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Exploiting Core-Shell Motif for New Synthetic Methodologies of Nanostructures

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Abstract

This thesis summarizes my postgraduate research on the exploration of basic mass transport phenomenon at nanoscale, the development of advanced assembly strategies for well-defined complex nanostructures, and the design of novel methodologies for the synthesis of hybrid nanostructures. The research was carried out based on core-shell nanostructures, where the metal core is encapsulated with amphiphilic diblock copolymer shell. In specific, metal-polymer core-shell nanoparticles were synthesized and then served as nanocarriers to elucidate the drug release kinetics. On the other hand, we studied the respond of polymer micelles to the environmental stimuli such as pH, solvent, and ultrasound, in particular, in the presence of embedded metal nanoparticles. It turned out that the structural transformation of polymer shell could facilitate the metal core assemble into a highly order manner.

In detail, we performed systematical investigation on the short-distance drug release in the presence of nanoacceptors (**Chapter 2**). The core-shell AuNP@PSPAA was chosen as nanocarriers, where the fluorescence of model drug (pyrene) could be quenched by the Au core. Thus, pyrene incorporated in AuNP@PSPAA can be readily distinguished from the one released into nanoacceptors or solution, offering a simple system for studying the release

kinetics via optical measurements. Introducing nanoacceptors (free PSPAA micelles) into the model system resulted in a rapid release of pyrene owing to concentration gradient. It is found that the rapid release fit well to the Fickian diffusion model and the diffusion of pyrene must be through the water, but not the rate-determining step. Thus, the release kinetics can be described by the radial diffusion from AuNP@PSPAA to free PSPAA micelles. The nanoacceptors were changed to both SDS and bovine serum albumin micelles to well represent cell membrane. The release kinetics was found to be similar in all cases.

Polymer micelles display rich structural variety, primarily including sphere, cylinder, and vesicle. These morphologies can transform among themselves in respond to the environmental stimuli. In **Chapter 3**, we studied the sphere-to-cylinder transformation of PSPAA micelles in response to pH, in particular, in the presence of embedded Au nanoparticles. It turned out that the rearrangement of the polymer shells promoted the assembly of Au nanoparticles into one-dimensional chains. This unconventional aggregation of nanoparticles followed the "chain-growth polymerization" mode, resulting in high structural selectivity which reduces the branches and irregulars. We found that the PSPAA shells played a crucial role in precise controlling the width of the nanoparticle chains and realizing the conversion from single-line chains to double-line chains.

Precise controlling the size and structure of metal nanoparticle assemblies could generate desirable optical properties. We developed a new method to control the size and structure of nanoparticle chains assisted by ultrasound (**Chapter 4**). The PSPAA encapsulated single-line nanoparticle chains broke into short ones as the result of the cavitation. By tuning the ultrasonic energy, the scission rate and the length of the chains were effectively controlled. Interestingly, the nanoparticle chains could reorganize into spherical nanoclusters under a higher ultrasonic energy. We provided the evidence that the formation of 3D nanoclusters involved the scission of the PSPAA shells and the cylinder-to-sphere transformation of the polymer micelles. Our studies showed the mobility and stretching ability of the surface ligand facilitated the organization of nanoparticle chains into 3D nanoclusters.

In **Chapter 5**, we demonstrate a scalable and facile method to the rational fabrication of "nanofish" via swelling the PSPAA micelles. In an emulsion system, the polymerization of oil droplets (divinylbenzen) took place in the polymer shell of AuNP@PSPAA and led to phase separation at nanoscale, thereafter resulting in the formation of anisotropic nanoparticles. The shearing force originated from stirring was regarded to be a critical factor. It controlled the diffusion of oil droplets from aqueous solution into the polymer shell of AuNP@PSAA, which further affected the swelling of polymer micelles and the degree of phase separation. As a result, various types of "nanofish-shaped"

nanoparticles were synthesized.

