with thiol-terminated polystyrene (PS-SH) to get

the hydrophobic AuNPs. The resulting hydrophobic AuNPs were then added into PSPAA/DMF solution, followed by introduction of water and HCl. Lastly, the mixture was heated at 100 °C for 1 h. The PSPAA self-assembled on the surface of hydrophobic AuNPs, with the PS chains on the surface of AuNPs and the hydrophilic PAA chains facing outside.⁹⁶ Other sizes or shapes of AuNPs could be encapsulated by such toroidal micelles as well, such as, 40 nm AuNPs, 70 nm AuNPs and Au nanorods (Figure 1-9c).³⁶

The interactions between the preformed NPs and the coronas also can make the NPs situate to the coronas of cylinders.¹⁰⁶ A typical example is shown in Figure 1-9(d) and (e). The NPs were rightly located in the quaternized P2VP coronas of cylindrical PFS-*b*-P2VP (polyferrocenylsilane-*b*-poly(2-vinylpyridine)) micelles.¹⁰⁶ The cylindrical micelles were generated by living self-assembly, where PFS-*b*-P2VP assembled at both ends of the quaternized micelles.¹⁰⁶ Selective localization of NPs was achieved by electrostatic interactions between the negatively charged AuNPs or PbS QDs and the positively charged P2VP blocks.

Figure 1-9. (a) TEM images and corresponding schematics of cylindrical micelles with nanoparticles precisely localized in the middle of the core; TEM images of toroidal micelles with embedded (b) 15 nm AuNPs, (c) 70 nm AuNPs; Dark-field TEM images show block co-micelles with the central block functionalized with (d) Au nanoparticles and (e) PbS quantum dots. (Reprinted with permission from ref.89, 36, and 106, respectively. Copyright 2011 from American Chemical Society; 2011 from Wiley-VCH Verlag GmbH & Co; 2007 from American Chemical Society.)

1.3.3 Vesicular Micelles with Embedded NPs

A common method for the preparation of vesicles with embedded NPs was co-assembly of amphiphilic BCPs and the preformed NPs in solution. The NPs and the hydrophobic chains would aggregate into the walls of vesicle due to hydrophobic interactions, and the hydrophilic blocks acted as coronas on the surface of both the inner and outer walls. By using this method, Lecommandoux *et al.* synthesized vesicular structures with Fe₂O₃ NPs randomly distributed inside by co-assembly of polybutadiene-*block*-poly(glutamic acid) (PB-*b*-PGA) copolymers and phosphoric diester modified Fe₂O₃ NPs.^{107,108}

Eisenberg and co-workers recently reported a general methodology to control the localization of preformed NPs into only the centre of vesicle walls.¹⁰⁹ The NPs used in the self-assembly were stabilized by amphiphilic copolymers of the same or similar composition as that of the vesicle-forming diblocks.¹⁰⁹ As shown in Figure 1-10(a), the Pb NPs were just located in the middle portion of the vesicles wall, and the corresponding schematic clearly illustrated this structure, in which each NP was located in the middle of the wall, with the corona chains stretched toward each interface. This symmetric way could reduce the high free energy associated with the asymmetric distribution.

Figure 1-10. TEM images and corresponding schematics of vesicles with nanoparticles precisely localized (a) in the middle of the core, (b) in the hydrophilic coronas, and (c) at the interface of vesicles. (Reprinted with permission from ref.109, 110, and 111, respectively. Copyright 2010 from American Chemical Society; 2009 from American Chemical Society; 2011 from American Chemical Society.)

Besides, the preformed NPs can also be controlled to locate in the hydrophilic coronas (Figure 1-10b)¹¹⁰ or at the interface of vesicles (Figure 1-10c)¹¹¹ with the aid of the electrostatic interaction among the preformed NPs and coronas.

1.4 Potential Applications

BCP aggregates and BCP aggregates with embedded NPs have appeared various applications in biotechnology, biomedicine, nanoreactor, etc. For example, spherical BCP micelles with embedded QDs in the core were emerging as a new class of fluorescent probes for diagnostic images.^{112,113} They effectively solved many problems encountered in use of QDs in biological environments, such as low water solubility, aggregation, and toxicity clearance, while retaining their unique optoelectronic properties.^{112,113}

Controlled clustering of magnetic NPs inside BCP aggregates results in high particle loading and a considerable increase in detection sensitivity in magnetic resonance imaging.¹¹⁴⁻¹¹⁶ The aggregates loaded with magnetic NPs and drugs can be directed to the drug-needing locations by magnetic fields.¹¹⁵ Drug release can be controlled through an oscillating magnetic field producing local hyperthermia in the magnetic particle loaded portion of the aggregates, which requires the precise localization of NPs in the corresponding parts of the aggregates, for example, micelle cores or vesicle walls.¹¹⁵

Core-shell AuNP@PSPAA was used as a new model delivery system, where pyrene was incorporated in the polymer shells of AuNPs and then released to nanoacceptors.⁴⁸ The fluorescence of pyrene was quenched in the vicinity of the AuNPs but reemerged upon its release, allowing in situ kinetics study by optical measurements.⁴⁸

The vesicle from the gemini amphiphiles worked as a nanoreactor in the synthesis of Au nanoflowers (AuNFs), and the spacer length of the gemini amphiphiles has an obvious control effect on the morphologies and the optical properties of the formed AuNFs.¹⁹ Recently, van Hest and co-workers reported that the preformed vesicles can be further re-engineered to perform a controlled shape transformation from a thermodynamically stable spherical morphology to a kinetically trapped stomatocyte structure.¹¹⁷ The stable bowl-shape stomatocyte morphology can be used to mimic micelles, entrapping the specific nanoparticles inside the stomatocytes.⁷²

1.5 Overall Objective

Most of the nanostructures reported in the literature are of single-component and generally have simple geometries such as sphere and cube. Sophisticated nanodevices or nanorobots in the future will certainly require complex components and assembly techniques. Due to the size limitation, self-assembly would be an appropriate technique to fabricate such complex nanodevices or nanorobots. Therefore, the mechanism understanding of the self-assembly process would be very helpful for designing and producing these nanodevices in future.

The specific aim of this thesis is trying to understand the mechanism behind the self-assembly of amphiphilic block copolymers, as well as fabricate

complicated hybrid nanostructures on the bases of the mechanism understanding. In this thesis, gold nanoparticles (AuNPs) were controlled to located fully or partially in spherical micells, cylindrical micelles, and vesicular micelles. These nanostructures are novel, and in the future, the properties and the applications of these nanostructures will be tested. Besides, triple-layered nanostructures (AuNP@Polymer@Metal nanoshell) were synthesized. When the metal nanoshell was silver, such nanostructures could be used as substrates for surface-enhanced Raman scattering (SERS) applications.

With these advances, the combination of metal nanoparticles with block copolymers self-assemblies holds promise for fabrication of functional materials with tailored structures, functionalities, and applications.

1.6 Bibliography

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Chapter 2 Toroidal Micelles of Polystyrene-*block*-Poly(acrylic acid)

2.1 Introduction

The self-assembly of amphiphilic molecules can generate micelles of different nanostructures including spheres, cylinders and vesicles.¹⁻⁴ Understanding their morphological transformation is of great importance owing to the relevance to the formation of lipid vesicles, which play a central role in the compartmentation of cellular reactants and support various biological functions. In comparison to small amphiphiles such as lipids, amphiphilic block copolymers are known for their slow molecular dynamics and, hence, offer a nice platform for studying micelle transformation.

Toroids are topologically different from the typical micelles. While achieving this unique topology is simple for macroscopic objects such as doughnuts, o-rings, and transformer cores, fabricating nano-toroids involves fundamentally different synthetic challenges, which has attracted great interest in nano-science.⁵⁻⁸ It has been known that certain amphiphilic diblock copolymers⁸⁻¹⁰ and triblock copolymers^{6,7,11-17} can self-assemble to give nano-toroids. In contrast, polystyrene-*block*-poly(acrylic acid) (PSPAA) is a

unique block copolymer in that a great variety of micelle morphologies have been synthesized from it: spheres, cylinders, lamellae, vesicles, large compound micelles, and large compound vesicles.¹⁸⁻²⁰ However, despite comprehensive study in this system, toroidal PSPAA micelles have so far not been reported. The most common method to induce the self-assembly of a block copolymer is to dissolve it in a good solvent for all blocks (e.g., DMF or THF) and then slowly add another solvent (e.g., water) to selectively exclude some of the blocks. In fabricating PSPAA micelles, it is known that the ratio of organic solvent to water is a key factor affecting both micelle morphology and the mobility of the glassy hydrophobic core.^{21,22} By slow addition of water to the solubilized PSPAA, it is not trivial to ensure sufficient time for PSPAA to fully assemble and evolve at the critical solvent ratio.

From the perspective of synthetic methodology, there are only limited means to create nanostructures of regular shapes. Other than the amphiphile self-assembly discussed above, nanocrystal synthesis²³⁻²⁵ is by far the most popular approach. Thus, combining the two approaches is an easy route for creating complex structures at nanoscale. In particular, inserting inorganic nanoparticles (NPs) into robust polymer nanostructures has attracted much interest.²⁶⁻³¹ The major challenges here are to make the two systems compatible and to insert NPs with site-selectivity.^{30,31} Hence, many factors are important, such as the colloidal stability of the NPs, the affinity between the NPs and the

polymer, the mobility and structural evolution of the polymer, etc.

Here, we report the synthesis of toroidal PSPAA micelles and the incorporation of AuNPs therein. In contrast to the traditional micellization method, we dissolved PSPAA in a precisely controlled DMF/water mixture, which was then treated with acid and heated to facilitate the micelle transformation. The PSPAA toroids can only be generated at a narrow window of DMF to water solvent ratio, which could be easily missed by using the traditional method. To create complex nanostructures, hydrophobically functionalized NPs can be selectively incorporated in the hydrophobic domains of the resulting toroids.

2.2 Materials and Methods

2.2.1 Materials and Characterization

All chemical reagents were obtained from commercial suppliers and used without further purification. Amphiphilic diblock copolymer polystyrene-*block*-poly(acrylic acid) (PS₁₅₄-*b*-PAA₄₉, $M_n = 16000$ for the PS block and $M_n = 3500$ for the PAA block, $M_w/M_n = 1.15$; PS₁₄₄-*b*-PAA₂₂, $M_n = 15000$ for the PS block and $M_n = 1600$ for the PAA block, $M_w/M_n = 1.11$), and thiol terminated polystyrene (PS-SH, $M_n = 2000$, $M_w/M_n = 1.15$) were purchased from Polymer Source, Inc.; Tetraethyl orthosilicate (TEOS) was purchased from Sigma Aldrich; the negatively charged, citrate-stabilized AuNPs

(15 nm, 40 nm, and 70 nm)³² and the positively charged, CTAB-stabilized AuNRs³³ were prepared following literature procedures. Copper specimen grids (200 mesh) with formvar/carbon support film (referred to as TEM grids in the text) were purchased from Beijing XXBR Technology co., ltd.

TEM images were collected from a JEM-1400 Transmission Electron Microscope (JEOL) operated at 120 kV. AFM characterization was carried out with a commercial AFM instrument (Dimension 3100 with Nanoscope IIIa controller, Veeco Instruments Inc., CA) equipped with a scanner (90×90 μm²) in tapping mode in air. Silicon tips with a resonance frequency of 330 kHz and spring constant of 42 N m⁻¹ (PP-NCH, Nanosensors, NanoWorld AG, Switzerland) were used.

Preparation of TEM samples (NH₄)₆Mo₇O₂₄ was used as the negative stain in all of the TEM images reported in this study. TEM grids were treated with oxygen plasma in a Harrick plasma cleaner/sterilizer for 1 min to improve their surfaces hydrophilicity. A drop of sample solution was carefully mixed with stain solution ((NH₄)₆Mo₇O₂₄, 1 wt %) on the surface of a Parafilm. Then, the hydrophilic face of the TEM grid was placed in contact with the stained sample solution, and a filter paper was used to wick off the excess solution on the TEM grid. After drying in air, it can be used for TEM characterization.

Preparation of AFM samples A piece of silicon wafer was cleaned thoroughly by using ultrasonication in water and ethanol, and then treated with

aqueous 3-aminopropyltriethoxysilane (0.1 wt %) for 15 min. After washing, the wafer was immersed in a purified sample solution for 1 h, washed with water to remove the excess sample and dried at room temperature. The NH₂-coated surface carries positive charges and could trap the negatively charged micelles. The samples remained dry during the characterization.

2.2.2 Preparation of Toroidal Micelles of PS-*b*-PAA

Synthesis of toroidal micelles of PS-*b*-PAA In a typical experiment for the single-toroids, DMF (380 μ L), PS₁₅₄-*b*-PAA₄₉ in DMF (40 μ L, 8 mg/mL), H₂O (145 μ L), and HCl aqueous solution (15 μ L, 0.1 M) were mixed in a vial in the sequence given above. The final solution contained PS₁₅₄-*b*-PAA₄₉ (0.55 mg/mL), HCl (2.59 mM), DMF (420 μ L), H₂O (160 μ L), and $V_{\text{DMF}}/V_{\text{w}} = 42/16$. The vial was then capped and incubated at 100 $^{\circ}\text{C}$ in a temperature-controlled oil bath for 1 h. After cooling down to room temperature, an aliquot of 50 μ L of the product solution was extracted and mixed with water (1400 μ L), and then the solution was centrifuged at 12500 rpm for 30 min to isolate the polymer micelles from DMF and excess reactants. The concentrated polymer micelles were used for TEM characterization.

For synthesis of PS₁₅₄-*b*-PAA₄₉ multi-toroids, the process was almost the same except that more H₂O (total 190 μ L) was added into the system. The final solution contained PS₁₅₄-*b*-PAA₄₉ (0.52 mg/mL), HCl (2.46 mM), DMF (420

μL), H_2O (190 μL), and $V_{\text{DMF}}/V_{\text{w}} = 42/19$. Note that the final concentrations of PSPAA and HCl were slightly different owing to the difference in dilution factors. We tried to keep the molar ratio of PSPAA to HCl ($m_{\text{PSPAA}}/m_{\text{HCl}}$) constant in this set of experiments. Typically, the HCl concentration has a large window as discussed above. In other experiments of $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$, the process remained unchanged except the quantity of reagents, the reaction temperature, and the reaction time were varied, respectively.

For preparation of toroidal micelles from $\text{PS}_{144}\text{-}b\text{-PAA}_{22}$, DMF (450 μL), $\text{PS}_{144}\text{-}b\text{-PAA}_{22}$ in DMF (60 μL , 8 mg/mL), H_2O (67 μL), and HCl aqueous solution (3 μL , 0.1 M) were mixed in sequence in a vial. The final solution contained $\text{PS}_{144}\text{-}b\text{-PAA}_{22}$ (0.83 mg/mL), HCl (0.52 mM), DMF (510 μL), H_2O (70 μL), and $V_{\text{DMF}}/V_{\text{w}} = 51/7$. The vial was then capped and incubated at 70 $^{\circ}\text{C}$ for 1 h to form toroidal micelles.

Synthesis of PSPAA-toroid@silica The aqueous solution of purified toroidal micelles (re-dispersed in 200 μL water) was added to a vial that contained ethanol (2 mL). After stirring for 10 min, $\text{NH}_3 \cdot \text{H}_2\text{O}$ (40 μL) was added. After stirring for another 10 min, 100 μL TEOS aqueous solution (2 μL TEOS in 98 μL water) was added in the solution. The volume of the final mixture was 2.34 mL ($V_{\text{ethanol}}/V_{\text{water}} = 2/0.34$). The mixture was gently stirred at room temperature overnight. The resulting PSPAA-toroid@silica was isolated by centrifugation at 13000 *rpm* for 20 min.

Incorporation of AuNPs in toroids and multi-toroids of PS₁₅₄-*b*-PAA₄₉

Synthesis of toroidal micelles with embedded AuNPs includes two steps. *First*, the AuNPs were modified with PS-SH to enhance the compatibility of AuNPs with the hydrophobic PS block in the copolymer. Typically, a solution of citrate-stabilized AuNPs ($d_{av} = 15$ nm, 1 mL) was concentrated to ~ 10 μ L by centrifugation at 14000 rpm for 15 min. The deep red solution collected at the bottom of the eppendorf tube was mixed with DMF (350 μ L) and PS-SH dissolved in DMF (20 μ L, 2 mg/mL). The mixture was then incubated at 60 °C for 2 h to ensure ligand exchange, giving PS-S-AuNPs. Finally, the PS-S-AuNPs solution was concentrated to ~ 10 μ L by centrifugation at 14000 rpm for 15 min for the subsequent use. The process of modifying citrate-stabilized AuNPs (40 nm, 70 nm) and CTAB-stabilized AuNRs was similar to the procedure above except different centrifugation speeds were used. *Second*, PS-S-AuNPs were incorporated in polymer micelles. In a representative experiment, DMF (370 μ L), PS₁₅₄-*b*-PAA₄₉ in DMF (40 μ L, 8 mg/mL), PS-S-AuNPs (15 nm, 10 μ L), H₂O (145 μ L), and HCl aqueous solution (15 μ L, 0.1 M) were mixed in sequence in a vial ($V_{DMF}/V_w = 42/16$). The vial was then capped and incubated at 100 °C for 1 h. For multi-toroids with embedded AuNPs and AuNRs, the experimental process was almost the same as above except the solvent ratio ($V_{DMF}/V_w = 42/19$).