Chapter 1 Introduction

1.1 Nanostructure Synthesis

Rational synthesis of nanostructures is a hot research field. Simple geometries such as sphere, cube, wire and plate have been extensively studied over the past decades. Notwithstanding these successes, the morphological variety of nanostructures is still very limited for future developments. Without synthetic dexterity of the basic building blocks, it would be nearly impossible to design and fabricate sophisticated superstructures. In biology, the complex interactions and mechanisms are supported by the rich structural variety of biomolecules. In macroscopic world, hardly any machine or device is made of simple components. Even simple nuts and bolts are too complex from the point of view of nanostructure synthesis.

In nanostructure synthesis, there are only two main origins: nanocrystals and polymers. Directly deriving shapes from molecular templates has so far been not very successful. The surface facets are responsible for the shapes of nanocrystals. By selecting ligands that have affinity for specific facets, one can obtain a variety of geometries such as cube, rod, and octahedron.^[1-6] The structural variety can be further enriched by employing site-specific growth and etching processes.^[7-10] On the other hand, polymer micelles are known to

display many types of morphology, including sphere, cylinder, toroid, vesicle, and compound structures.^[11-18] Both the fields of nanocrystal growth and polymer morphology have been well established. Thus, exploring the intersection between these two fields should offer opportunities in obtaining new structures and more importantly, new synthetic methodologies.^[19-29]

There are a few ways through which nanocrystal and polymer can be combined. The simplest forms are to encapsulate one component with another, or to place two components side by side (Figure 1-1). The former can be classified as core-shell structure whereas the latter as Janus (two-faced) structure.^[30-33] If such structures can be consistently and reproducibly synthesized, combining different materials provides a basic way to enrich the complexity of nano-objects. Exploiting the unique characteristics of core-shell or Janus particles, a great variety of complex structures can be synthesized. In addition to nanocrystals and polymers, different materials such as amorphous oxides can be included in the synthesis. There are multiple ways to combine three or more components: they can be concentrically stacked together in a multi-layer conformation or juxtaposed to give a well-defined cluster. Moreover, the core-shell and Janus motives can also be combined.^[34-38] Interestingly, by exploiting the synergistic effects among different components, nanoscale chains, rings, and even double helices can be synthesized in pure form.^[39-42]

In general, the potential energy landscape of multi-component systems is

much more complex than that of single-component ones. While this richer landscape poses great synthetic challenges, it can offer opportunities to achieve structures that are otherwise impossible. Given the complex nature of such systems, understanding the underlying mechanisms becomes essential for rational design and synthesis.

1.2 Formation Mechanism of Core-Shell Nanostructures

In 1970, Mason et al. studied the possible conformations of two immiscible liquid droplets in a third immiscible liquid using optical microscope.^[43] In general, when combining two phases (1 and 3) in a medium 2, three interfaces are formed (Figure 1-1); their interfacial energies can be expressed as σ_{12} , σ_{23} , and σ_{13} (phase 1 is selected such that $\sigma_{12} > \sigma_{23}$). Depending on the relative strength of the interfacial energies, droplet 3 can completely, partially, or not engulf droplet 1. The conditions for these scenarios are listed in Figure 1-1, where $S_i = \sigma_{jk} - (\sigma_{ij} + \sigma_{ik})$ are the spreading coefficient. Simply put, if the droplet 1 “hates” the solvent 2 ($S_3 > 0$, or $\sigma_{12} > \sigma_{23} + \sigma_{13}$), it would be more favorable for the droplet 3 to completely engulf the droplet 1. If the droplet 1 “hates” the droplet 3 ($S_2 > 0$, or $\sigma_{13} > \sigma_{12} + \sigma_{23}$), engulfing cannot occur. In between these two extremes, the droplet 3 partially engulfs the droplet 1 to minimize the overall interfacial energies.