2.3 Results and Discussions

2.3.1 Synthesis of Toroidal Micelles from PS₁₅₄-*b*-PAA₄₉

Toroidal PSPAA micelles were discovered by screening for a right DMF/water ratio ($V_{\text{DMF}}/V_{\text{w}}$) with the addition of an appropriate amount of acid: Typically, PS₁₅₄-*b*-PAA₄₉ was first dissolved in a DMF/water mixture ($V_{\text{DMF}}/V_{\text{w}} = 42/16$), which was added HCl (2.5 mM), heated at 100 °C for 1 h, and followed by cooling. The solutions were heated in capped vials to minimize solvent evaporation (estimated solvent loss < 0.3 wt %). The PS_m-*b*-PAA_n self-assembly forced the hydrophobic PS blocks in the micellar cores (Figure 2-1a), while leaving the hydrophilic PAA blocks dissolved in the solvent as a coronal layer. After the synthesis, the solution was diluted by a large amount of water to remove DMF from the cores. The de-swelling reduced the mobility of the hydrophobic blocks, trapping the micelles in kinetically stable states.^{20,34,35}

Figure 2-1. (a) Schematics showing the preparation and structure of toroids; (b, c) TEM and AFM image of $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$ toroids and cylinders, $V_{\text{DMF}}/V_{\text{w}} = 42/16$, the cross-sectional height profile along the white line is shown in the inset of c; (d) TEM image of $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$ multi-toroids, $V_{\text{DMF}}/V_{\text{w}} = 42/19$; (e) toroids and cylinders of $\text{PS}_{144}\text{-}b\text{-PAA}_{22}$, $V_{\text{DMF}}/V_{\text{w}} = 51/7$.

Figure 2-1 shows the transmission electron microscopy (TEM) images of typical toroids. At $V_{\text{DMF}}/V_{\text{w}} = 42/16$, PS₁₅₄-*b*-PAA₄₉ micelles in the forms of both rings and cylinders were observed (Figure 2-1b). An ionic negative stain, (NH₄)₆Mo₇O₂₄, was used so that after drying it formed a layer of dark matter around the micelles. These micelles had similar contrast against the stained background. In particular, stain deposited inside the rings, providing an initial indication that the rings were circles of cylindrical micelles (toroids), and not 2-dimensional (2D) projection of spherical vesicles with enclosed cavity. At slightly higher water content ($V_{\text{DMF}}/V_{\text{w}} = 42/19$) during the synthesis, however, the resulting product of the same polymer became mostly multi-toroids (Figure 2-1d). They were often symmetric, with sometimes mirror symmetry. This phenomenon was previously observed in a different polymer system and explained by a tendency to balance the internal free energy.^[2d] Despite the difference in shapes, the cylinders, toroids, and multi-toroids were quite uniform in width (39 ± 5 nm).

Since TEM images only give 2D projection of 3D objects, it is not an ideal tool for distinguishing toroids from vesicles. Atomic force microscopy (AFM) was thus used to provide additional information on their topology (Figure 2-1c). The height profile across the diameters of typical ring structures showed that the centre of the rings was lower than the edges, which had similar heights of about 30 nm. Hence, the ring-like structures were indeed toroids. The heights of

the edges measured by AFM were somewhat less than their width measured by TEM, presumably due to slight deformation of the polymer structure upon drying and adsorption onto the substrate.

Figure 2-2. TEM image showing (a) the silica encapsulated $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$ toroidal micelles (toroids@ SiO_2) and (b) enlarged image in (a).

Besides, in order to provide direct evidence for the nanostructure of the assembly, silica was used to encapsulate the micelles.³⁶ As shown in Figure 2-2, obviously, silica layer deposited on the shell-layer of the toroidal micelle, and formed the ring-shaped silica nanostructure. The arrows in Figure 2-2(b) marked the polymer part and silica part, respectively. This result directly confirmed the ring-shaped structures (toroids) of PSPAA micelles.

At a slightly higher DMF ratio while keeping other conditions unchanged, the formed PS₁₅₄-*b*-PAA₄₉ micelles were mostly spherical (Figure 2-3a, $V_{\text{DMF}}/V_{\text{w}} = 42/11$); whereas at a lower DMF ratio, lamellar and some vesicular micelles were observed (Figure 2-3b, $V_{\text{DMF}}/V_{\text{w}} = 42/23$). Repeated experiments have confirmed that these results were reproducible, even in the presence of gold nanoparticles (AuNPs, *vide infra*). Thus, the window of solvent ratio for each form of micelles was quite narrow. Since DMF is hygroscopic, it is important to ensure precise solvent ratio by using dry DMF for preparing the solutions.

The morphological evolution of PSPAA micelles has been systematically studied. In general, with increase of water content or acid concentration, the micellar morphology changed from sphere to cylinder, to lamella, and then to vesicle.^{21,22} In our system, control experiments were also carried out to verify the effect of acid. In the absence of HCl while keeping $V_{\text{DMF}}/V_{\text{w}} = 42/16$, only spherical micelles were obtained.³⁷ With pronounced increase in HCl

concentration, different morphologies were observed, following the trend of cylinders (0.86 mM, Figure 2-3c), toroids (2.5 mM, Figure 2-1b), lamellae, and vesicles (7.8 mM, Figure 2-3d). It should be noted that the centre of the vesicles often appeared white in the presence of stain, probably because the ionic stain solution did not penetrate the hydrophobic PS wall. We also used HNO₃ to replace HCl and similar results were obtained. The acid protonated the PAA chains and reduced the repulsion among them. This promoted their aggregation and induced morphological transformation. Hence, our results here were in general agreement with the literature, except that two new forms, single- and multi-toroid, were added in between cylinder and lamella in the series.

However, in the literature, most of the PSPAA micelles were “crew-cut” micelles, where the hydrophilic PAA blocks were significantly shorter than the PS blocks (e.g., PS₂₀₀-*b*-PAA₁₅ and PS₅₀₀-*b*-PAA₅₈).¹⁸⁻²⁰ In comparison, the *relative* PAA content in PS₁₅₄-*b*-PAA₄₉ was much higher, giving “long-hair” micelles. As such, the polymer morphology appeared to evolve very slowly: addition of HCl to PS₁₅₄-*b*-PAA₄₉ solution at $V_{\text{DMF}}/V_{\text{w}} = 42/16$ at room temperature caused the micelles to fuse and form chain-like aggregates (Figure 2-4a). But the fusion was not extensive: the aggregates still maintained the shape of joined spheres, and their width was only about 15 nm. At elevated temperature of 60 °C, their surface became slightly smoother and their width increased to 36 nm (Figure 2-4b), which was still less than the 39 nm width of

the cylinders/toroids. Even after prolonged incubation at 60 °C (14 h, Figure 2-4c), the resulting polymer structures still have many open ends. Hence, it appeared that the high temperature (100 °C) was important for promoting the morphological evolution of the PSPAA micelles: the polymer morphology evolved to cylinders and toroids after 1 h (Figure 2-1b), to multi-toroids after 3 h (Figure 2-4d), and then to vesicles after 9 h (Figure 2-4e). In our other experiments that were carried out under different solvent ratios and [HCl], heating was also found to be essential.

Figure 2-3. Different morphologies of PS₁₅₄-*b*-PAA₄₉ micelles obtained at: (a) $V_{\text{DMF}}/V_{\text{w}} = 42/11$; (b) $V_{\text{DMF}}/V_{\text{w}} = 42/23$; (c) $[\text{HCl}] = 0.86 \text{ mM}$, (d) $[\text{HCl}] = 7.8 \text{ mM}$. Scale bars: 200 nm.

Figure 2-4. Different morphologies of $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$ micelles obtained at: (a) RT, 24 h, (b) 60 °C, 1 h, (c) 60 °C, 14 h, (d) 100 °C, 3 h, (e) 100 °C, 9 h; (f). $\text{PS}_{144}\text{-}b\text{-PAA}_{22}$ vesicles obtained under the conditions used for the $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$ toroids. Scale bars: 200 nm.

In comparison to PS₁₅₄-*b*-PAA₄₉, PS₁₄₄-*b*-PAA₂₂ has relatively lower PAA content and its micelles appeared to evolve faster. Under the conditions used for the PS₁₅₄-*b*-PAA₄₉ toroids, only vesicles of PS₁₄₄-*b*-PAA₂₂ were obtained owing to extensive micelle transformation (Figure 2-4f). The optimal solvent ratio for PS₁₄₄-*b*-PAA₂₂ toroids occurred at $V_{\text{DMF}}/V_{\text{w}} = 51/7$, at which condition 1 h incubation at 70 °C was sufficient (Figure 2-1e). In comparison to Figure 2-1(b), the PS₁₄₄-*b*-PAA₂₂ toroids and cylinders had much less side branches.

2.3.2 Synthesis of Toroidal Micelles Embedded with AuNPs

The PSPAA self-assembly was compatible with hydrophobically functionalized NPs.^{30,31} Previously, it has been shown that PSPAA was able to form spherical shells on a variety of different NPs.^{26-28,38,39} With the appropriate modification of the self-assembly conditions, we can now incorporate NPs inside PSPAA toroids.

Figure 2-5. TEM images of cylindrical and toroidal PS₁₅₄-*b*-PAA₄₉ micelles obtained at $V_{\text{DMF}}/V_{\text{w}} = 42/16$ with embedded AuNPs: (a-e) 15 nm, (f) 40 nm, and (g) 70 nm; (b) schematic illustration and TEM images of toroids formed by thinning the center of lamellar disks; (c, d, and e) schematic illustration and TEM images of toroids formed by end-to-end, end-to-side and side-to-side aggregations, respectively. Scale bars for (b-e): 50 nm.

More specifically, citrate-stabilized AuNPs (15 nm) were first incubated with a hydrophobic ligand (thiol-ended polystyrene, abbreviated as PS-SH) in DMF at 60 °C for 2 h. The PS-SH coordinated to the Au surface rendering it hydrophobic. The resulting AuNPs were then isolated from the solution, added to PSPAA dissolved in DMF/water mixture, before the addition of HCl and

heating at 100 °C for 1 h. The PSPAA self-assembled on the ligand-functionalized Au surface, with the hydrophobic PS blocks facing the surface and the hydrophilic PAA blocks facing the solution.²⁷ Apparently, the morphology evolution of the PSPAA micelles was not greatly affected by the presence of AuNPs. At $V_{\text{DMF}}/V_{\text{w}} = 42/16$, we obtained cylindrical and toroidal micelles with AuNPs incorporated in the center of the hydrophobic domain (Figure 2-5a to 5e). This was true for all types of locations including the tips, arms, and Y junctions of the toroids and cylinders. At $V_{\text{DMF}}/V_{\text{w}} = 42/19$, AuNP-incorporated multi-toroids were obtained (Figure 2-6a).

Other types of NPs could also be incorporated, for example, 40 and 70 nm AuNPs (Figure 2-5f and 5g) and Au nanorods (AuNRs, Figure 2-6b to 6d). The AuNPs were citrate-stabilized whereas the AuNRs were stabilized by hexadecyltrimethylammonium bromide (CTAB). After ligand exchange with PS-SH and purification, these NPs were all compatible with PSPAA self-assembly. In the composite toroids, the thickness of the polymer shells on the large 70 nm AuNPs was around 10 nm, similar to those obtained by direct encapsulation,^{27,28} but the width of toroids remained at 39 nm. This result indicated that the Au-polymer interfacial energy played a significant role in determining the local polymer structure. The polymer shells on the 15, 40, and 70 nm AuNPs were similar in thickness, and so the surface of the toroids often bulged out at the location of AuNPs. In general, the AuNPs and the residue

excess ligands did have some influence on the general morphology of the micelles in a sample, but there still exists a general agreement: toroids and cylinders were obtained at $V_{\text{DMF}}/V_{\text{w}} = 42/16$ (Figure 2-5) and multi-toroids were obtained at $V_{\text{DMF}}/V_{\text{w}} = 42/19$ (Figure 2-6).

Figure 2-6. TEM images of multi-toroidal $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$ micelles obtained at $V_{\text{DMF}}/V_{\text{w}} = 42/19$ with embedded AuNP or AuNRs: (a) AuNPs (15 nm), and (b-d) AuNRs. Scale bar: 200 nm.

2.3.3 Mechanism Study for Toroidal Micelles Formation

Figure 2-7. (a) TEM images of PS₁₅₄-*b*-PAA₄₉ micelles obtained at 100 °C, 0.5 h; (b-f) magnified images of typical micellar structures in sample (a). Scale bars: (b-f), 50 nm.

It was proposed that toroids could be formed by two pathways: (a) thinning at the center of lamellar disks; and (b) end-to-end or end-to-side aggregation of cylindrical micelles.^{6,7,9,12} In our system, both scenarios could be discerned.

Figure 2-7 shows the intermediate PS₁₅₄-*b*-PAA₄₉ micelles after 0.5 h heating at 100 °C. Small circular lamellae were found with a dent at their center (Figure 2-7b and 7d). They were likely intermediates before the perforation to make toroids. Some toroids have smaller diameter of inner circle than that of their circular cross-section (e.g., Figure 2-7c). It seems unlikely that they could be formed by end-to-end aggregation of very short cylinders bent at a very high curvature. Indeed, we never observed any cylindrical micelle with higher ring curvature than that observed in these small toroids. On the other hand, cross cylinders were found with long arms (Figure 2-7e and 7f). Considering the small size of typical lamellar micelles, it seemed unlikely that these crosses should be derived from thinning of lamellar micelles. They were more likely generated via end-to-end, end-to-side, or side-to-side aggregation of cylindrical micelles.

Corroborating results were obtained with AuNP-embedded toroids and cylinders. Figure 2-5(b) to (e) show some typical examples of toroids resulted from either of two mechanisms mentioned above, along with the corresponding schemes. Toroids with very small inner circle were observed and some appeared as precursors to toroids with a dent at their center (Figure 2-5b). For large toroids, sometimes more than one AuNPs were observed in each toroid (Figure 2-5c) and sometimes they had braching arms (Figure 2-5d and 5e). Since we know that the AuNPs cannot have extensively aggregated under these

conditions before the PSPAA encapsulation,^{27,28} it is likely that the cylinders and toroids should arise from the aggregation of individual micelles. In toroids, the observation of multiple separated clusters of AuNPs provided support that they were derived from AuNPs originally embedded in spheres or cylinders.

Obviously, the final PSPAA micelles obtained in our system after the removal of DMF and acid were metastable and kinetically trapped.⁴⁰ But even in the preparative solution at 100 °C, the swelled PS₁₅₄-*b*-PAA₄₉ cylinders and toroids were found to continuously evolve, indicating that they were only kinetic products. After 9 h at 100 °C, they finally turned into vesicles (Figure 2-4e), which still retained a cylindrical tail in quite a number of cases. Merging the tail into the vesicle would require the polymer to be redistributed not only to the outer surface, but also to the inner surface. Hence, it must involve moving PSPAA through the vesicle wall. The remnant of cylindrical moieties on these atypical vesicles came in direct contrast to the regular PS₁₄₄-*b*-PAA₂₂ vesicles formed under similar conditions (Figure 2-4f). The PS₁₅₄-*b*-PAA₄₉ vesicles had not reached their lowest surface to volume ratio (S/V) and, hence, must also be kinetic products.

A thermodynamic driving force is required for any micelle transformation, even if the product is kinetically trapped. From a thermodynamic point of view, PSPAA micelles are driven towards the lowest S/V of the hydrophobic PS domain, which decreases in the order: sphere, cylinder, toroid, lamella, and

vesicle.³ Many types of interaction potentials favour the reduction in the S/V of the core, which can be grouped as a general term $U_{S/V}$. In our system, the interactions are the hydrophobic and van der Waals interactions among the PS blocks, but in the literature they could also include H-bonding,⁴¹ Coulombic^{42,43} or dipolar interactions,⁴¹ π - π stacking,⁴⁴ additional packing interactions resulted from the order or crystallinity within the core,^{45,46} etc. In general, these interaction potentials in the core are maximized if the S/V is minimized. The counteractive force, however, is the steric and charge repulsion among the coronal blocks, which is weaker at high curvature. Thus, the repulsion potentials favour high S/V towards spherical micelles.

At equilibrium, the structure of a surfactant micelle is a result of the balance between the $U_{S/V}$ and the repulsion potentials. Any condition that leads to a dominant $U_{S/V}$ favours the formation of vesicles. Reducing the repulsions is one way to achieve that: for example, use of PSPAA with shorter PAA chains,³⁵ protonation of the PAA chains by acid,²⁰ inclusion of homo-polystyrene in the PSPAA cores,¹⁸ increasing ionic strength for more effective shielding,¹⁹ mixing of cationic and anionic surfactants.⁴² Alternatively, increasing the inter-actions in the core can also lead to a dominant $U_{S/V}$: for example, increasing water content in the solvent (stronger interfacial tension),^{21,22} use of polymer blocks with more hydrophobic nature, and use of polymer that packs well in the core.

In our system, acid was used to protonate the PAA chains and reduce their

repulsion. Under this condition, vesicles were the favored form as they were the dominant species after long incubation (Figure 2-4e). Hence, the cylindrical and toroidal micelles were the trapped intermediates as spherical micelles transformed en route to vesicles. The high curvature ends of cylinders are thermodynamically unfavorable,^{7,47} and thus, they can be removed by aggregation or contraction.^{6,7} Annealing the polymer structures typically led to reduced side arms and end caps (Figure 2-1).

From a kinetic perspective, it is critical to understand the source of the barrier in the rate-determining micelle transformation. In the literature, it is known that the PS/PAA ratio is more important than the absolute length of the PS or PAA blocks.³⁵ Thus, we hypothesize that both PS and PAA blocks were involved: as two spherical micelles aggregated to make a cylinder, the interface between the spheres could trap some PAA blocks in the fused PS domain. Removing the ionic or polar PAA blocks through the nonpolar PS domains would conceivably require significant kinetic energy. Hence, PSPAA molecules with longer PAA blocks should encounter a higher activation barrier. As cylinders further transformed to give toroids, lamellae, and vesicles, the increase in size and decrease in S/V suggested that these transformations must have also involved the fusion of micellar interfaces, trapping PAA blocks in similar processes. Thus, PSPAA molecules with shorter PAA blocks led to higher mobility of the overall micelles which evolved faster.

Large polymer aggregates such as cylindrical and vesicular micelles must have evolved from smaller bodies by step-wise aggregation and structural transformation. Thus, the final PS₁₅₄-*b*-PAA₄₉ structure depended on the rate of both processes. Given the long PAA chains, it is unlikely that overall equilibrium was ever reached and the system was non-ergodic.^{40,48} As such, different individual micelles in a same solution may have very different trajectory. On the other hand, drifting of polymer molecules within individual micelles should require less kinetic energy, so long as it does not involve moving PAA through the PS domains. The *near* equilibration of individual micelles is supported by the uniform radii/thickness of the PS domains, and by the high symmetry of vesicles, single- and multi-toroids.

2.4 Conclusion

There is a dilemma in studying the self-assembly of amphiphilic polymers: If the polymer structure is highly mobile, it evolves quickly in a solution and typically reaches stable states under normal conditions. However, it is very difficult to trap and characterize the resulting micelles as they may evolve during the stirring, centrifuging, drying or freezing processes associated with sample preparation for microscopy methods. On the other hand, if the polymer structure has low mobility, it is difficult to induce its self-assembly and ensure sufficient structural transformation. Up to now, most of the reports in the

literature used high mobility polymer micelles which were often characterized by cryo-TEM. In contrast, we used low mobility polymer micelles which were known to be very stable in water at room temperature,³⁴ and this allowed us to use simple TEM characterization to screen a large number of samples formed under different conditions. We used heating and high organic solvent ratio to improve the polymer mobility during the micelle formation; the intermediates were conveniently trapped by adding water to deswell the polymer micelles. Most importantly, the extremely low mobility of the polymer micelles proved to be invaluable as we were able to follow their structural evolution in the time scale of hours. With the understanding in the structural transformation of PSPAA micelles, we can readily incorporate NPs in their hydrophobic domains. The NP-incorporated micelles can aggregate and evolve from spheres to cylinders and toroids, giving rise to unique nanocomposites.

Understanding the micelle transformation is of critical importance. A large number of experimental variables are important, particularly because of the rich kinetics of this process. We tried to give rationales for each variable to avoid random search in the multi-dimensional variable space. We believe that in-depth understanding and rational design of experimental setups are critical for developing structural controls of surfactant micelles and their nanocomposites.

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Chapter 3 Bridging the Gap in the Micellar Transformation from Cylinders to Vesicles

3.1 Introduction

Controlling the shape of nanostructure in self-assembly is a major challenge in nanoscience and a crucial step in exploring new applications. Nanocrystals exhibit a variety of shapes depending on the stability of their surface facets. On the other hand, polymer micelles can take many forms including sphere, cylinder, and vesicle, but these shapes are governed by a different set of rules. Understanding these rules is essential for controlling polymer micelles and exploring new polymer-nanocrystal composites.¹⁻⁴

Vesicles are fluid-filled sacs that have a bilayer wall assembled from amphiphilic molecules or copolymers.⁵⁻⁸ The most notable examples are the various membrane-enclosed sacs in biology (e.g. cells). Thus, studying the formation of vesicles and their behavior is of critical importance for understanding biomembranes. Moreover, vesicles are ideal artificial nanoscale compartments, which are being explored as nanoreactors and drug delivery vehicles.^{1,9-12}

Micellar evolution involves three distinct but intertwined processes: the

micellization of amphiphiles once they reach critical micelle concentration (CMC); the “growth” in size via micellar aggregation; and the morphological evolution after the aggregates reach a critical size.¹³⁻¹⁵ Vesicles can directly emerge from the phase-segregation (micellization) of agglomerated amphiphiles¹⁶⁻¹⁹ or sequentially evolve from pre-existing micelles,^{6,17,20} depending on the property of the amphiphile and the initial condition. In the latter scenario, it is well known that the micellar evolution goes from spheres to cylinders, to lamellae, and then finally to vesicles. While these four distinct states can be characterized by electron microscopy and optical spectroscopy,^{5,20-22} they merely represent isolated “snapshots” in the overall micellar transformation.

Indeed, most of the mechanistic studies in the literature focused on the thermodynamic driving forces, for example, by analyzing the packing parameters^{6,23,24} or the surface-to-volume ratio (S/V) of the specific micelle types.¹⁵ As such, how the detailed processes occur in between the static states remains elusive, and this can be largely attributed to the lack of experimental evidence. For example, the transformation from lamella to vesicle is nothing but trivial, involving complicated processes of folding and “stitching” a flat membrane into an enclosed sac, where the intermediates were rarely observed.^{20,21} This gap of missing intermediates has led to a gap in understanding micellar transformation, particularly regarding to the principles

underlying the kinetic pathways.

In the literature, the PSPAA micelles with short PAA chains (“crew-cut” micelles) rarely gave morphological intermediates,^{5,8,13,25,26} suggesting that their growth in size was the rate-limiting step (slower than their morphological evolution). Recently, we reported the synthesis of PS₁₅₄-*b*-PAA₄₉ toroids in DMF/H₂O mixture at elevated temperature.¹⁵ In this report, we explored the same system for the transformation pathways from cylinders/toroids to vesicles. With the “long-hair” PAA chains, the morphological evolution of PSPAA micelles was sufficiently slow, allowing the intermediates to be continually trapped. Analysis of these intermediates revealed the main barriers in the kinetic pathways and thus, the underlying principles.

3.2 Materials and Methods

3.2.1 Materials and Characterization

Amphiphilic diblock copolymer polystyrene-*block*-poly(acrylic acid) (PS₁₅₄-*b*-PAA₄₉, M_n =16000 for the polystyrene block and M_n =3500 for the poly(acrylic acid) block, M_w/M_n =1.15), and PS₁₄₄-*b*-PAA₂₂, M_n =15000 for the polystyrene block and M_n =1600 for the poly(acrylic acid) block, M_w/M_n =1.11) were purchased from Polymer Source, Inc.. Deionized water (resistance>18.2 M Ω •cm⁻¹) was used in all of our experiments. Copper specimen grids (200 mesh) with formvar/carbon support film (referred to as TEM grids in the text)

were purchased from Beijing XXBR Technology co., ltd.

TEM images were collected from a JEM-1400 Transmission Electron Microscope (JEOL) operated at 100 kV.

Preparation of TEM samples $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ was used as the negative stain in all of the TEM images reported in this study. TEM grids were treated with oxygen plasma in a Harrick plasma cleaner/sterilizer for 1 min to improve their surfaces hydrophilicity. A drop of sample solution was carefully mixed with stain solution ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 1 wt %) on the surface of a Parafilm. Then, the hydrophilic face of the TEM grid was placed in contact with the stained sample solution, and a filter paper was used to wick off the excess solution on the TEM grid. After drying in air, it can be used for TEM characterization.

3.2.2 Preparation of Micelles from PS-*b*-PAA

Synthesis of bowl-like intermediates from PS₁₅₄-*b*-PAA₄₉. The PS₁₅₄-*b*-PAA₄₉ diblock copolymer was first dissolved in anhydrous DMF (dimethylformamide, a good solvent for both PS and PAA blocks) at room temperature. Then, an appropriate amount of water, a selective solvent for PAA block, was added into the polymer solution in one shot to reduce polymer solubility and induce micellization. Lastly, acid was injected into the mixture to reduce the charges among the PAA corona, thus inducing the aggregation of the micelles. Heating was necessary to form vesicles and bowl-like intermediates.

In a typical experiment, 380 μL DMF, 40 μL of $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$ in DMF (8 mg/mL), 145 μL of H_2O , and 15 μL of HCl aqueous solution (0.1 M) were mixed in sequence in a glass vial. The final solution contains $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$ (0.55 mg/mL), HCl (2.59 mM), DMF (420 μL), H_2O (160 μL), and the $V_{\text{DMF}}/V_{\text{w}} = 42/16$. The vial was then capped to minimize solvent evaporation (estimated solvent loss < 0.3 wt %) and incubated at 100 °C in a temperature-controlled oil bath. After incubating the solution for 6 to 9 hours, the bowl-like micelles were formed. Due to the light scattering of the micelles, the resulting solution appeared slightly opaque with a bluish tinge. After cooling down to room temperature, 50 μL of the product solution was extracted and mixed with 1400 μL water. This caused a significant decrease of the DMF content in the solution and thus, the deswelling of the polymer domain. This solution was centrifuged at 12500 rpm for 30 min to isolate the polymer products and remove the residue DMF (residue amount of DMF could influence the quality of TEM images). The polymer products can be directly used for TEM characterization. For synthesizing other types of self-assembled morphologies of $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$, the experimental process was similar to that for bowl-like micelles except that the incubation time and temperature were different.

Control experiments: Synthesis of $\text{PS}_{144}\text{-}b\text{-PAA}_{22}$ vesicles. 380 μL of DMF, 40 μL of $\text{PS}_{144}\text{-}b\text{-PAA}_{22}$ in DMF (8 mg/mL), 125 μL of H_2O , and 5 μL of aqueous HCl solution (0.1 M) were mixed in sequence in a vial. The final

solution contains PS₁₄₄-*b*-PAA₂₂ (0.58 mg/mL), HCl (0.91 mM), DMF (420 μ L), H₂O (130 μ L), and the $V_{\text{DMF}}/V_{\text{w}} = 42/13$. The vial was then capped and incubated at 100 °C in a temperature-controlled oil bath for 1 hour to form vesicular micelles. Additional experiments were also attempted, by lowering the incubation temperature, reducing the acid quantity, etc, but we were unable to obtain bowl-like intermediates.

Evidence for lack of polymer exchange among micelles. 84 μ L DMF and 32 μ L aqueous solution of PS₁₅₄-*b*-PAA₄₉ encapsulated gold nanoparticles chain (Figure 3-10a, Au-Chain@PSPAA,²⁷) were added into the as-prepared solution of cylindrical micelles (Figure 3-10b), and then the solution was reheated at 100 °C for 6 hours. The addition of additional DMF into the solution was to keep the volume ratio of DMF to H₂O unchanged.

3.2.3 Interpretation of the TEM Images.

(NH₄)₆Mo₇O₂₄ was used as the negative stain to improve the contrast of the polymer aggregates in all TEM images reported in our study. The salty stain solution often formed a thin layer when dried on a TEM grid. In TEM images, because the polymer domains scatter electrons less efficiently than a dried layer of (NH₄)₆Mo₇O₂₄ salt, they appeared whiter with clear contrast against the background. From the TEM images, we can find that the distribution of the stain layer varied from place to place in a sample, because it is highly

dependent on the drying of stain solution during the sample preparation (forming randomly distributed puddles in the final stage of drying).

For a bowl-like micelles embedded inside a stain layer (Figure 3-1a), electron beam transmitted through the polymer was less scattered as compared to the beams transmitted through the stain layer. Therefore, the polymer shell normally appears white in contrast to the dark stain layer. Specifically, the stain layer can surround the micelles, go in the bowls, deposit on the tethered cylinders, as well as deposit on the TEM grid, where the thickness of the stain layer are quite different, as illustrated in Figure 3-1a. The corresponding brightness in the TEM image decreases in the following order (with reducing thickness of polymer and/or increasing thickness of stain): the side wall of the bowl (1); the tethered cylinder (3); the central wall of the bowl (2); and the background (4).

For a bowl-like micelle left out of the stain puddle (Figure 3-1b), due to capillary effect in the drying process, the stain solution can leave a dark outline surrounding the micelle. In addition, the stain solution can go into the cavity of the bowl, and form a thin layer on the surface of the TEM substrate. Thus, the corresponding brightness in the TEM image decreases in the following order: the polymer domains except the central wall of the bowl (1,3) have similar brightness as the background (4); the outline surrounding the micelle (5); and the central cavity region of the bowl (2).

Figure 3-1. Interpretation of the bowl-like micelles with different contrast in TEM images. The bowl-like micelles could be buried inside a stain puddle (a), or left outside of it (b).

3.2.4 Calculation of Surface-to-Volume Ratio (S/V)

Calculation of S/V (without rims). As shown in Scheme 3-1, the diameter or the thickness of the hydrophobic core is limited by the physical length of

coiled hydrophobic blocks, which are unfavourable when stretched. In the literature, it is known that the diameter of the sphere, the diameter of the rod, the thickness of the lamella, and the shell thickness of the vesicle are slightly (but consistently) different. In our calculation, we set them as the same for convenience of comparison. To remove the effect of the rims, the length of the rod and the size of the lamella are set to infinite.

Scheme 3-1. Schematics showing sphere, rod, lamella, and vesicle.

Under the idealized conditions above:

$$S/V_{\text{sphere}} = 4\pi r^2 / [(4/3) \pi r^3] = 3/r$$

$$S/V_{\text{rod}} = 2\pi r l / (\pi r^2 l) = 2/r \text{ (ignore the end caps)}$$

$$S/V_{\text{lamella}} = 2ab / (ab \cdot 2r) = 1/r \text{ (ignore the rim)}$$

A vesicle of infinite size should have the same S/V as a lamella of infinite size. Thus, under the idealized conditions, the S/V decreases in this order: sphere, cylinder, and vesicle.

If a vesicle is not infinite, and has an overall radius of R :

$$\begin{aligned} S/V_{\text{vesicle}} &= [4\pi R^2 + 4\pi(R+2r)^2] / [(4/3) \pi(R+2r)^3 - (4/3) \pi R^3] \\ &= [1.5/r] \{R^2 + (R+2r)^2\} / [R^2 + (R+2r)^2 + R(R+2r)] < 1.5/r \end{aligned}$$

$$\text{since } [R^2+(R+2r)^2]/[R^2+(R+2r)^2 + R(R+2r)] < 1$$

$$S/V_{\text{vesicle}} < 1.5/r$$

Calculation of S/V (caps and rims are included). We use the following principles in the calculations: (a) For convenience of comparison, we set the diameter of the cylinders and the shell thickness of the lamella to be the same as that of the vesicle (27 nm). (b) The volume of the rod, lamella, and concave lamella, was set to be the same as that of vesicle, which can be calculated from the measured values from TEM images. More specifically, we measured hundreds of vesicles from TEM images, and obtained the average diameter of the vesicles (86 ± 4 nm) and the average diameter of the cavity (32 ± 3 nm) in the vesicles. With these values, we can obtain the volume of a typical vesicle (100548π , see below). (c) The length of rod, the diameter of lamellar disk, and the diameter of the inner cavity of partial vesicle, respectively, were set as the only variables; they can be calculated once the total volume is given (100548π , the same as that of vesicle).

Given these principles, we solved the following equations to obtain a value for the unknown variables, and calculated the overall S/V . The detailed calculation process was shown in Scheme 3-2. The calculation results showed that S/V of the hydrophobic domain decreases monotonously in this order: cylinder, lamella, concave lamella and vesicle (Figure 3-6e and 6f).

Geometric drawing	Calculation method	S/V values
Vesicle	<p>$R=16 \text{ nm}$ (Measured), $2r=27 \text{ nm}$ (Measured)</p> <p>$S = 4 \pi R^2 + 4 \pi (R+2r)^2 = 8420 \pi$</p> <p>$V = (4/3) \pi (R+2r)^3 - (4/3) \pi R^3 = 100548 \pi$</p> <p>The volume of following structures and the diameter of the cylinder/the thickness of the partial vesicle were supposed to be the same with those of vesicles.</p> <p>These known data was substituted into following equations. After solving these equations, we can get the unknown variable, and thus the S/V.</p>	0.0837
(a) cylinder	<p>$2r=27 \text{ nm}$</p> <p>$a=533.7 \text{ nm}$ (unknown variable, Calculated)</p> <p>$V = \pi r^2 a + (4/3) \pi r^3 = 100548 \pi$</p> <p>$S = 2 \pi r a + 4 \pi r^2$</p>	0.1506
(b) lamella Side view top view	<p>$2r=27 \text{ nm}$</p> <p>$b=50.53 \text{ nm}$ (unknown variable, Calculated)</p> <p>$V = \pi b^2 2r + (1/2) \pi r^2 [(4r/3 \pi) + b] \cdot 2 \pi = 100548 \pi$</p> <p>$S = 2 \pi b^2 + \pi r [(4r/3 \pi) + b] \cdot 2 \pi$</p>	0.09770
(c) partial vesicle : $\alpha < 90^\circ$ Side view	<p>$2r=27 \text{ nm}$, $R-R'=27 \text{ nm}$,</p> <p>$H = R - R \cos \alpha$, $H = R' - R' \cos \alpha$,</p> <p>$L = (R' + r) \sin \alpha + (4r/3 \pi) \cos \alpha$</p> <p>$R'$: unknown variable, depend on the α</p> <p>$V_{\text{total}} = SR/3 - S'R'/3 + \pi r^2/2 \cdot 2 \pi L = 100548 \pi$</p> <p>$S_{\text{total}} = S + S' + S'' = 2 \pi RH + 2 \pi R'H' + \pi r \cdot 2 \pi L$</p>	<p>$\alpha = 30^\circ$: 0.09743</p> <p>$\alpha = 45^\circ$: 0.09707</p> <p>$\alpha = 60^\circ$: 0.09651</p>
(d) half vesicle	<p>$2r=27 \text{ nm}$</p> <p>$c=24.2 \text{ nm}$ (unknown variable, Calculated)</p> <p>$V = (2/3) \pi (c+2r)^3 - (2/3) \pi c^3 + (1/2) \pi r^2 (c+r) 2 \pi = 100548 \pi$</p> <p>$S = 2 \pi c^2 + 2 \pi (c+2r)^2 + \pi r (c+r) 2 \pi$</p>	0.09558
(e) partial vesicle : $90^\circ < \alpha < 180^\circ$	<p>$2r=27 \text{ nm}$, $R-R'=27 \text{ nm}$</p> <p>$H = R - R \cos \alpha$, $H = R' - R' \cos \alpha$,</p> <p>$L = (R' + r) \sin \alpha - (4r/3 \pi) \cos \alpha$</p> <p>$R'$: unknown variable, depend on the α</p> <p>$V_{\text{total}} = (4 \pi R^3/3 - SR/3) - (4 \pi R'^3/3 - S'R'/3) + \pi r^2/2 \cdot 2 \pi L = 100548 \pi$</p> <p>$S_{\text{total}} = S + S' + S'' = (4 \pi R^2 - 2 \pi RH) + (4 \pi R'^2 - 2 \pi R'H') + \pi r \cdot 2 \pi L$</p>	<p>$\alpha = 120^\circ$: 0.09171</p> <p>$\alpha = 135^\circ$: 0.08965</p> <p>$\alpha = 150^\circ$: 0.08725</p>

Scheme 3-2. Schematics showing the detailed calculation of S/V values for the intermediates in between cylinder and vesicle.