At nanoscale, the interfacial energies should always be proportional to the

interfacial area. Because the above analysis is based on geometric considerations,^[43] it is independent on the size of the phases. Thus, the formation of core-shell and Janus nanostructures can be similarly analyzed. However, it is often difficult to measure the interfacial energies at nanoscale, which are highly dependent on the nature and density of surface ligands (or of interfacial surfactants). Qualitatively, the interfacial energies can be tuned by adjusting the lattice match/mismatch of two materials, the hydrophilicity/hydrophobicity of the surface ligands, and the ligand density.^[44] Thus, the relative conformation of two nanomaterials can be rationally designed.

Figure 1-1 Equilibrium configurations determined by the relative strength of interfacial energies. Specifically, this can be applied to the conformations of two immiscible droplets 1 and 3 in solvent 2. Reproduced with permission from ref 43.

The underlying assumption for the above analysis is that at least one of the

phases can flow freely to adjust the contact area in between. Thus, the final conformations are thermodynamically controlled as dictated by the conditions in Figure 1-1. When both phases are solid, the obtained conformation is not necessarily at minimal-energy state. The kinetic pathways leading to the nanostructures can also play an important role. The stable conformations may form, either because the structure eventually reaches equilibrium or because its formation always follows a minimal-energy pathway.

1.3 Basic Core-Shell Systems

Core-shell structure is probably the most basic motif in two-component nanomaterials. It has been extensively studied and summarized in several recent reviews.^[45-47] From the point of view of interfacial energies, the key issue is to exchange the surface ligands on the core nanoparticles (NPs), so that the subsequently formed shell can form a wetting layer around the core. The systematic development of such encapsulation processes will provide a foundation for synthetic advancements.

1.3.1 Shells of Amphiphilic Block Copolymers

Broad studies on polystyrene-*block*-poly(acrylic acid) (PSPAA), which comprises of covalently linked hydrophilic and hydrophobic fragments, have been carried out in relation to their self-assembly.^[18] In a polar solvent, the polystyrene (PS) blocks tend to aggregate together to minimize their contact

with solvent. However, the resulting aggregates cannot grow indefinitely because the tethered poly(acrylic acid) PAA blocks strongly repel one another. Thus, the copolymers can form micelles like small surfactant molecules. If any NP can be functionalized with a suitable hydrophobic ligand, it would be possible to self-assemble the polymer around it (Figure 1-2a).^[48]

In the conventional approach, the self-assembly of amphiphilic block copolymer is induced by the slow addition of a nonsolvent (e.g., water) to a good solvent (e.g., DMF) containing the polymer.^[18] The gradual decrease in solubility causes the polymer to be excluded from the solution, forming micelles. Using this approach, Taton and coworkers pioneered the encapsulation of several types of NPs in PSPAA shells.^[49-51] In our works, we adapted a more facile process, whereby NPs were directly mixed with PSPAA in a suitable solvent mixture (e.g., DMF/water = 4:1), which was then heated to assist the polymer self-assembly.^[16, 48] After assembly, quick addition of water can remove DMF from the polymer shell, de-swelling it and locking the resulting nanostructure (Figure 1-3a-c).

In general, a ligand is suitable if it has strong affinity for the NP surface and possesses a hydrophobic tail. During PSPAA self-assembly, the PS blocks adsorb on the ligand-coated NP surface, driven by van der Waals and hydrophobic interactions. The PS domain is highly swollen by DMF and thus, it is sufficiently mobile to adopt a minimal-energy conformation with respect to

Figure 1-2 Schematics showing the three basic approaches for synthesizing core-shell nanostructures. Three types of shell materials were used (a) amphiphilic block copolymer; (b) conductive polymer; (c) silica. Reprinted with permission from ref 48, and 53, respectively. Copyright 2008 from Wiley-VCH Verlag GmbH & Co; and 2009 from the Royal Society of Chemistry.

the NP. Once functionalized with a hydrophobic ligand, the NP-PS interfacial energy can be greatly reduced to give core-shell structure. Like in empty micelles, the size of the PS domain is limited by the repulsion among the PAA

blocks dissolved in water. Moreover, the PS domain is limited in thickness by the physical length of the PS blocks, which are entropically unfavorable when stretched. Thus, the shell thickness is thermodynamically controlled, that is, too thick or too thin shells are unstable.

1.3.2 Shells of Conductive Polymers

Typical conductive polymers include polyaniline (PANI), polypyrrole (PPy),^[52, 53] and polythiophene (PTh).^[54] They can be easily synthesized by the direct polymerization of the respective monomers, but the resulting polymer chains do not repel one another. Thus, directly encapsulate NPs by *in situ* polymerization often leads to aggregation.

Our group developed a simple approach by introducing a surfactant, sodium dodecylsulfate (SDS). The monomers were polymerized *in situ* in the presence of NPs and SDS. The following is a typical example: When aniline adsorbs on the surface of AuNPs, the AuNPs become more hydrophobic and tend to aggregate. In the presence of SDS, it self-assembles on the aniline-coated AuNPs, endowing them with negative charges and preventing aggregation. When the oxidant $(\text{NH}_4)_2\text{S}_2\text{O}_8$ is added, aniline is continually oxidized to PANI. Because of the crosslinking of PANI, the polymer domain cannot flow and it grows by layer-by-layer deposition of PANI, forming core-shell nanostructure (Figure 1-2b, 1-3d). Thus, the shell thickness is kinetically controlled, unlike

the above PSPAA system.

With minor modifications, this method can be used to encapsulate a variety of metal NPs in shells of PANI, PPy, or PTh, forming core-shell structures. The oxidant plays an important role: For example, when weak oxidant AgNO_3 was used to oxidize aniline, the lack of crosslinking in PANI led to liquid-like polymer domain, giving Janus structures. When $\text{H}_2\text{O}_2/\text{FeCl}_3$ were used to oxidize thiophene, over-oxidation of the resulting PTh gave hydrophilic segments (poly(thiophenesulfone)), leading to the formation of vesicles.

1.3.3 Silica

In addition to polymer shells, silica has been frequently used for building core-shell nanostructures. It is essentially a highly crosslinked inorganic polymer, but it is chemically more robust than organic ones. In addition, it is porous and optically transparent.

There are two main methods to deposit silica on the surface of NPs: In the Stöber method, $\text{NH}_3\cdot\text{H}_2\text{O}$ catalyzes the hydrolysis of tetraethoxysilane (TEOS) in water/alcohol mixture.^[34, 35] Alternatively, with the help of surfactants, TEOS hydrolysis can occur in the aqueous droplets of water-in-oil emulsion.^[55-58] Because the silica domain is highly crosslinked, it cannot flow once it is formed. Thus, silica shell can be continually grown by the layer-by-layer deposition (Figure 1-2c, 1-3e); the shell thickness can be easily tuned by modulating the

Figure 1-3 TEM images of typical core-shell nanostructures (a-c) PSPAA encapsulated (a) AuNPs; (b) Au nanorods; (c) Au-Ag dimers; (d) AuNP@PANI; (e) AuNP@SiO₂; (f) multilayer (Au@SiO₂)@PSPAA. Reprinted with permission from ref 53, and 15, respectively. Copyright 2009 from the Royal Society of Chemistry; and 2012 from Wiley-VCH Verlag GmbH & Co.

extent of silica deposition. The main issue is to modify the NP surface with a suitable ligand, so that silica can form a wetting layer on the NP surface (i.e., minimize the NP-silica interfacial energy). In general, ligands should have -Si-OH or -COOH terminal groups to render the NP amenable for silica adsorption (e.g., using 3-aminopropyltriethoxysilane (APTES) and 4-mercaptobenzoic acid (MBA), respectively).^[59, 60] Many literatures have been reported on these well-established methods for synthesizing core-shell NPs.