3.3 Results and Discussion

3.3.1 Fabrication of Bowl-like Intermediates

The synthesis of PS₁₅₄-*b*-PAA₄₉ micelles was as follows: The copolymer (0.55 mg/mL) was first dissolved in a DMF/water mixture (27 % *v/v* water), where the DMF content was selected such that the swollen micelles²⁷⁻³⁰ were sufficiently mobile but their transformation was not too fast. After adding HCl (2.59 mM) to reduce the charges on the PAA chains, the solution was heated at 100 °C for a given period of time ($t = 1-12$ h) to promote micellar transformation.³¹ After cooling, a large amount of water was added to remove DMF from the polymer domains. The deswollen PSPAA micelles were known to be kinetically frozen.^{32,33} In this system, both high-temperature and high DMF content of the solution were critical for the micellar transformation. The intermediates can be easily trapped by depriving them of these conditions.

After centrifugation, the isolated intermediates can be directly dried at room temperature and characterized by transmission electron microscopy (TEM). At $t = 1$ h, cylinders and toroids were obtained (Figure 3-2b);¹⁵ and at $t = 3$ h, some of them merged into multi-toroids, where small lamellar phase can be identified at the toroidal junctions (Figure 3-2c, black arrows). After prolonged incubation ($t = 12$ h), most of the micelles turned into vesicles (Figure 3-2d). Study of the transition period ($t = 3-12$ h) revealed that the bowl-like micelles were the

common intermediates (Figure 3-2f, 2g, and Figure 3-3),³¹ which were essentially partial-vesicles with tethered remnant cylinders. Such a motif can be frequently observed, either at the tip of a cylinder, at the U-shaped corner of a bent cylinder, or at the lamellar junction of a multi-toroid (Figure 3-4).

Figure 3-2. (a) Schematics illustrating the structure of a bowl-like micelle (for convenience, the “long-hair” PAA chains were represented only as spheres). TEM images of (b) PS₁₅₄-*b*-PAA₄₉ cylinders and toroids (*t* = 1 h); (c) PS₁₅₄-*b*-PAA₄₉ toroids and multi-toroids with lamellar junction (*t* = 3 h); (d) PS₁₅₄-*b*-PAA₄₉ toroids and multi-toroids with lamellar junction (*t* = 3 h); (e) PS₁₅₄-*b*-PAA₄₉ toroids and multi-toroids with lamellar junction (*t* = 3 h); (f) PS₁₅₄-*b*-PAA₄₉ toroids and multi-toroids with lamellar junction (*t* = 3 h); (g) PS₁₅₄-*b*-PAA₄₉ toroids and multi-toroids with lamellar junction (*t* = 3 h).

PS₁₅₄-*b*-PAA₄₉ vesicles that have remnant cylindrical tails even after prolonged incubation ($t = 12$ h); (e) “clean” vesicles made from PS₁₄₄-*b*-PAA₂₂ ($t = 1$ h); (f, g) Selected images of the bowl-like intermediates ($t = 9$ h) from a same sample. The black arrows indicate the lamellar junction and the subsequent bowls; the white arrows indicate the bowls at the U-junctions. Scale bar: 200 nm.

Figure 3-3. TEM image of the PS₁₅₄-*b*-PAA₄₉ bowl-like intermediates (shallow bowls) and lamellar junction with protruding cylinders ($t = 6$ h).

Figure 3-4. Selected TEM images showing the different types of bowl-like micelles: (a) at the tip of cylinders; (b) at the U-shaped turn of cylinders; (c) at the inner corner of toroids; (d) at the U-shaped turns in a sample with prolonged heating (after 12 h incubation at 100 °C or 1h incubation at 130 °C, most of the micelles in this sample turned into vesicles).

3.3.2 Assignment of Bowl-like Structures

We applied aqueous $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ as the negative stain in preparing our TEM samples. When the PSPAA micelles were embedded in a layer of stain, the polymer domains appeared white against the dark background (Figure 3-4c4, 3-4d); for those left outside the stain “puddle” (Figure 3-4a, 3-4b, 3-4c1 to 4c3), the small amount of residue stain tended to adsorb on the boundary of the micelles, forming dark outline against the grayish white background. The

solution was able to enter the bowls due to capillary effect, but not the enclosed cavities of the vesicles. As a result, the central wall of a bowl (top/bottom membrane) always appeared darker than its side wall, because the stain inside the bowl cavity always scatters electron beam more efficiently than a polymer domain of equal thickness (the electron beam is perpendicular to the substrate surface; see the Schematics in 3.2.3 Interpretation of the TEM Images). In Figure 3-2(f) and 2(g), the brighter side walls of the bowls can be easily distinguished from the cylinders and flat lamellae nearby, because the polymer walls create a thicker “spacer” in the path of electron beam.

Our assignment of the bowl structure was based on the follows: (a) the above analyses of the TEM contrast; (b) being a “partial-vesicle”, the bowls have uniformly curved walls, which are different from the tethered cylinders/toroids; (c) in tilted images (Figure 3-5a and b), the diameter of the bowl remained unchanged, suggesting that they were roughly symmetrical around the bowl axis, whereas the height of the bowls was variable depending on the viewing angle; (d) for the bowls derived from multi-toroids (Figure 3-2f, 3-4c1 and 4c2), cylinders were found tethered not only to the side walls, but also to the central walls, proving the existence of such top/bottom wall; (e) after silica encapsulation,³⁴ both the inner and outer surfaces of the bowls were coated with silica (Figure 3-5c), which was distinctively different from the closed vesicles with uncoated inner surface (Figure 3-5d).

Figure 3-5. Selected TEM images showing (a, b) the bowls before and after tilting the TEM grid by $\pm 25^\circ$ (see the schematics on the top panel). The ruler indicates that (a) the diameter of the bowl remains unchanged but (b) the height of the bowl varies depending on the viewing angle. After silica encapsulation, (c) four layers can be identified in a typical bowl: 1. silica shell on the outer surface; 2. polymer layer; 3. silica shell on the inner surface; and 4. open cavity; in contrast, (d) typical vesicles only present 1, 2, and 4, indicating the uncoated inner surface.

Because polymer structures are usually soft, one would expect a bowl-like micelle to be flattened during the drying of the sample on a flat TEM substrate. This would be particularly problematic for shallow bowls. In comparison to the literature examples, we attribute our ability to trap bowl-like micelles to the rigidity of the de-swollen PSPAA domains at room temperature.^{32,33}

3.3.3 Structure Analysis of Bowl-like micelles

In the literature, it is reported that the diameter of the cylinders is close to but always larger than the thickness of the corresponding vesicle walls.²⁰ This trend was recognizable in the TEM images, particularly by comparing the vesicles (27 ± 3 nm in thickness) to the tethered cylinders (37 ± 5 nm in width) (Figure 3-4). The wall thickness of the bowl-like micelles (29 ± 4 nm) was close to that of vesicles, suggesting that they were indeed partial-vesicles.

Thus, it appeared that the bowls were derived from and seamlessly connected to the remaining cylinders. Their walls should have the same double-layered structure as that of vesicles, with the hydrophobic PS chains packed in the center and the hydrophilic PAA chains covering both the inner and outer surfaces (Figure 3-6a and 6b). This assignment was consistent with the full coverage of silica on the exposed polymer surfaces (Figure 3-5c and 5d). Hence, these domains can match with the hydrophobic/hydrophilic domains of the tethered cylinders, though at their junction there is a significant decrease

from the cylinder diameter to the wall thickness. Such a seamless connection is critical for the “flow” of polymer in the following hypothesis.

Figure 3-6. (a) Schematics showing the transformation process from cylinder to vesicle. (b) The cross-section view of the corresponding structures in (a), where a section is highlighted in purple to show the flowing polymer domain. (c, d) Selected TEM images showing the typical intermediates during cylinder-to-vesicle transformation. (e, f) Calculated S/V ratio of cylinder, lamella, vesicle, and bowl-like intermediates, showing its monotonous decrease during the transformation.

Analysis of the various intermediates trapped during the cylinder-to-vesicle transformation revealed a holistic pathway. Selected TEM images are shown in Figure 3-6c and 6d to support the progressive evolution of bowl-like micelles towards vesicles (each sample contained a mixture of products but the trend was clear³¹). The initial step is always associated with the "flattening" of a cylindrical section to give a small lamellar domain.²⁰ This lamellar domain then depresses to give a shallow bowl, which gradually grows as the polymer in the tethered cylinder continuously flows into the lamella. In our system, large lamellar domains were rarely observed, and the small lamellae almost always had a dent at their center. With the growth of bowls, they rarely increase in overall size (judging from their cavity diameter) but always increase in depth. These observations suggested that the growth of lamellar disks (likely via flow of polymer from the tethered cylinder) was slower than the transformation into deeper bowls, leading to their narrow size distribution. When they pass the stage of half-vesicle, the rim of the bowls then tightens, before eventually merging together to give closed vesicles. The fact that we were able to trap a large percentage of the micelles as partial-vesicles (Figure 3-2f and 2g) suggests that the subsequent step is the main barrier.

The trapped intermediates provided detailed "snapshots" in the transformation process. Their structural analysis may help validate the above pathway. The packing parameters^{6,8} of the constituent amphiphiles cannot be

extracted from the bowl-like micelles. By setting all micelles to have a same volume and a same diameter/thickness (27 nm), we can easily compare their respective S/V . Typically, larger S/V means a larger area of the unfavorable polymer-solvent interface and less amount of the favorable internal interactions (van der Waals and hydrophobic) within the polymer domains. It is well known that the S/V decreases from cylinder to lamella and to vesicle, and this trend was easily confirmed (Figure 3-6e). When a lamellar disk slightly depresses to form a shallow bowl, the surface of the central portion increases but the disk contracts, giving a smaller rim perimeter. Hence, there is an overall decrease of S/V , indicating that the depression of lamella is energetically favored. Indeed, our calculations of the intermediates showed that the S/V decreases monotonously from a lamellar disk to a vesicle (Figure 3-6f).³¹

3.3.4 Mechanism Analysis

The analysis of S/V gives no obvious barrier in the transformation process. Should micelles transform freely, one would expect any micelle to adapt a lowest energy structure once it reaches a critical size. However, in our system, most of the bowls with tethered cylinders obviously have sufficient size, but they did not turn into vesicles. Hence, relying on the structural analysis of the overall shapes can be misleading. Moreover, while shape analysis provides a trend of the thermodynamic driving force, it does not touch on the core of the

question of how the transformation occurs. More often than not, this question is a question on the kinetics.

An interesting characteristic of the observed pathway is that it mostly involves the “flow” of polymer domains without significant mingling of the hydrophobic and hydrophilic domains. Such mingling step is unfavorable but often necessary. From this perspective, the kinetic barrier probably lies in the movement of amphiphiles and the merging of the polymer domains, which are not represented in the static analysis of S/V or packing parameters. For example, slightly depressing a lamella will induce compressive strain in the inner layer and tensile strain in the outer layer of the bilayer membrane. Geometric requirements thus dictate that the constituent amphiphiles must move from the inner to the outer layer, creating an extra step that must be considered.

The kinetic barriers can be analyzed from the point of view of interactions: In general, hydrophilic groups have polar interactions with each other, and hydrophobic groups have van der Waals and hydrophobic interactions with each other. But there is little interaction between hydrophilic and hydrophobic groups. On these bases, there should be low barriers for an amphiphilic molecule to move parallelly within a micelle (Figure 3-7a) and for an entire domain to “flow” back and forth (Figure 3-7b), as long as the interactions within the hydrophobic and hydrophilic domains are not disrupted. In contrast, moving an amphiphilic molecule directly across a bilayer membrane must

disrupt the hydrophobic domain because its hydrophilic head group will interact poorly with its surrounding (Figure 3-7c). Similarly, the merging of two micelles (Figure 3-7d), the severing of cylindrical micelles (the reverse process of Figure 3-7d), and the closing of partial vesicles (Figure 3-7e) should be unfavorable because these processes require the formation of a huge cavity in the hydrophobic domains or the mingling of the hydrophilic and hydrophobic domains.

Under these proposed principles, an optimal pathway would maximize the “easy” processes and minimize the “difficult” ones. In the above example of depressing a lamella, flowing polymer from the inner to the outer layer would be more favorable than transferring molecules directly across the membrane. In forming a vesicle, it would be more favorable to reshape the micelles by flowing, via bowl-like intermediates as shown in Figure 3-6(b). The final step of closing the bowl (Figure 3-6b) must involve the merging of micellar fronts (Figure 3-7e), making it the rate-limiting step.

Figure 3-7. Analysis of kinetic barriers from the point of view of molecular interactions: (a) moving amphiphiles parallelly within a micelle; (b) “flowing” of an entire domain back and forth; (c) moving amphiphiles directly across a hydrophobic domain; (d) merging of micellar fronts (the reverse process is the severing of micelles); (e) elimination of pinhole at the final stage of vesicle formation. a and b should encounter lower barriers than c-e.

Using the principles, we can further compare across different polymer systems. In general, having a smaller or less charged head group would reduce the barriers involved in mingling hydrophilic and hydrophobic domains (Figure 3-7c to 7e), thus promoting the structural evolution of micelles. This explains the common use of acids^{5,15} and polyamines^{35,36} in neutralizing the negatively charged head groups and facilitating micellar evolution. In Eisenberg's system, the "crew-cut" micelles with relatively short PAA chains underwent rapid evolution, with the transformation process taking only a few minutes.^{14,20} The short PAA chains also facilitated the movement of PSPAA molecules directly across vesicle walls, redistributing molecules with longer PAA chains on the outer surface of the vesicles and those with shorter PAA chains on the inner surface.³³ In our system, when we used PS₁₄₄-*b*-PAA₂₂ instead of PS₁₅₄-*b*-PAA₄₉, with the similar experimental recipe to that of Figure 3-2(b), vesicles were formed from the self-assembly of PS₁₄₄-*b*-PAA₂₂ in the DMF/H₂O mixture after incubating at 100 °C for 1 h (Figure 3-2e, Figure 3-8), and bowl-like intermediates were rarely observed. The time was much shorter than that needed for forming PS₁₅₄-*b*-PAA₄₉ vesicles (Figure 3-2d, 12 h), indicating that the polymer with relatively shorter PAA chains evolves much faster during the self-assembly process. We cannot capture bowl-like intermediates from the self-assembly of PS₁₄₄-*b*-PAA₂₂, even after lowering the incubation temperature and reducing the acid quantity (Figure 3-9). As shown in Figure 3-9, both

cylinders and vesicles were obtained in a same sample, suggesting that the conditions were suitable, but no bowl-like micelle was observed. Moreover, the vesicles did not have any tethered cylinders, suggesting that severing of their junction became easier. These results further supported our proposed principles.

Figure 3-8. Large area TEM image of the “clean” PS₁₄₄-*b*-PAA₂₂ vesicles in Figure 3-2(e), which formed after incubating at 100 °C for 1 h.

Figure 3-9. TEM image of the vesicles and cylinders made from the self-assembly of PS₁₄₄-*b*-PAA₂₂, which formed after incubating at 70 °C for 1 h.

The exchange of polymer chain among micelles seemed rarely happened. To prove it, gold nanoparticle chains encapsulated in PS₁₅₄-*b*-PAA₄₉ shells²⁷ (Figure 3-10a, Au-Chain@PSPAA; AuNPs were essentially markers for the polymer shells) were purified and then dispersed in 32 μL of water and 84 μL of DMF. This solution was quickly added into the as-prepared solution of cylindrical micelles (Figure 3-2d, 3-10b), and then the mixture was heated at 100 °C for 3-6 hours. The additional DMF was to keep the volume ratio of DMF to H₂O unchanged. Thus, two types of polymer micelles were mixed in a

same solution, one type of micelles has embedded gold nanoparticles whereas the other has not. After incubation, the initial cylinders/toroids were converted to bowl-like intermediates but none of the Au nanoparticles were separated/transferred, suggesting that there were little transfer of polymer among the two types of micelles. We can further rule out that (a) the PSPAA disassemble and reassemble rapidly and (b) the aggregation (growth) of micelles occurred extensively in this system.

Figure 3-10. TEM images of (a) Au-chain@PSPAA, (b) PS₁₅₄-*b*-PAA₄₉ cylinders and toroids, (c) after heating the mixture of the purified sample (a) and sample (b) at 100 °C for 6 h, the initial Au-Chain@PSPAA cylinders changed to vesicle/bowl-like micelle with protruding cylinder. The images in (c)

were all from one sample.

Hence, the apparent “mobility” of polymer micelles depends on the kinetic barriers in moving amphiphiles and merging polymer domains, which in turn depends on factors such as polymer chain length, solvent quality, and temperature. In our system, the mobility was not changing because these factors were kept constant. It would be more difficult to study the transformation kinetics when these factors are variable, for example, during the slow addition of water into an organic solution of polymer,^{14,17,33} or during the slow in situ polymerization of amphiphilic polymers.^{21,37,38} Indeed, with these key factors changing over time, even the thermodynamically favored end-point (cylinders or vesicles) is shifting, making it harder to distinguish whether the obtained products were thermodynamically or kinetically controlled.

3.3.5 Comparison to Other Systems

In the literature, several different methods were commonly used to prepare micelles from amphiphilic molecules and block copolymers. With the preparative conditions different, it is important to understand their mechanistic implications, *i.e.*, how the thermodynamic force and kinetic barrier of the micelle transformation are affected.

a) Typically, a good solvent (*e.g.*, DMF or THF) is selected to dissolve both the hydrophobic and hydrophilic blocks of the block copolymer (*e.g.*, PSPAA).