1.3.4 Other Core-Shell Nanoparticles

In the literature, there are several other core-shell systems.^[45] In metal-metal and semiconductor-semiconductor core-shell NPs, the interfacial energy between the two materials are mainly determined by the extent of their lattice mismatch.^[61-65] The different growth modes have been summarized in several excellent reviews.^[47, 66] Conversely, the polymer or oxide can be encapsulated with a metal shell.^[67-69] But in this system, the metal-solvent interfacial energy is typically higher than the oxide-solvent energy and thus, the deposited metal often forms granular islands in order to reduce the metal-oxide interface. The usual approach is to decorate the core surface with a large number of small metal seeds, so that they can grow into each other forming a continuous shell.

The lattice-matching requirement brings limitation to the choice of both core and shell materials. Ouyang Min et al. developed a nonepitaxial growth method to fabricate metal-semiconductor NPs (Figure 1-4).^[70, 71] Concentric Ag shell was firstly grown on Au core via intermediate-phase assisted phase exchange and reaction. Under certain conditions, the Ag shells can be modified to form silver compound shells (Ag_2X) with an amorphous structure, which is independent of the lattice-matching principle. When the exchange of cations occurred, the amorphous layer transformed into crystalline shell.

Figure 1-4 Non epitaxial growth process and mechanism of hybrid core-shell nanostructures with substantial lattice mismatches. (a) Schematic of growth process. (b) Series of high-resolution TEM images highlighting different synthetic stages of Au-CdS growth. Scale bar, 5 nm. (c) Corresponding XRD patterns of different stages illustrated in (b). Reprinted with permission from ref 71. Copyright 2010 from AAAS.

1.3.5 Janus and Multi-layer Nanostructures

Janus NPs can be created by modulating the core-shell interfacial energy, and several strategies are available.^[72-78] For example: self-assembly, masking and phase separation.^[32] In the AuNP@PSPAA system, the Au-PS interfacial energy can be easily tuned by ligand exchange on the Au surface.^[36] We found that several types of hydrophobic (L_A) and hydrophilic ligands (L_B) can bind

competitively on the surface of AuNPs, forming phase-segregated domains. Because the PS- L_B interfacial energy is large, the PS domain cannot engulf the L_B -coated surface. Thus, PSPAA selectively self-assembled on the hydrophobic surface, giving Janus NPs (Figure 1-5a, b). The scope of this process has been shown to be broad: a variety of hydrophobic and hydrophilic ligands, along with different NPs such as Ag nanocubes and Au nanorods, were shown to work, giving partial PSPAA shells.^[79] Furthermore, the idea of ligand competition can also be applied to the silica system: MBA and PAA₈₆ were shown to competitively bind to AuNPs. These PAA chains were long enough to prevent silica adsorption and thus, silica selectively deposited on the MBA-coated surface, forming Janus AuNP@SiO₂ NPs (Figure 1-5c, d).^[37]

In the PANI system, the direct reaction between AgNO₃ and aniline at room temperature caused the simultaneous formation of Ag and PANI layers. Because AgNO₃ is a weaker oxidant than (NH₄)₂S₂O₈, the resulting PANI domain was not sufficiently crosslinked and thus, it acted like a liquid droplet. As a result, the PANI domain moved to one side of the metal core in order to minimize the overall interfacial energies, thus giving rise to Janus Au-PANI nanostructure (Figure 1-5e).^[38] The fact that this configuration was favored suggested that $\sigma_{\text{Ag-PANI}} > \sigma_{\text{PANI-water}} + \sigma_{\text{Ag-water}}$ (Figure 1-1). We verified that the mobility of the PANI domain was the critical factor: higher reaction temperature (80 °C) or stronger oxidant led to higher degree of crosslinking in PANI, giving complete

Figure 1-5 Three approaches to Janus nanoparticles: (a-b) self-assembly of PSpAA on AuNPs, where the competitive binding of hydrophobic and hydrophilic ligands causes partial polymer encapsulation; (c-d) selective deposition of SiO_2 on one side of AuNP by exploiting competitive ligand binding; (e-f) formation of eccentric Au@Ag@PANI NP and the subsequent growth of Ag on the metal core. Reprinted with permission from ref 36, 37, and 38, respectively. Copyright 2008 from American Chemical Society; 2010 from American Chemical Society; and 2010 from American Chemical Society.

encapsulation.