Then, a selective solvent (*e.g.*, water) that only dissolves the hydrophilic block is slowly added into the solution, inducing micellization. In this system, different solvent ratio leads to different end state (thermodynamics) of the micelle morphology.¹³ Moreover, the solvent ratio affects the swelling of the polymer domain and thus, the barrier for molecular reorganization. In other words, both the thermodynamic and kinetic factors are changing during the slow addition of water into the solution. Once the water content is over a critical level, the micelles are kinetically frozen. It is difficult to study the ideal solvent ratio and to ensure sufficient time for the copolymers to fully assemble and evolve. Thus, the preparative conditions create additional problems for studying the kinetic pathway in micellar transformation.

b) An alternative method is to fully dissolve a block copolymer (*e.g.*, polybutadiene-*block*-polyethylene oxide, PB-*b*-PEO) in an organic solution, which is then dried into a film, often with vacuum and/or annealing.^{39,40} Then, the film is rehydrated in water. In doing so, it breaks into pieces which then evolve into various types of micelles. Typically, the polymers used in this method have high mobility, so that they can evolve in pure water. But this also means that when a sample is dried or concentrated it will continue to evolve. Hence, cryo-TEM must be used to characterize the micelles. In this system, the starting state can only be a film and the high mobility of polymer makes it difficult to trap intermediates. Moreover, freezing has its own problems as a

means of trapping intermediates. It is an inhomogeneous process that can concentrate the solutes and it is known to affect nanostructures.⁴¹

c) Recently, vesicle intermediates were observed during the direct polymerization of amphiphilic copolymer in water.²¹ In this system, the monomer is a potential swelling agent and the polymer growth occurs in an inhomogeneous system (solvent and the various form of micelles). As the monomer is consumed and the polymer blocks grow longer, both the thermodynamic morphology and the mobility of the polymer are changing. It is challenging to study the kinetic pathway in such a complex system.

d) In our system, the block copolymer (PSPAA) is dissolved in a solution with selected and unvarying DMF/water ratio. The solution is then treated with acid and heated for a sufficient long period of time. Both the DMF and the high temperature facilitate the micelle transformation, and they also lead to facile sample preparation. Thus, all variables are locked except the reaction time, and they can be individually tuned to study their effects. Such a clean system allows easy distinction of thermodynamic and kinetic factors, facilitating our mechanistic study.

3.4 Conclusion

In summary, during the cylinder-to-vesicle transformation, the reduction of surface-to-volume ratio provides the thermodynamic driving force whereas the

main kinetic barrier arises from the molecular reorganization within the micelles. Hence, the apparent "mobility" of polymer micelles depends on the barriers in moving amphiphiles and merging polymer domains, which in turn depend on factors such as polymer chain length, solvent quality, and temperature. Our experimental conditions are selected such that these key factors are kept constant, reducing the ambiguity in assigning the kinetically controlled intermediates.

The pioneering mechanistic studies by analyzing the packing parameters and S/V have granted tremendous insights in the thermodynamic driving forces of micellar evolution. Understanding the kinetics of this process is of equal importance, if not more. Our study is only an initial step in probing the nature of kinetic barriers and the governing principles, which will permit rational design of vesicles and grant insights in biomembrane behavior.

3.5 Bibliography

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Chapter 4 Dumbbell-like Nanostructures Made from Polystyrene-*block*-Poly(acrylic acid) and AuNPs

4.1 Introduction

Amphiphilic diblock copolymers (BCPs) can self-assemble into various micelles in selective solvents, such as spherical, cylindrical, lamellar, and vesicular micelles, large compound micelles, and large compound vesicles.¹⁻⁷ These controllable self-assembly morphologies provide excellent templates for constructing organic/inorganic hybrid nanomaterials, such as metal, magnetic, or semiconducting nanoparticles embedded in the polymer domains.⁸⁻¹⁴ The produced hybrid nanomaterials generally possess not only the properties of the organic and inorganic parts but also some new characteristics, which were generated by the specific spatial and size distribution of the inorganic NPs.

Two main approaches have been used to incorporate nanoparticles (NPs) into the BCP aggregates. One is the *in situ* synthesis of NPs on the BCP aggregates, which means that metal ions are first linked to or adsorbed into the aggregates, and then transformed to metal NPs by chemical reductions.^{12,14} One typical example is using polystyrene-*block*-polyvinylpyridine (PS-*b*-P4VP) as the

template to incorporate the inorganic precursors selectively into P4VP part followed by a chemical reduction step to obtain metallic nanoparticles.¹⁴ However, one disadvantage of this method is that it is not suitable for the as-prepared BCP aggregates NPs incapable of linking the metal ions. The other method is the *ex situ* synthesis of NPs on the BCP aggregates, in which the NPs stabilized by the organic ligands on the surface are first prepared, followed by the co-assembly of the NPs and BCPs in solution.¹⁵⁻²⁰ This method does not need the post chemical reduction within the aggregates. Besides, after surface modification of the as-prepared NPs, they can be linked to or embedded into the selective segments of the BCP aggregates, which means the controllable located position of the NPs in the BCP aggregates. For example, Wooley and co-authors reported the AuNPs stripes periodically distributed along the cylindrical micelles of amphiphilic triblock copolymers.²¹ They firstly prepared the cylindrical micelles with periodic PAA layers by self-assembly of poly(acrylic acid)-*block*-poly(methyl acrylate)-*block*-polystyrene (PAA-*b*-PMA-*b*-PS) in THF/H₂O mixed solvent, followed by addition of amine-modified AuNPs into the solution to make the amine-modified AuNPs incorporate into the PAA layers by electrostatic attractions.

In our previous work, by using the *ex situ* synthesis method, the location of the AuNPs (gold nanoparticles) was well controlled by introducing the ligands into the self-assembly of amphiphilic copolymer poly(acrylic

acid)-*block*-polystyrene (PAA-*b*-PS) in DMF/H₂O mixed solvent: concentric AuNP@PSPAA was obtained when hydrophobic ligand was added, while the Janus AuNP@PSPAA was obtained when hydrophobic ligand and hydrophilic ligand was added together.^{17,18,22,23} This control was achieved by a function of the binding competition between the hydrophobic ligand and the hydrophilic ligand.

Figure 4-1. TEM images and the corresponding schematics of the dumbbell-like nanostructures in which the BCP domains are (a) spherical micelles, (b) cylindrical micelles, and (c) vesicular micelles, respectively.

However, herein, we found that without addition of any ligand into the self-assembly solution, the located position of the AuNPs in the BCP aggregates of PS-*b*-PAA can be controlled to dumbbell-like nanostructures, as shown in Figure 4-1. This hybrid possesses three characteristics: a) the structure is highly symmetric; b) the BCP domains generally locate at the two opposite spherical caps of the AuNP, and the other parts of the AuNP is exposed to the outside environment; c) the BCP domains can be spherical micelles (Figure 4-1a), cylindrical micelles (Figure 4-1b), and vesicular micelles (Figure 4-1c). In this

chapter, we will focus on the mechanism behind the formation of these dumbbell-like nanostructures.

4.2 Materials and Methods

4.2.1 Materials and Characterization

All chemical reagents were obtained from commercial suppliers and used without further purification. Amphiphilic diblock copolymer polystyrene-*block*-poly(acrylic acid) (PS₁₅₄-*b*-PAA₄₉, $M_n = 16000$ for the PS block and $M_n = 3500$ for the PAA block, $M_w/M_n = 1.15$; PS₁₄₄-*b*-PAA₂₂, $M_n = 15000$ for the PS block and $M_n = 1600$ for the PAA block, $M_w/M_n = 1.11$), and thiol terminated polystyrene (PS-SH, $M_n = 2000$, $M_w/M_n = 1.15$) were purchased from Polymer Source, Inc.; 2-dipalmitoyl-sn-glycero-3-phosphothioethanol (PS-H) was purchased from Avanti Polar Lipids; the negatively charged, citrate-stabilized AuNPs (15 nm and 40 nm)²⁴ were prepared following literature procedures. Copper specimen grids (200 mesh) with formvar/carbon support film (referred to as TEM grids in the text) were purchased from Beijing XXBR Technology co., ltd.

Characterization. TEM images were collected on a JEM-1400 (JEOL) Transmission Electron Microscope operated at 100 kV.

Preparation of TEM samples (NH₄)₆Mo₇O₂₄ was used as the negative stain in all of the TEM images in this study. TEM grids were treated with oxygen

plasma in a Harrick plasma cleaner/sterilizer for 1 min to improve their surfaces hydrophilicity. A drop of sample solution was carefully mixed with stain solution ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 1 wt %) on the surface of a Parafilm. Then, the hydrophilic face of the TEM grid was placed in contact with the stained sample solution, and a filter paper was used to wick off the excess solution on the TEM grid. After drying in air, it can be used for TEM characterization.

4.2.2 Synthesis of Different Shapes of AuNP@PSPAA

Synthesis of concentric AuNP@PSPAA with hydrophobic ligand
addition 3 mL Sodium citrate stabilized AuNPs solution ($d_{\text{av}} = 15 \text{ nm}$, 4.75 nM) was centrifuged at 14,000 *rpm* for 15 min, and the deep red solution collected at the bottom of the tubes was diluted with H_2O to get 200 μL aqueous AuNPs solution. Then, DMF (820 μL), $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$ in DMF (80 μL , 8 mg/mL), aqueous AuNPs solution (200 μL), and PS-H (hydrophobic ligand) in ethanol (40 μL , 2 mg/mL) were mixed in a vial in the sequence given above. The vial was then capped and incubated at 110 $^\circ\text{C}$ in a temperature-controlled oil bath for 2 h. After cooling down to room temperature, an aliquot of 50 μL of the product solution was extracted and mixed with water (1400 μL), and then the solution was centrifuged at 12,500 *rpm* for 30 min to isolate the AuNP@PSPAA from DMF/ H_2O and excess reactants. The concentrated polymer micelles were used for TEM characterization.

Synthesis of dumbbell-like AuNP@PSPAA with two spherical micelles

ends For synthesis of dumbbell-like AuNP@PSPAA with two spherical micelles ends, the process was similar to that for the concentric one except that no ligand and a small amount of HCl was added into the system. Typically, 0.5 mL sodium citrate stabilized AuNPs solution ($d_{av} = 15$ nm, 4.75 nM) was centrifuged at 14,000 *rpm* for 15 min, and the deep red solution collected at the bottom of the tube was diluted with H₂O to get 145 μ L aqueous AuNPs solution. Then, DMF (380 μ L), PS₁₅₄-*b*-PAA₄₉ in DMF (40 μ L, 8 mg/mL), aqueous AuNPs solution (145 μ L), and HCl (15 μ L, 0.1 M) were mixed together in a vial in the sequence given above. The vial was then capped and incubated at 100 °C in a temperature-controlled oil bath for 1 h. For the experiment without HCl, longer reaction time (5 h) or higher temperature (130 °C) was needed to get these dumbbell-like nanostructures.

Synthesis of dumbbell-like AuNP@PSPAA with two cylindrical micelles

ends For synthesis of dumbbell-like AuNP@PSPAA with two cylindrical micelles ends, the process was similar to the synthesis of dumbbell-like AuNP@PSPAA with two spherical micelles ends except that more H₂O was added into the system.

Synthesis of AuNP@PSPAA-vesicle Synthesis of PSPAA vesicular micelles with fully embedded AuNPs includes two steps. *Firstly*, the sodium citrate stabilized AuNPs were modified with PS-SH to enhance the compatibility of

AuNPs with the hydrophobic PS block in the copolymer. Typically, a solution of citrate-stabilized AuNPs ($d_{av} = 15$ nm, 1 mL) was concentrated to ~ 10 μ L by centrifugation at 14,000 *rpm* for 15 min. The deep red solution collected at the bottom of the eppendorf tube was mixed with DMF (350 μ L) and PS-SH dissolved in DMF (20 μ L, 2 mg/mL). The mixture was then incubated at 60 °C for 2 h to ensure the ligand exchange completed, giving hydrophobic PS-S-AuNPs, which could be used directly in the subsequent process. *Secondly*, PS-S-AuNPs were incorporated in PSPAA vesicular micelles. In a representative experiment, DMF (260 μ L), PS₁₄₄-*b*-PAA₂₂ in DMF (60 μ L, 8 mg/mL), PS-S-AuNPs (15 nm, 100 μ L), H₂O (125 μ L), and HCl aqueous solution (5 μ L, 0.1 M) were mixed in sequence in a glass vial. The vial was then capped and incubated at 70 °C for 1 h.

Synthesis of dumbbell-like AuNP@PSPAA with two vesicular micelles

ends 1 mL sodium citrate stabilized AuNPs solution ($d_{av} = 15$ nm, 4.75 nM) was centrifuged at 14,000 *rpm* for 15 min, and the deep red solution collected at the bottom of the tubes was about 10 μ L. Then, DMF (360 μ L), PS₁₄₄-*b*-PAA₂₂ in DMF (60 μ L, 8 mg/mL), citrate stabilized AuNPs (15 nm, 10 μ L), H₂O (125 μ L), and HCl aqueous solution (5 μ L, 0.1 M) were mixed in sequence in a glass vial. The vial was then capped and incubated at 70 °C for 1 h.

4.3 Results and Discussion

4.3.1 Synthesis of Dumbbell-like Nanostructures

In our previous work, we found that concentric AuNP@PSPAA could be obtained when a thiol-ended hydrophobic ligand (PSH) was added into the reaction system (Figure 4-2a and 2b). The hydrophobic ligand (PSH) exchanged the sodium citrate on the surface of the AuNPs and formed the hydrophobically functionalized AuNPs, which could highly improve the compatibility between the AuNPs and hydrophobic part of the copolymer. When one more hydrophilic ligand was added into the above system, Janus AuNP@PSPAA could be obtained.¹⁸ This control was achieved by a function of the binding competition between the hydrophobic ligand and the hydrophilic ligand. However, in this work, we found that when no ligand but HCl was added into the system, the obtained nanostructures were similar to that with both hydrophilic/hydrophobic ligands: the AuNP was partially embedded into the BCP domains, as shown in Figure 4-2(c) and 2(d). Most of these hybrid nanostructures were highly symmetric, and the two opposite spherical caps of the AuNP were generally embedded into the BCP domains while the other parts of AuNP were exposed to the outside environment. This finding was surprised because it cannot be explained by our previous theory. So, the principle behind the formation of these dumbbell-like nanostructures is needed to further explore.

Figure 4-2. TEM images of (a, b) concentric AuNP@PSPAA obtained as hydrophobic ligand was added; (c, d) dumbbell-like AuNP@PSPAA with spherical BCP ends obtained as no ligand but HCl was added; (e, f) exposed AuNPs and empty PSPAA micelles obtained as no HCl was added.

When the system was without HCl, the result was quite different from that with HCl (Figure 4-2c and 2d): almost all of the AuNPs were exposed to the outside environment, not encapsulated by BCP domains, and copolymer PSPAA only self-assembled into the empty spherical micelles in DMF/H₂O mixed solvent (Figure 4-2e and 2f). HCl seemed to play an important role during the formation of such dumbbell-like nanostructures.

4.3.2 Investigation of the Influence Factors

Considering the significant influence of HCl on the final morphology, more controlled experiments and analysis have been done. It is reported that some residual Au ions still existed on the surface of the AuNPs or AuNWs (gold nanowires),^{25,26} which were synthesised by the reduction of the Au precursor. We proposed that the chloride ions (Cl⁻) from the HCl could neutralize the Au ions to change the surface property of the AuNPs, which made it more compatible to the hydrophobic part of the PSPAA. Based on this analysis, if this propose was right, when HCl was exchanged to NaCl or NaBr in this experiment, similar result would be obtained. However, as shown in Figure 4-3, no matter which substitute (NaCl or NaBr) was added into the reaction, most of the AuNPs were exposed, and PSPAA only self-assembled into empty spherical micelles in both experiments. This result revealed that chloride ion is not the key factor during the formation of the dumbbell-like nanostructures.

Figure 4-3. TEM images of the exposed AuNPs and empty PSPAA micelles obtained under the conditions with different anions: (a, b) NaCl, (c, d) NaBr, respectively.

Figure 4-4. TEM images of dumbbell-like AuNP@PSPAA with spherical ends obtained when different acid was added: (a) HNO_3 , and (b) H_2SO_4 , respectively.

The other possibility for the key factors that influenced the formation of the dumbbell-like nanostructures was the hydrogen ions donated by HCl. According to this consideration, some other acids (HNO_3 , H_2SO_4) were used instead of HCl in the experiment. As shown in Figure 4-4, the same dumbbell-like nanostructures were synthesised in both cases. Maybe due to the purification problem, plenty of empty PSPAA micelles were observed from TEM images (Figure 4-4a). Therefore, hydrogen ion seems to be the key factor in the formation of the dumbbell-like nanostructures.

However, for the system without addition of any acid, when the reaction time was prolonged to 5 h (reaction temperature: $100\text{ }^\circ\text{C}$) or the reaction temperature was elevated to $130\text{ }^\circ\text{C}$ (reaction time: 1 h), the same dumbbell-like nanostructures were formed, as shown in Figure 4-5(a) and 5(b). This finding

was quite different from the previous result under the same experimental condition (without acid) but different reaction time or temperature (1 h heating at 100 °C): exposed AuNPs and empty PSPAA spherical micelles (Figure 4-2e and 2f).

Figure 4-5. TEM images of dumbbell-like AuNP@PSPAA with spherical ends obtained when NO acid was added but (a, b) prolonging the reaction time to 5 h at 100 °C, or (c, d) elevating the reaction temperature to 130 °C, respectively.

4.3.3 Mechanism Analysis

The dumbbell-like nanostructures can be synthesized under these conditions:

(a) with hydrogen ion (H^+), 1 h heating at 100 °C; (b) without hydrogen ion (H^+), 5 h heating at 100 °C; and (c) without hydrogen ion (H^+), 1 h heating at 130 °C. So, several questions appeared in our mind: why the results obtained from the longer time heating or higher temperature heating are the same with that from the system with hydrogen ion? What is the relationship between the "hydrogen ion" and "long time heating or heating at elevated temperature"?

To solve these questions, careful analysis has been done. The final solution contained DMF, PSPAA, sodium citrate stabilized-AuNPs, H_2O , HCl (in case (a)). High temperature (100 °C or 130 °C) was required in all of these experiments. On one hand, the high temperature could increase the mobility of the copolymer chain and promote the morphological evolution of the micelles during the self-assembly process;⁸ on the other hand, the high temperature could speed up the decomposition or aging of some component in the solution. From DMF MSDS (material safety data sheet), we know that DMF is not stable in the presence of strong bases like sodium hydroxide or strong acids such as hydrochloric acid or sulfuric acid, and it would hydrolyze back into formic acid (FA) and dimethylamine (DMA) (Scheme 4-1), especially at an elevated temperature. Therefore, in case (a), because of the existence of acid, DMF was more likely to decompose to FA and DMA than that in case (b) and (c). And

thus, shorter heating time was needed in case (a) while longer time or higher temperature was required for the decomposition of DMF in case (b) or (c).

Scheme 4-1. Decomposition of DMF into DMA and FA.

The AuNPs used in this system were synthesized from the reduction of HAuCl_4 precursor by sodium citrate (NaCt), which acted both as reducing agent and stabilizer in this reaction. In the synthesis of dumbbell-like nanostructure process, the hydrogen ion donated by HCl or FA could reduce the charge repulsion on the surface of the NaCt-AuNPs as well as transform the NaCt on the surface of AuNPs to citric acid (CA) possibly. We tested the solubility of NaCt and CA in DMF, and found that NaCt did not dissolve in DMF while CA dissolved well in DMF. Therefore, DMF/ H_2O mixed solvent was a good solvent for CA, but a poor solvent for NaCt. As a result, it is highly possible that CA can leave the surface of the AuNPs and dissolve in the mixed solvent, resulting in the partially naked surface of AuNPs. Besides, DMA decomposed from DMF can bind to the surface of AuNPs, forming a hydrophilic layer, which would prevent PSPAA from attaching on it.¹⁸

The amphiphilic block copolymers PS-*b*-PAA were used in this system. When they dissolved in a good solvent (DMF), the copolymer chains were

unimolecularly dispersed and existed as the random coils conformation. As water, a good solvent for the hydrophilic PAA block, was added into the solution, the solubility of the hydrophobic PS part became worse. At some critical point, the PS blocks started to aggregate to form small spherical micelles to minimize the interfacial energy between the PS part and the outside solvent.^{27,28} In our experiments, since a large amount of water was added into the polymer solution in one shot, the critical micellization concentration (CMC) of the copolymer became very low, and almost all of the polymer chains involved in the micellization.^{27,29}

Scheme 4-2. The schematics showing the mechanism for the formation of the dumbbell-like nanostructures from AuNPs and PSPAA.

Based on the above analysis, the mechanism for the formation of the dumbbell-like nanostructures was hypothesized. As shown in Scheme 4-2, two processes occurred simultaneously in the first stage: (1) some ligands left the surface of AuNP and made part of the AuNPs naked while some others

exchanged with the stronger hydrophilic ligand (DMA), and (2) PSPAA amphiphilic block copolymer self-assembled into spherical micelles; in the second stage, the naked part of the AuNP, which was energetically unfavourable, tended to be embedded in spherical micelles while the other part of the AuNP with hydrophilic ligands could not be encapsulated with the BCP micelles.¹⁸

In order to test the correctness of this mechanism, the following control experiments have been designed.

(1) In these experiments, FA and DMA were added into the system directly in the last step instead of HCl, considering that DMF could decompose into FA and DMA gradually at elevated temperature. The reaction temperature (100 °C) and time (1 h) were the same as the normal one (Figure 4-2c). Just as expected, similar dumbbell-like AuNP@PSPAA with spherical ends were obtained, as shown in Figure 4-6. Maybe because it was difficult to ensure that the amount of FA and DMA added into the experiment was the same with that generated spontaneously from DMF in the normal experiment, the size of the BCP spherical micelles was different from that in Figure 4-2(c), and there were more empty micelles. However, if FA and DMA were added into DMF/PSPAA solution before the addition of AuNPs aqueous solution, aggregation of AuNPs would occur once the AuNPs solution was injected into the solution in the following step. The reason was that in the former case (FA and DMA were added in the last step), the presence of excess AuNPs plus PSPAA in the

solution would consume or react with the FA and DMA once they were introduced, while in the latter case, when AuNPs solution was added, the excess FA and DMA in the solution would reduce the charge repulsion on the surface of AuNPs suddenly and result in the aggregation of AuNPs.

Figure 4-6. TEM images of dumbbell-like AuNP@PSPAA with spherical ends obtained when FA and DMA were added into the system directly.

(2) When FA was added into the system directly instead of HCl in the last step, AuNPs could be partially encapsulated by BCP micelles too, as shown in Figure 4-7(a). However, the morphology of the BCP domains was cylindrical micelles, maybe due to the relatively large quantity of the FA in the solution, which would reduce the charge repulsion among the PAA corona and make the morphological transformation from spherical micelles to cylindrical micelles.^{2,30}

(3) When DMA was added into the system, whether in the first step or in the

last step, almost all of the AuNPs could not be encapsulated by BCP micelles (Figure 4-7b). This result also can be explained by our mechanism. The excess DMA in the solution could exchange the NaCt on the surface of the AuNP and form a hydrophilic layer surrounding the AuNP. The density of hydrophilic DMA on the surface of AuNPs should be very high because there was no other competing ligand in the mixture, which would prevent the hydrophobic block PS from coating on the surface of AuNPs.¹⁸

Figure 4-7. TEM images of (a) the nanostructures of AuNP@PSPAA when FA was added into the reaction instead of HCl, (b) the naked AuNPs and PSPAA micelles obtained when DMA was added into the reaction instead of HCl, respectively.

(4) When additional NaCt was added into the reaction (this reaction was without direct addition of any acid like HCl, HNO₃), in order to make AuNPs embed into BCP domains, more acid would be needed to transform NaCt to CA

(citric acid) according to our proposed mechanism. The more acid needed meant that longer reaction time was required to decompose more DMF into DMA and FA. As a result, a lot of DMA was produced in the solution, and thus, AuNPs were partially encapsulated with BCP micelles or not encapsulated with BCP micelles at all because of the existence of excess DMA in the system. As shown in Figure 4-8(a), when additional 0.3 % NaCt (20 μ L or 50 μ L) was added into the reaction, unlike the system without additional NaCt (Figure 4-5a), 6 h reaction time was not enough to produce the dumbbell-like nanostructures of AuNP@PSPAA. Even after prolonging the reaction time to 14 h, most of AuNPs in the system with more NaCt (50 μ L) were still not encapsulated with BCP micelles (Figure 4-8c), while in the system with less NaCt (20 μ L), most of the AuNPs were partially encapsulated and the other AuNPs were exposed (Figure 4-8b and 8d). Moreover, it is obvious that the exposed area of the AuNPs in this control experiment (Figure 4-8d) was larger than that in the system without additional NaCt addition (Figure 4-8e), since in this experiment, more DMA was produced from the decomposition of DMF after long time heating, which could endow larger surface area of the AuNP with hydrophilicity.

From above analysis, we can find that the proposed mechanism was readily supported by these control experiments.

Figure 4-8. TEM images of the nanostructures obtained when additional NaCt was added into the system without acid: (a) the naked AuNPs and PSPAA micelles, 0.3 % NaCt: 20 μ L or 50 μ L, 100 $^{\circ}$ C, 6 h; (b) dumbbell-like structures and Janus AuNP@PSPAA, 0.3 % NaCt: 20 μ L, 100 $^{\circ}$ C, 14 h; (c) Janus AuNP@PSPAA, the naked AuNPs and PSPAA micelles, 0.3 % NaCt: 50 μ L, 100 $^{\circ}$ C, 14 h; (d) the magnified images from (b) showing that the exposed area of the AuNP was larger than that in (e), which were synthesized from the system without additional NaCt addition.

4.3.4 Complex Nanostructures Based on this System

Complex nanostructures were synthesized based on our understanding of this system. Firstly, dumbbell-like nanostructures of AuNP@PSPAA where the BCP domains were cylindrical micelles were produced as a little more water was added into the system, as shown in Figure 4-9. It is known that the solvent volume ratio (e.g. DMF/H₂O) can influence the morphology of the self-assembly of BCPs in the solution significantly.^{8,31,32} When more H₂O was added into the system, the interfacial energy between the hydrophobic PS core and the outside solvent would increase. To minimize the interfacial energy, the surface/volume ratio tended to decrease. One way to decrease the surface/volume ratio was increasing the size of the spherical aggregates. However, the size of the aggregates could not increase unlimitedly due to the limitation from the certain length of the hydrophobic chain. When the size of the aggregates approached the transition boundary, they would transform to cylindrical micelles, which had lower surface/volume ratio. That was the reason for the formation of dumbbell-like nanostructures of AuNP@PSPAA with cylindrical ends in our system.

Figure 4-9. TEM images of dumbbell-like AuNP@PSPAA with cylindrical ends obtained from the system with more H₂O addition.

Secondly, dumbbell-like nanostructures of AuNP@PSPAA (PS₁₄₄-*b*-PAA₂₂), where the BCP domains were vesicular micelles and AuNPs were partially exposed, were synthesized when the NaCt stabilized AuNPs were used instead of the thiol-ended polystyrene stabilized AuNPs (PS-S-AuNPs) in the system of fabricating AuNP@PSPAA vesicles. As shown in Figure 4-10(a) and 10(b), AuNP@PSPAA vesicles were obtained when PS-S-AuNPs were added into the PS₁₄₄-*b*-PAA₂₂/DMF solution, followed by addition of H₂O and HCl. There were two characteristics for AuNP@PSPAA vesicles: (a) one vesicle generally possessed one AuNP (yield > 90 %), (2) AuNP was fully embedded in the wall of the vesicle. When NaCt stabilized AuNPs were introduced into the reaction instead of PS-S-AuNPs, dumbbell-like nanostructures of AuNP@PSPAA, where the BCP domains were vesicular micelles and AuNPs were partially

exposed, were obtained, as showing in Figure 4-10(c) and 10(d).

Figure 4-10. TEM images of (a, b) vesicular micelles with fully embedded AuNP, (c, d) dumbbell-like nanostructures with partially embedded AuNP in both vesicular ends, respectively.

4.4 Conclusion

In summary, we have developed a novel and facile method to produce the dumbbell-like nanostructures from the self-assembly of PSPAA on the surface of AuNPs. Unlike the previous report, no ligand was needed for the formation of such "Janus" structures. The formation of dumbbell-like nanostructures in our system can be attributed to FA and DMA decomposed from the solvent (DMF): FA can neutralize the NaCt and PAA chains while DMA can bind to the surface of the AuNP to form a hydrophilic layer. Based on the understanding of this system, more complex dumbbell-like nanostructures such as cylindrical and vesicular ones were synthesized. These results not only provide a new method for the formation of the dumbbell-like nanostructures, but also call attention to the influence of the solvent decomposition on the final nanostructures.

4.5 Bibliography

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Chapter 5 General Method for Growing Metal Nanoshells on AuNP@PSPAA Core

5.1 Introduction

The synthesis of nanoparticles and other nanostructures has received considerable attention in these years, since their properties, such as optical, mechanical, and chemical properties, depend strongly on their size, their geometric structures and components, which are quite different from that of bulk materials.¹⁻⁵ Because of this dependence, researchers can prepare specific nanomaterials with desired properties, and also can endow the nanomaterials with multiple properties or new properties by combining nanomaterials with different properties together.⁶⁻⁹

One typical example is metal nanoshells, which are a new class of nanoparticles with highly tunable optical properties, consisting of a dielectric (silica, Polystyrene, Au₂S, Fe₂O₃, et al.) core (40-250 nm radius) surrounded by a thin (10-30 nm) metallic shell (Au, Ag).⁹⁻¹³ Metal nanoshells exhibit a strong plasmon-derived optical resonance, which can shift to much longer wavelengths than that of the corresponding metal nanoparticles, allowing materials to be specifically designed to match the wavelength required for a particular

application, for instance to fall within near infrared (NIR) regions where light penetration through tissue is optimal.⁹

There are several methods for preparation of such metal nanoshell. The most commonly used one was developed by Halas and co-workers, who made gold nanoshells on silica nanoparticles (NPs) with Au nanoseeds attached.¹⁴⁻¹⁷ Typically, silica NPs were first modified with (3-aminopropyl) trimethoxysilane, followed by immobilizing the small Au nanoseeds (2 nm) onto the surface of silica NPs. The Au nanoseeds could act as the nucleation sites for the Au deposition in the following process of reducing the Au precursor. The other method exploited the direct overgrowth of Au nanoshell on a hybrid nanostructures consisting of AuNPs and polystyrene (PS) NPs without the self-nucleation of Au.¹⁸ However, in these reported method, the core materials were generally limited to silica, PS, Au₂S and Fe₂O₃.

In our previous work, we have successfully synthesized AuNPs encapsulated with amphiphilic block copolymer PS_m-*b*-PAA_n (AuNP@PSPAA), which has the AuNP as the core, PS as the shell, and PAA as the corona dissolved in the solvent.^{5,19-22} The shell thickness was about 10 nanometers. We wonder that maybe some new properties would present if the metal nanoshell was growing surround the surface of AuNP@PSPAA. Based on this thinking, in this chapter, a general method was developed to make various metal nanoshells growing on the surface of AuNP@PSPAA. And also, the corresponding optical properties of

this AuNP@PSPAA@metal nanoshell was tested.

5.2 Materials and Methods

5.2.1 Materials and Characterization

All chemical reagents were used as purchased without further purification. Hydrogen tetrachloroaurate(III) hydrate (HAuCl_4 , 99.9 %, metal basis Au 49 %), sodium palladium tetrachloride (Na_2PdCl_4), hydrogen tetrachloroplatinate(II) (H_2PtCl_4), and ruthenium chloride (RuCl_3) were purchased from Alfa Aesar; Indium(III) nitrate hydrate ($\text{In}(\text{NO}_3)_3 \cdot x\text{H}_2\text{O}$), silver nitrate (AgNO_3), cetyl trimethylammonium bromide (CTAB), sodium citrate (NaCt), polyvinylpyrrolidone (PVP, K-30), tetrakis(hydroxymethyl)phosphonium Chloride (THPC), and poly(allylamine hydrochloride) (PAH) were purchased from Sigma-Aldrich. Amphiphilic diblock copolymer polystyrene-*block*-poly(acrylic acid) ($\text{PS}_{154}\text{-}b\text{-PAA}_{49}$, $M_n = 16000$ for PS block and $M_n = 3500$ for PAA block, $M_w/M_n = 1.15$) was purchased from Polymer Source, Inc.. Sodium hydroxide (NaOH) was purchased from Sinopharm Chemical Reagent Co. Ltd. 2-Dipalmitoyl-sn-glycero-3-phosphothioethanol (PS-H) was purchased from Avanti Polar Lipids. The negatively charged, citrate-stabilized AuNPs (15 nm and 40 nm)²³ were prepared following literature procedures. Deionized water (resistance > 18.2 M Ω /cm) was used in all reactions. All other chemicals were

purchased from Sigma Aldrich. Copper specimen grids (200 mesh) with formvar/carbon support film (referred to as TEM grids in the text) were purchased from Beijing XXBR Technology co. ltd.

Characterization. TEM images were collected on a JEM-1400 (JEOL) Transmission Electron Microscope operated at 100 kV.

Preparation of TEM samples $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ was used as the negative stain in all of the TEM images in this study. TEM grids were treated with oxygen plasma in a Harrick plasma cleaner/sterilizer for 1 min to improve their surfaces hydrophilicity. A drop of sample solution was carefully mixed with stain solution ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 1 wt %) on the surface of a Parafilm. Then, the hydrophilic face of the TEM grid was placed in contact with the stained sample solution, and a filter paper was used to wick off the excess solution on the TEM grid. After drying in air, it can be used for TEM characterization.

5.2.2 Methods

As shown in Figure 5-1, the synthesis of AuNP@PSPAA@MNS includes the following steps:

Step 1: Synthesis of AuNPs@PSPAA@NS.

(a) Synthesis of AuNPs@PSPAA. The method used here was similar to our previous report on single-encapsulation of gold nanoparticles with minor modification.²⁴ Typically, two 1.5 mL tubes of AuNPs solution ($d_{\text{Au}} = 15$ nm)

were concentrated to $\sim 10\ \mu\text{L}$ by centrifugation at 14 000 *rpm* for 15 min. The deep red solution collected at the bottom of the tubes was redispersed in 190 μL H_2O . Then the mixture was added into 900 μL of polymer solution which was prepared by mixing 820 μL DMF with $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$ in DMF (80 μL , 8 mg/mL). Finally, hydrophobic ligand PS-H in EtOH (40 μL , 2 mg/mL) was added into the mixture. The mixture was heated at 110 $^\circ\text{C}$ for 2 h and then allowed to slowly cool down to room temperature. The similar method was used to encapsulate the 50 nm and 70 nm AuNPs.

Figure 5-1. Schematics showing a general Method for growing Metal Nanoshells (MNS) on AuNP@PSPAA Core.

(b) Surface Modification of AuNP@PSPAA. The surface modification process was performed using poly(allylamine hydrochloride) (PAH) as a positive ligand to attach on the surface of AuNP@PSPAA via electrostatic adsorption. To purify the AuNP@PSPAA, four tubes of the solution of AuNP@PSPAA (0.5 mL AuNP@PSPAA solution mixed with 1 mL H_2O) were

centrifuged at 14,000 *rpm* for 20 min. After being washed 2 times with H₂O, the AuNP@PSPAA was re-dispersed in water (2 mL, pH 10), followed by mixed with PAH aqueous solution (4 mL, 1 %) and stirring overnight. After purification by centrifugation, the solution was re-dispersed in 2 mL H₂O for further use.

(c) Attachment of Gold Nanoseeds (NS). NS solution was first prepared as follows: tetrakis-hydroxymethyl-phosphonium chloride (THPC, 12 μ L, 80 %) and NaOH (0.25 mL, 2 M) were added to DI water (45 mL). The mixture was stirred vigorously for 5 min before HAuCl₄ (2.0 mL, 1 %) was quickly injected in one shot. An immediate color change to dark brown was observed. This solution was stored in a lightproof container with stirring overnight.