Multi-layered nanostructures can be easily constructed, typically by sequential deposition of two materials. In each encapsulation step, the core

surface should have suitable functional groups (e.g., -OH, -NH₂, or -COOH), so that the core-shell interfacial energy is sufficiently low. The fabrication of triple-layer structure is easy if two of the materials are similar in composition, such as Au@Ag@PPy^[52] and Au@silica@silica.^[34]

Mutual encapsulation of silica and polymer is more challenging. Because silica surface is negatively charged with polar Si-OH groups, neither PS nor PAA block of PSPAA can attach to it due to lack of interactions. One solution is to functionalize silica shells with positively charged amino groups using APTES, so that PAA blocks can absorb via electrostatic and H-bonding interactions. Hence, PSPAA can self-assemble into uniform shells around the modified silica NPs (Figure 1-3f). If the PAA blocks are short (e.g., PAA₄₉), silica can also nucleate on its surface forming a shell. These two encapsulation processes can be repeated to create silica/PSPAA multilayer structures.^[35]

Yolk-shell is a special type of multi-layer nanostructure, where a void layer separates the core and shell. The most straightforward synthetic strategy is to selectively remove the intermediate layer of triple-layer NPs. Such a multi-step process is often tedious, unless the starting triple-layer NPs are easy to make and the selectivity for the intermediate layer is high.^[52, 80-82] An alternative strategy is to exploit the intrinsic inhomogeneity present in the silica shells prepared by the Stöber method.^[34] The inner shell section could be selectively removed to obtain yolk-shell Au@SiO₂ NPs. This method is facile by directly

etching double-layer NPs. Starting from multi-layer Au@silica@silica@silica, nano-matryoshkas can be easily fabricated.

By a similar principle, yolk-shell nanostructures were synthesized in the PANI system by exploiting chemically induced inhomogeneity: The outer section of the oligoaniline (OANI) shells on AuNPs can be partial oxidized to PANI. Thus, Au@PANI yolk-shell nanostructures can be synthesized by selectively dissolving the inner shell section with 2-propanol.^[83]

1.4 Shells as a Means of Preservation

In the synthesis of nanostructures, shells play important roles: It can keep the ligands and NPs together, and prevent the undesired aggregation, thus, enhancing colloidal stability of NPs. The shell encapsulation allows isolating and highly purifying the NPs, which provides an effective route to trap aggregation intermediate for mechanistic studies.

1.4.1 Preservation of Surface Ligands

In certain applications, for example, surface-enhanced Raman scattering (SERS), preventing ligand dissociation and exchange is of great importance. The number of ligands on the surface of NPs is a critical factor for its SERS intensity. However over long periods of time, these ligands can dissociate. Even a strong thiol ligand could dissociate from Au surface, not to mention ligands bound by weak interactions. By using a polymer or silica shell,^[59, 84] the ligands

are locked inside the shell and isolated from the environment. It has been demonstrated that SERS-active ligands can be used to modify the surface of Au/Ag NPs, rendering them amenable for PSPAA encapsulation.^[84] The

Figure 1-6 (a) SERS active-ligands used in SERS probes; (b) SERS spectra of three different nanoprobe using (i), (ii), and (iii) as ligands, respectively. Reprinted with permission from ref 84. Copyright 2009 from Wiley-VCH Verlag GmbH & Co.

resulting core-shell NPs can be used as SERS probes (Figure 1-6). Because the shells ensure the colloidal stability of the NPs, a wider variety of ligands can be used, including weak and insoluble ligands. Moreover, the shells present similar surface features for general applications. When using multiple types of SERS NPs, completely preventing ligand exchange among them is a necessity for multiplexed sensing and detection.

1.4.2 Preservation of Nanoparticle Assemblies

A general problem in characterizing colloidal nanostructures is that the samples have to be dried before being studied by electron microscopy (TEM/SEM). However, the act of drying concentrates NPs as well as salts, often leading to serious aggregation. It is not always clear whether the observed structure is formed in solution or during the drying process of sample preparation.