By combining 2 mL of PAH coated AuNP@PSPAA solution with 2.5 mL NS solution, the NS could be attached onto AuNP@PSPAA surface after 12 hours' curing.

Step 2: Synthesis of AuNPs@PSPAA@MNS. The AuNP@PSPAA@NS solution was mixed with the surfactant (sodium citrate, CTAB, or PVP) solution, followed by adding metal precursor (aqueous solution of AgNO₃, HAuCl₄, H₂PtCl₄, Na₂PdCl₄, RuCl₃ or In(NO₃)₃) and reducing agent (ascorbic acid or NaBH₄).

5.3 Results and Discussion

5.3.1 Synthesis of AuNP@PSPAA@NS

First, the AuNP@PSPAA was synthesized according to the method reported in previous literature.^{19,22} Citrate-stabilized AuNPs (50 nm) were first modified with hydrophobic ligand, 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol (PS-H). The amphiphilic block copolymer PSPAA then self-assembled on the surface of AuNP, forming a uniform PSPAA shell (Figure 5-2a). The AuNP@PSPAA was purified 2 times by centrifugation to remove the empty PSPAA micelles and excess DMF. To get the positively charged AuNP@PSPAA, the purified nanoparticles were then re-dispersed in H₂O (pH 10), followed by addition of positive PAH aqueous solution. After incubating the mixture at RT overnight to facilitate the ligand attachment, the positively charged AuNP@PSPAA was formed. The positively charged AuNP@PSPAA was purified 2 times again by centrifugation to remove the excess PAH, and re-dispersed in H₂O for the further use. Finally, to attach the Au nanoseeds (NS) onto the surface of AuNP@PSPAA, the negatively charged NS were prepared separately and then mixed with the aqueous solution of the positively charged AuNP@PSPAA in a desired ratio, and allowed to age for a controlled time. After that, the AuNP@PSPAA@NS was formed and can be used in the following step after purification by centrifugation. As shown in Figure 5-2(b)

and 2(c), the small dark dots (NSs) attached on the PSPAA shell can be clearly observed and the size of NSs size was around 1 nm. The NSs distributed uniformly on the PSPAA shell with high density.

Figure 5-2. TEM images of (a) AuNP@PSPAA, (b) AuNP@PSPAA@NS, (c) enlarged image of the marked area in (b); (d) UV-vis absorption spectra of (a) AuNPs@PSPAA and (b) AuNPs@PSPAA@NS.

The UV-vis absorption spectra of the AuNP@PSPAA and AuNP@PSPAA@NS in water were measured. As shown in Figure 5-2(d), there was no obvious change of absorption between these two samples, which meant

that during the preparation process, there was no aggregation of AuNP@PSPAA occurred. However, because of the loss of AuNP@PSPAA during 4 times of purification process, the intensity of the absorption was decreased sharply from AuNP@PSPAA to AuNP@PSPAA@NS.

5.3.2 Synthesis of Au Nanoshell on AuNP@PSPAA

When CTAB was used as the surfactant in the synthesis of Au nanoshell on AuNP@PSPAA, it is difficult to get the continuous shell on the AuNP@PSPAA. Typically, AuNP@PSPAA@NS solution was mixed with CTAB solution, followed by adding HAuCl₄ solution and reducing agent ascorbic acid into the mixture. The color of the solution changed quickly from light red to light purple once the ascorbic acid was injected into the mixture, since Au⁰ was produced via the reduction of HAuCl₄.

Figure 5-3. TEM images of (a, b) one AuNP satellite growing on one AuNP@PSPAA (15 nm AuNP) core under the gentle experimental condition, (c, d) several AuNP satellites growing surround one AuNP@PSPAA (15 nm AuNP) core under the harsh experimental condition, respectively.

When the size of AuNP (AuNP@PSPAA) was 15 nm, two types of nanostructures were achieved: 1. one AuNP satellite on one AuNP@PSPAA (Figure 5-3a), which was synthesized under the gentle experimental condition: The reaction solution was gently shook with hand after addition of all reactants;

2. several AuNP satellites on one AuNP@PSPAA which was synthesized under the harsh experimental condition: The reaction solution was vigorously shook by vortex after addition of all reactants. Maybe because the size of AuNP@PSPAA was too small, its high curvature was not favorable for the formation of continuous shell. We used larger size AuNP (70 nm) instead of the small one, and found that similar structures were formed: plenty of AuNP satellites surrounded the AuNP@PSPAA core, as shown in Figure 5-4. Enlarged TEM image showed that the size of AuNP satellites was around 20 nm, and the density of the satellites was very high. So, under this experimental condition, we could not obtain the AuNP@PSPAA with continuous shell. From the literature, it is known that the CTAB has good affinity to the [110] facet of AuNP.^{25,26} Maybe due to this reason, the AuNP satellites could not fuse together to form the continuous shell easily.

When negatively charged sodium citrate was used as surfactant in this system, continuous Au nanoshell could grow on the surface of AuNP@PSPAA, as shown in Figure 5-5. The AuNP core and Au nanoshell appear black in TEM image while the color of PSPAA domain in between them was grey, because the polymer part can create a “spacer” in the path of the electron beam. From the magnified TEM image (Figure 5-5b), it is obvious that the Au nanoshell is a continuous smooth shell. In addition, a lot of smaller AuNPs were synthesized from the self-nucleation process, which was hard to prevent in this system.

Figure 5-4. TEM images of (a) plenty of AuNP satellites growing surround the AuNP@PSPAA (70 nm AuNP) core, and (b) enlarged area of (a) and corresponding schematic.

Figure 5-5. TEM images of Au nanoshell growing on the AuNP@PSPAA (50 nm AuNP) core.

Figure 5-6. TEM images of AuNP@PSPAA with rough Au nanoshells, which was achieved by adding additional ligand into the reaction: (a,b) 4-MBA, 1 mM, 10 μ L, (c,d) 4-MBA, 10 mM, 10 μ L, (e,f) 4-MBA, 10 mM, 30 μ L.

The surface roughness of the Au nanoshell on the AuNP@PSPAA can be controlled by adding additional ligand into the reaction. As shown in Figure 5-6(a) and 6(b), when a small amount of 4-MBA (4-mercaptobenzoic acid) was added into the system, the Au nanoshell with rough surface was easily achieved. The more ligand added, the rougher surface the Au nanoshell presents (Figure 5-6b to f). The phenomenon can be explained by the theory reported in our previous paper:²⁷ the strong binding property of 4-MBA in this system forced selective deposition of Au at the ligand-deficient interface between Au NSs and PSPAA domain to form dense Au nanoworms, resulting in the rough surface.

5.3.3 Synthesis of Ag Nanoshell AuNP@PSPAA

For the synthesis of Ag nanoshell on AuNP@PSPAA, AuNP@PSPAA@NS solution was first mixed with sodium citrate solution, followed by adding AgNO₃ solution and reducing agent ascorbic acid. The color of the solution changed quickly from light red to yellowish-brown once the ascorbic acid was injected into the mixture. Different amount of the AgNO₃ precursor resulted in different density of AgNP/Ag cluster, as shown in Figure 5-7(a) to 7(e): at a low concentration of AgNO₃ (10 μ L), only several AgNPs were produced attaching on the surface of AuNP@PSPAA (Figure 5-7a); as the concentration of AgNO₃ increased, the density of AgNPs around AuNP@PSPAA also enhanced (Figure 5-7b to 7e).

UV-vis extinction spectra were used to monitor this reaction under the experimental condition of Figure 5-7(b). As Figure 5-7(f) showed, the initial solution containing AuNP@PSPAA@NS showed one band (before, black line): Au transverse absorption at around 560 nm. After introduction of AgNO₃, once the ascorbic acid was added into the solution, reduction reaction of AgNO₃ occurred, which was indicated on UV-vis extinction spectra. As shown in Figure 5-7(f), at 2 min, Ag transverse plasmon absorption appeared around 420 nm, Au transverse absorption disappeared, and Au-Ag longitudinal absorption appeared around 625 nm; as reaction time prolonged, Ag plasmon absorption shifted to 410 nm and its shoulder increased²⁸ due to the increased thickness/density of Ag domain, and Au-Ag longitudinal absorption red shifted to 640 nm. The final colloid appeared turbid owing to the more efficient scattering of the Ag domain.

Figure 5-7. TEM images of AuNP@PSPAA (50 nm AuNP) with AgNP satellites/Ag shell synthesized under different condition: (a) AgNO₃ 10 μ L, (b) AgNO₃ 40 μ L, (c) AgNO₃ 70 μ L, (d) AgNO₃ 100 μ L, (e) AgNO₃ 200 μ L; (f) UV-vis absorption spectra collected during the reduction of AgNO₃ in (b).

Figure 5-8. SERS spectra of the AuNP@PSPAA@Ag Nanoshell resulting from reducing AgNO_3 in AuNP@PSPAA@NS solution with different amount of 4-MBA: (a) 4-MBA, 1 mM, 10 μL , (b) 4-MBA, 1 mM, 5 μL , (c) 4-MBA, 0.1 mM, 10 μL , (d) 4-MBA, 0.01 mM, 10 μL , (e) 4-MBA, 0.001 mM, 10 μL ; Corresponding TEM images of (a) and (d) AuNP@PSPAA@Ag nanoshell.

It is known that silver nanostructures can be used as substrates for surface-enhanced Raman scattering (SERS) applications.^{29,30} The AuNP@PSPAA@Ag nanoshell synthesized in our system was attractive for use as a SERS substrate. In order to evaluate the performance of this structure for SERS application, 4-MBA was used as probe molecule because it could be adsorbed on the surface of AgNP easily and had enormous intensity enhancement.¹⁰ A certain amount of 4-MBA was introduced into the solution before the addition of AgNO₃ and ascorbic acid. The addition of 4-MBA did not have significant influence on the final morphologies, as shown in Figure 5-8(a) (the 4-MBA amount: 1 mM, 10 μ L) and 8(d) (the 4-MBA amount: 0.01 mM, 10 μ L). When the concentration of 4-MBA in the solution reached 1 μ M (Figure 5-8, SERS-c), the highest SERS enhancement was achieved. Above this critical concentration, the SERS signal kept unchanged. It is known that SERS exceptionally strong in the gap between plasmon-coupled metal nanoparticles (NPs), which is called SERS “hotspots”.³¹⁻³³ In our system, the "Ag nanoshell" was constructed from a lot of AgNPs. Therefore, plenty of SERS “hotspots” existed in between the AgNPs, which could employ as the container for the 4-MBA. Maybe due to the limited capacity of such "container", the higher concentration of the 4-MBA did not result in the stronger SERS signals.

5.3.4 Synthesis of Other Metal/Metal Oxide Nanoshells on AuNP@PSPAA

Besides the growth of Au and Ag nanoshells on AuNP@PSPAA, we can extend this method to grow other metal/metal oxide nanoshells (e. g. Pt, Pd, Rh, In(OH)₃) on AuNP@PSPAA.

For the synthesis of AuNP@PSPAA@Pt nanoshell, AuNP@PSPAA@NS solution was first mixed with H₂PtCl₄ solution, followed by addition of reducing agent ascorbic acid. The color of the solution changed from light red to grey after 10 min of the addition of ascorbic acid at room temperature. As shown in Figure 5-9(a) and (b), the thin Pt nanoshell consisted of plenty of small Pt nanoparticles. In this case, the concentration of AuNP@PSPAA@NS must be very high, or Pt nanoshell cannot be produced. From the literature, it is known that Pt required higher temperature to be reduced from the precursor H₂PtCl₄, since the primary nucleation of Pt nanoparticles needed to conquer significant energy barrier.^{34,35} However, because of the existence of NSs (Au nanoseeds) in our system, the growth of Pt on NSs was favoured even at room temperature.³⁶

For the synthesis of AuNP@PSPAA@Pd nanoshell, AuNP@PSPAA@NS solution was mixed with sodium citrate solution, followed by addition of Na₂PdCl₄ solution and reducing agent ascorbic acid. Figure 5-9(c) and (d) showed the typical TEM images of the AuNP@PSPAA@Pd nanoshell. The Pd

nanoshell possessed loose nanostructures and the shell thickness was around 30 nm. The decrease of the addition amount of Na_2PdCl_4 solution would lead to the thinner Pd nanoshell.

For the synthesis of $\text{AuNP@PSPAA@Rh/In(OH)}_3$ nanoshell, the surfactant and reducing agent were changed to PVP and NaBH_4 (a stronger reduce agent than ascorbic acid) respectively. AuNP@PSPAA@NS solution was first mixed with PVP solution, followed by addition of $\text{RuCl}_3/\text{In(NO}_3)_3$ solution and NaBH_4 aqueous solution. Figure 5-9(e) showed the typical TEM image of AuNP@PSPAA@Rh nanoshell, where the Rh nanoshell consisted of hair-like nanostructures, as shown in the inserted enlarged image in Figure 5-9(e). The yield of this product was relatively low, since plenty of RhNPs were formed from the self-nucleation process. Besides, TEM images of $\text{AuNP@PSPAA@In(OH)}_3$ nanoshell³⁷ were shown in Figure 5-9(f). The enlarged image showed that there were plentiful white dots in the In(OH)_3 nanoshell, indicating that the nanoshell was mesoporous. The shell thickness was around 60 nm.

Figure 5-9. TEM images of AuNP@PSPAA (50 nm AuNP) with different metal nanoshells: (a, b) Pt; (c, d) Pd; (e) Rh, (f) In(OH)₃.

5.4 Conclusion

We have developed a facile and general method for preparation of various metal/metal oxide nanoshells on AuNP@PSPAA core. Because of the existence of the Au nanoseeds, the experimental condition for reducing the metal precursor was gentle, usually at room temperature. The shell thickness and roughness were controlled by several factors, such as the type of surfactant, the ligand concentration, the quantity of the precursor, and the type of reducing agent. We believe that the present method could be applied to fabricate more complex and anisotropic particles containing various inorganic cores/amphiphilic copolymer domain/metal nanoshell, which thus enriches the diversity of nanocomposites for the potential applications.

5.5 Bibliography

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Chapter 6 Exploiting Charge Interactions for Facile TEM Characterization of Colloidal Species

6.1 Introduction

As a powerful technique to characterize materials down to nanometer scale, TEM has been widely used to capture the morphologies of specimens in various fields, such as biological field, nanotechnology, macromolecules, and so on.¹⁻⁵ It is generally accepted that the results obtained from TEM characterization are significantly influenced by the quality of sample preparation. A good sample preparation method should make the captured images reflect the real situation of the samples. However, taking colloidal solution sample for example, two common problems encountered in TEM characterization are the aggregation and uneven distribution of specimens, which are generally caused by the solvent evaporation (drying process) during the sample preparation. Therefore, some artifacts like aggregates in the TEM images are easily mistaken for the real features in the colloidal solution, and thus, it is difficult to distinguish the aggregation induced by drying process from that really existed in the solution. Besides, because the sample only covers part of the surface of TEM grid, a lot

of time would be wasted in looking for samples during TEM characterization. Thus, a lot of effort has been done to solve these problems, such as the development of cryo-TEM.⁶⁻⁸ However, while the better results can be obtained from cryo-TEM, due to its high cost and relatively complex operation, it is not widely applicable.

Due to the significant influence of sample preparation on the quality of TEM images, the development of new preparation method is particularly important. Herein, a simple and efficient TEM sample preparation method for negatively charged colloids was developed by introducing amino-group (amino-group on APTES) on the TEM grid, which made the specimens adsorb on the TEM grid by chemical interaction, rather than the general physical adsorption. APTES was chosen because of its unique property: it could form a covalently attached self-assembled monolayer (SAM) on a wide range of substrate like mica and silicon wafer,^{9,10} and thus supply amino terminal group to attract DNA microarray,¹¹ protein arrays,¹² sol-gels,¹³ negative molecules,⁹ and so on. By using APTES-modified TEM grid, many kinds of negative colloidal specimens could chemically adsorb on all the surface of TEM grid to form a monolayer with monodispersed specimens, which made the captured TEM images more representative and saves a lot of characterized time.

6.2 Materials and Methods

6.2.1 Materials

Copper specimen grids (200 mesh) with carbon coated Formvar support film (TEM grids) were purchased from Beijing XXBR Technology co., ltd. 3-aminopropyltriethoxysilane (APTES, 98 %, Alfa Aesar) and $(\text{NH}_4)_2\text{Mo}_7\text{O}_{24}$ (99 %, Alfa Aesar) were used without further purification. Ultrapure water (resistance $> 18 \text{ M}\Omega \text{ cm}^{-1}$) was used for all solution preparation. The colloidal specimens like gold nanoparticles (AuNPs),¹⁴ polystyrene₁₅₄-*b*-poly(acrylic acid)₄₉ encapsulated AuNPs (AuNPs@PSPAA),^{15,16} multi-toroids self-assembled from PSPAA,¹⁷ and polypyrrole coated AuNPs (AuNPs@PPy)¹⁸ were synthesized according to the literature.

TEM characterization of the samples were performed at ambient temperature using JEM 1400 transmission electron microscope (JEOL), operated at 120 kV.

6.2.2 The method for TEM Sample Preparation

Common method for TEM sample preparation As shown in Figure 6-1(a), a drop of colloidal specimen solution (if the specimens contain polymer, stain would be mixed with it to enhance the contrast) was dripped on the surface of TEM grid, and a filter paper was used to wick off the excess solution on the TEM grid. After drying in air, it can be used for TEM characterization. It is needed to point out that in general, the aggregation of colloids would occur to

leave “coffee ring”^{19,20} behind due to the solvent evaporation in the drying process, and the distribution of samples was also uneven.

Improved method for TEM sample preparation As shown in Figure 6-1(b), compared to the common method, the TEM grids were first treated with oxygen plasma to improve their surface hydrophilicity before using in the following step. This step would strongly decrease the colloidal solution contact angle on the surface of TEM grid, and increase the adsorption between the colloids and the surface. Thus, better result would be obtained. However, aggregation of colloids still cannot be totally avoided because of the drying process.

New improved method for TEM sample preparation Our developed method for sample preparation included two steps, as shown in Figure 6-1(c).

Step 1, modified TEM grids with APTES: firstly, the surface of the TEM grid was covered by 20 μL APTES aqueous solution (0.1 %, v/v) for 10-15 min. After this treatment, the grid surface was covered with an aminopropyl silane film²¹ since the reaction between APTES and the Formvar surface of TEM grid proceeded with the hydrolysis of alkoxy groups followed by the covalent adsorption of the hydroxysilane product. Then, the TEM grid was rinsed with ultrapure water several times to remove the excess APTES molecules, and the APTES-modified TEM grid (APTES-TEM grid) was ready for the further use.

Figure 6-1. Schematics showing the TEM sample preparation processes: (a) common method: use the TEM grid as received; (b) improved method: use the TEM grid treated with oxygen plasma to improve the surface hydrophilicity; (c) the new improved method to prepare monodispersed specimen: prepare the APTES-modified TEM grid first, and then drop the negative colloidal specimens on the APTES-modified TEM grid.

Step 2, attached the negative colloidal specimens on the APTES-modified TEM grid: 20 μL colloidal solution was dripped on the surface of APTES-modified TEM grid. After 40-60 min, the TEM grid was removed from the sample solution, rinsed with water several times to clear the excess specimens and dried in air at room temperature. For citrate stabilized AuNPs colloids, after drying, it could be used in TEM characterization. However, for those colloidal specimens containing polymers, one more step was needed to

obtain better TEM images: $(\text{NH}_4)_2\text{Mo}_7\text{O}_{24}$ was used as the negative stain to make the polymer shells white against the stained background. The stain aqueous solution (1 wt %) was in contact with the above TEM grid, and then, filter paper was used to blot the excess stain solution. After drying in air, the sample could be used in TEM characterization.

6.3 Results and Discussion

6.3.1 Mechanism Study

The most common used TEM grid is the carbon coated Formvar grid, which is known for its high beam stability and relatively low cost. Structurally, this carbon coated Formvar grid is a copper grid coated with a support film, named Formvar (polyvinyl formal), and a carbon film. The polyvinyl formal is synthesized by the acetalization of polyvinyl alcohol (PVA) with formaldehyde. Since the degree of acetalization is limited, the unreacted hydroxyl groups on the support film could react with APTES to coat aminopropyl silane film (ASF) on the surface of the TEM grid. Besides, the hydroxyl groups produced by the aging of Formvar film also involved in this reaction.

6.3.2 Uniform Distribution of AuNPs on APTES-TEM grid

Figure 6-2(a) shows the typical TEM image of large-area monodispersed colloidal AuNPs on APTES-modified TEM grid. The AuNPs were negatively charged since they were synthesized by citrate reduction of gold chloride.

Therefore, the carboxylic AuNPs could couple with the amino groups from the surface of modified TEM grid, and meanwhile the electrostatic repulsions among AuNPs in the solution could prevent them from aggregating. Thus, the whole surface of the TEM grid could be attached with monodispersed evenly distributed AuNPs (Figure 6-2b, high magnification), and almost no aggregates appeared, which would save a lot of characterizing time and get more representative results. It seems that the carbon film on the Formvar film has little effect on the covalent adsorption of the hydroxysilane product into the grid surface since the distribution of AuNPs on the different side (the right side : carbon monolayer, the plasma treated right side: increase the hydrophilicity of carbon monolayer, and the reverse side: copper grid and Formvar membrane) of TEM grid modified with APTES was quite similar: even distribution and almost no aggregation (Figure 6-3). In comparison, the TEM image of the same AuNPs sample made from common method (Figure 6-2c) shows many aggregates as marked by dotted circle, and the TEM grid was only partially covered with the AUNPs. In fact, it is quite difficult to avoid these problems when solvent evaporates during dry process.

Figure 6-2. (a) Large-area TEM image of monodispersed AuNPs; (b) high magnification of AuNPs in (a); (c) TEM image of AuNPs prepared via common sample preparation, the dotted circles show the appeared aggregates.

Figure 6-3. Large-area TEM image of monodispersed AuNPs on the reverse side of the TEM grid after APTES modification.

6.3.3 Uniform Distribution of Negatively Charged AuNPs@polymer on APTES-TEM grid

We think that the interaction between carboxylic group on AuNPs and amino group on the surface of the TEM grid should be the force to make the citrate stabilized AuNPs stick on it. However, the amino group can directly cap with AuNPs to form Au-N bond,²² which also could make AuNPs attach on the APTES-modified TEM grid. So, in order to make sure which interaction was predominant, PS₁₅₄-*b*-PAA₄₉ was used to encapsulate AuNPs to form the

core-shell structures with AuNP core and PSPAA shell (carboxylic groups surrounded the colloids), which could prevent amino-group from contacting the surface of AuNPs.

As shown in Figure 6-4(a) and 4(b), the colloids AuNPs@PSPAA were monodispersed on the whole surface of APTES-modified TEM grid as well, and no aggregation occurred. In addition, the anionic surfactant, sodium dodecyl sulfate (SDS), stabilized AuNPs@PPy could also evenly distribute on the modified TEM grid (Figure 6-5). However, the distance between two particles was longer than that of AuNPs and AuNPs@PSPAA. There may be two reasons: First, the excess SDS molecules in solution might consume the amino groups on the APTES-modified TEM grid; second, the lower surface tension of the solution made less solution deposit on the TEM grid, resulting in fewer and more sparsely distributed nanoparticles. So, the predominant force between the colloidal specimens and the surface of APTES-modified TEM grid must be the interaction of the negative charged specimens and the positive ASF on the surface of TEM grid.

Figure 6-4. (a) Large-area TEM image of evenly distributed AuNP@PSPAA on APTES-TEM grid; (b) high magnification of AuNP@PSPAA in (a); (c) evenly distributed dense AuNP@PSPAA on APTES-TEM grid induced by adding salt into the solution; (d, e) TEM images of the same sample in (c), but prepared

with the common method.

Figure 6-5. TEM images of SDS stabilized AuNPs@PPy on APTES-TEM grid.

6.3.4 The Unique Advantage of this Method and its Application

The grey background in Fig. 6-4(a) was the thin layer of the stain left behind as the solvent evaporated while the white area was not covered by stain. The thickness of the stain layer seemed quite uniform, and the stain did not influence the final dispersity of the colloids. However, when the stain solution was mixed with colloids in common method, some unwanted aggregation would occur due to the salty property of the stain, especially when it became concentrated as drying on the TEM grid. It is known that when a droplet with

suspended particles dried on a solid surface, the “coffee ring” was commonly left.^{19,20} This phenomenon was quite normal in daily life, as well as in normal SEM (Scanning Electron Microscopy) and TEM sample preparation (Figure 6-6). When a colloidal solution was spilt onto the TEM grid, the extent of its spreading depended on the attraction between the water molecules and the molecules on TEM grid. More wetting produced more attraction, that was, more spreading, and thus less aggregating. That is why the TEM grid was treated with oxygen plasma to enhance its surface hydrophilicity before use in some cases. Here in our case, the hydrophilicity of TEM grid was greatly enhanced after modification by APTES, and therefore, the thickness of the stain layer formed in last step was relatively uniform.

If the colloidal solution contained high density salt, the advantage of this developed method was greatly emerged. For example, when AuNPs@PSPAA was dispersed into NaCl solution (0.5 M), the electrostatic repulsion among the particles was decreased, and as a result, the density of AuNPs@PSPAA on APTES-modified TEM grid was obviously increased and a small portion of the particles aggregated, as shown in Figure 6-4(c). In comparison, the TEM images from common method showed the sparse particles (Figure 6-4d) and the aggregated particles (Figure 6-4e) with the un-uniformed stain layer. Besides, a lot of salt crystals (Figure 6-4d) were formed since it became concentrated as the solution evaporated on the surface of TEM grid.

Figure 6-6. TEM images of the “coffee ring” of AuNPs@PSPAA appeared in common TEM sample preparation.

Besides, for the complex micellar structures, like the multiple toroids self-assembled from the PSPAA in DMF/H₂O mixed solvent, when the TEM sample was prepared via common method, net-work structures were observed from the TEM image (Figure 6-7a). However, when our new developed method was employed for the sample preparation, as shown in Figure 6-7(b), the micellar structures were the multiple toroids factually, and the net-work structure in Figure 6-7(a) was just pseudo image, which was formed by overlapping multiple toroidal structures during sample preparation process. Thus, with this new method, we can get more representative results.

Figure 6-7. TEM images of the same complex PSPAA micelles prepared by different methods: (a) common method, and (b) new improve method.

6.4 Conclusion

In summary, the APTES-TEM grid could make the negatively charged specimens (e.g., citrate-AuNPs, AuNP@PSPAA) evenly distribute on all the surface of TEM grid through charge interaction between the specimens and the positively charged amino-groups. By using this method, the problems usually encountered in common method such as aggregation and unevenly distribution of samples could be well avoided. We think that it is one alternative preparation method to well characterize the negatively charged sample with TEM method, and would be applicable to many other specimens in nanoscience.

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Chapter 7 Summary and Outlook

7.1 Summary

From the perspective of synthetic methodology, there are only limited methodologies to create nanostructures of regular shapes. One popular method is the self-assembly of amphiphilic block copolymers, which can generate micelles with different nanostructures including spheres, cylinders and vesicles. Another way is nanocrystal synthesis, which is by far the most popular approach. Thus, combining the two approaches is an easy route for creating complex structures at nanoscale. The major challenges here are to make the two systems compatible and to insert metal NPs into the BCP assemblies with site-selectivity. In this thesis, the main goal of my research is trying to understand the principles behind the BCP self-assembly and explore new methodologies for preparation of complex hybrid nanostructures.

In this thesis, two main works were done: (1) exploration of basic principles behind the micelles transformation during the block copolymer (BCP) self-assembly process; and (2) the fabrication of complex hybrid organic/inorganic nanostructures based on the block copolymer self-assembly. These studies were based on the self-assembly of amphiphilic block copolymer

in solution. Specifically, block copolymer can self-assemble into various morphologies such as spheres, cylinders, lamella, vesicles, et al, in solution. When inorganic NPs were introduced into this system, hybrid organic/inorganic nanostructures with specific morphologies could be achieved. Besides, more complicated hybrid nanostructures containing metal core, polymer shell (first layer) and metal nanoshell (second layer) were synthesized by a general method.

To investigate the mechanism behind the micelles transformation during BCP self-assembly process, PSPAA was chosen since it was a representative amphiphilic block copolymer, and a great variety of micellar morphologies have been synthesized from it: spheres, cylinders, lamellae, vesicles, large compound micelles, and large compound vesicles. To our surprise, the unique toroidal micelles of PSPAA and various vesicle intermediates (bowl-like micelles) were first synthesized in our system. We ascribed this finding to three factors: 1. the "long-hair" PSPAA copolymer (relatively long PAA block) used in this system, which made the micelle transformation process very slow, allowing the intermediates to be trapped easily; 2. the special experimental setup: mixed all ingredients together at a critical solvent ratio, giving sufficient time for PSPAA to fully assemble and evolve at a higher temperature; 3. adjusting the charge repulsion among the PAA coronas, for example by adding acid into the reaction, which would facilitate the aggregation of micelles and

morphological evolution.

While the arguments of packing parameter focuses on the shape of an average molecule, analyzing the overall micelles for their S/V would provide more direct, but equivalent, arguments. Not considering the small variation in the thickness of hydrophobic domain ($2l$), the S/V decreases in the following order: sphere ($3/l$) > cylinder ($2/l$) > vesicle ($1/l$). Hence, vesicle would be favoured if there is no competitive driving force. Most importantly, the shape of an average molecule cannot be easily measured, but the S/V of micelles can be calculated, not only for spheres, cylinders, vesicles, but also for the kinetically trapped intermediates between their transitions.

On the basis of S/V analysis, the common factors affecting the shape of micelles can be easily understood. Longer PAA blocks or higher charge density (higher degree of deprotonation) would leads to stronger repulsion, thus driving the transformation to the spherical micelles. Neutralizing the surface charge or shield the charge repulsion (by addition of salt or surfactant/additive of opposite charge) driving the transformation to the vesicular micelles. Longer PS blocks would lead to less surface density of PAA blocks and thus favoring the vesicular micelles. In general, improving the internal interactions in the hydrophobic domain means that the reduction of S/V would bring about a larger driving force, thus favoring the vesicular micelles.

When metal nanoparticles were introduced into the above BCP self-assembly

system, dumbbell-like nanostructures were easily synthesized. These hybrid dumbbell-like nanostructures possessed three characteristics: a) the structure was highly symmetric; b) the BCP domains generally located at the two opposite spherical caps of the gold nanoparticle (AuNP), and the other part of the AuNP was exposed; c) the BCP domains could be controlled to be spherical micelles, cylindrical micelles, and vesicular micelles. Unlike the previous report, no ligand was needed for the formation of such "Janus" structures. The formation of dumbbell-like nanostructures in our system can be attributed to FA and DMA decomposed from the solvent (DMF): FA can neutralize the NaCl and PAA chains while DMA can bind to the surface of the AuNP to form a hydrophilic layer. Based on this system, more complex dumbbell-like nanostructures such as cylindrical and vesicular ones were synthesized. These results not only provide a new method for the formation of the dumbbell-like nanostructures, but also call attention to the influence of the solvent decomposition on the final nanostructures.

Then, based on the previous work, a general and facile methodology for the synthesis of various metal nanoshell on the surface of AuNP@PSPAA was developed. The Au nanoseeds were first attached on the surface of AuNP@PSPAA by layer-by-layer assembly. Because of the existence of the Au nanoseeds, the experimental condition for reducing the metal precursor was gentle, usually at room temperature. The shell thickness and roughness were

controlled by several factors, such as the type of surfactants, the ligand concentration, the quantity of the precursors, and the type of reducing agents. We believe that the present method could be applied to fabricate more complex and anisotropic particles containing various inorganic cores/BCP shells/metal nanoshells, which thus enriches the diversity of nanocomposites for the potential applications.

Finally, to facilitate the TEM characterization, an effective and facile strategy to prepare TEM samples for negatively charged colloids was developed by modifying the TEM grids with 3-aminopropyltriethoxysilane (APTES). The APTES-modified TEM grid could make the negatively charged specimens (e.g., citrate-AuNPs, AuNP@PSPAA) evenly distribute on TEM grid through charge interaction between the negatively charged specimens and the positively charged amino-groups. By using this method, the problems usually encountered in common method such as aggregation and unevenly distribution of samples could be totally avoided. We think that it is one alternative method of negatively charged sample preparation for TEM characterization, and would be applicable to many other specimens in nanoscience.

7.2 Outlook

Based on the understanding of the principles behind BCP self-assembly system, as well as the strategies developed in the synthesis of complex hybrid

nanostructures, several future works were proposed in the following:

1. The Application of AuNP@Vesicle: nanoreactor. We have synthesized highly uniform AuNP@Vesicle in previous work, as shown in Figure 7-1(a) and 1(b). In 90 % cases, one vesicle was embedded with only one AuNP, and AuNP located in the center of the hydrophobic BCP domain. The high yield and its unique structure endowed AuNP@Vesicle with plentiful applications in the future. For example, the cavity of the vesicle can be used as nanoreactor (Figure 7-1c) to precisely control the reaction scope as well as avoid aggregation during the synthesis of nanoparticles. Besides, by changing the reaction condition, the location of AuNP in the vesicle might be controlled: AuNP was half-embedded into the internal wall of the vesicle or the outside wall of the vesicle (Figure 7-1c). The exposed AuNP can play as the seed to facilitate the synthesis of other nanoparticles on it, and as a result, the new nanoparticles can grow on the exposed surface of AuNP either in the cavity or outside, as shown in Figure 7-1(c).

Figure 7-1. (a, b) TEM images of AuNP@Vesicle, (c) schematics showing the application of such AuNP@Vesicle: nanoreactor.

We have used the dumbbell-like nanostructures (in Chapter 4) to test this proposal, and the results were shown in Figure 7-2: Ag nanopaticles only grew on the exposed surface of AuNPs, and a more complicated nanostructure was achieved. This is only the initial work, and more studies would be done in the future. We believe that the cavity of the vesicle can play as the nanoreactor to control the reaction speed as well as restrict the morphology of the product.

Figure 7-2. TEM images of (a) dumbbell-like nanostructures, (b) growth of Ag on the exposed surface of the AuNP.

2. Use of PSPAA self-assemblies as templates to fabricate silica tube with specific shape. Amphiphilic diblock copolymers PSPAA can self-assemble into various micelles in selective solvents, such as spherical, cylindrical, toroids/multitoroids, vesicular micelles, and et al. These controllable self-assembly morphologies provide excellent templates for constructing other nanomaterials, such as silica nanotube, as shown in Figure 7-3. The top schematic in Figure 7-3 shows the proposed steps for the synthesis of silica nanotube: PSPAA micelles are first encapsulated with silica; then, after removing the PSPAA micelle by dissolving it in organic solvent or by high temperature calcination, the silica nanotube with specific shape can be achieved easily.

Figure 7-3. Top schematic showing the proposed steps for the synthesis of silica nanotube; TEM images of (a) PSPAA toroidal micelles (toroids), (b) Toroids@Silica, and (c) hollow silica toroid and silica tube formed after removing PSPAA template.

The initial results were shown in Figure 7-3 (a, b, and c). This method was facile, and various types of silica nanotube would be obtained, depending on the shape of the initial PSPAA templates. However, one big problem encountered here was that the reproducibility of removing the PSPAA domain in the last step was quite low. More works will be done in the future to solve this problem. This type of silica nanotube can be used as a new template to direct the metal growth. For example, the toroidal silica tube can act as nanoreactor for the reduction reaction of AgNO_3 or some other metal precursor, resulting in the formation of

Ag nanoring or other metal nanoring. These metallic nanorings can functionalize as plasmonic cavities which may present high quality factor for sensing applications.

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