With the protection of shells, the solution form of the NPs can be better preserved. In our group, we have shown that the act of polymer encapsulation did not cause significant change in the plasmon absorption spectra of the samples.^[53, 85] Once the solution species are clearly identified, the response in SERS or UV-vis signals can be better interpreted. Particularly for SERS studies,^[84] knowing the population of the aggregated NPs is essential because the SERS hotspots thereof can have much stronger SERS intensity than the individual NPs. In our group, we have demonstrated the purity of non-aggregated NPs^[84, 86] and of Au-Ag dimers using this method.^[44]

The random aggregation of NPs in a colloidal solution is in certain degree similar to the polymerization of organic monomers, because the NPs have to find each other via diffusion and random collision, which involve similar principles. We used PSPAA encapsulation to preserve the AuNP assemblies and attempted to fit their “polymerization” kinetics.^[87] With the polymer shells

preventing aggregation and dissociation, the assemblies can be readily characterized, purified and applied for SERS studies.^[87-89]

Figure 1-7 (a) TEM image of enriched dimers of AuNPs; inset: separation result of Au nanoclusters in high-density CsCl solutions via differential centrifugation; (b-c) TEM images of PANI protected single-line chains, which were synthesized via freezing the aqueous solution of AuNPs. Reprinted with permission from ref 89, and 90, respectively. Copyright 2009 from American Chemical Society; and 2011 from American Chemical Society.

Similarly, shell encapsulation can be applied to larger assembled structures. When freezing of an aqueous solution of AuNPs, the NPs were trapped in the ice veins forming long and non-branched chains.^[90] However, these chains were fragile and easily broken. After *in situ* polymerization to form PANI coating on the chains, the mechanical strength of the chains was greatly improved and thus, we were able to isolate and purify the single-line chains (Figure 1-7b, c).

1.4.3 Purification of Nanostructures

Purifying NPs and their assembly is as important for nanoscience as purifying chemicals is for organic chemistry. We discovered that differential centrifugation using high-density CsCl solutions is an effective method for separating NP clusters.^[89] However, colloidal NPs are generally not stable in salt solutions. Typical NPs would easily aggregate under physiological salt concentrations (~ 0.45 mM), not to mention near-saturated CsCl solutions (11 M). After PSPAA encapsulation, the colloidal stability of the resulting core-shell NPs was greatly improved. At $\text{pH} > 10$, their surface is covered with long ionic PAA chains in extended conformation, introducing charge and steric repulsion against aggregation. Thus, using PSPAA shells as a means of protection, we were able to purify dimers of AuNPs to about 95% purity (Figure 1-7a).

This method was further used to prepare dimers and trimers of AgNPs with 85% and 70% purity, respectively. The structural uniformity of the hotspots in these clusters allowed improved calculation and interpretation of the respective SERS enhancement factors.^[88]

In the hetero-assembly of NPs discussed above, PSPAA encapsulation was applied to allow the purification of the resulting nanoclusters $\text{AB}_n\text{@PSPAA}$ ($n = 2-4$).^[91] For the smaller AB_n ($n = 1-2$) clusters, Ag can be selectively deposited on the exposed A-NPs, making them much heavier than all other species in the

reaction mixture. They can be easily isolated by centrifugation and then the Ag domain was removed by etching.

1.5 Use of Core-Shell Motif for Advanced Nanostructures

With controlled synthesis of core-shell nanostructures, more complex structural features can be built from them. Core-shell NPs possess two components with distinctive properties, both of which can be exploited for unique growth or assembly modes.^[92]

1.5.1 Unconventional Crystal Growth

In comparison to the synthesis of single-component nanocrystals, nucleation and growth of nanocrystals in multi-component systems have been much less studied. The complex interactions among the components are a great challenge in terms of synthetic design. From this perspective, well-controlled core-shell nanostructures are an important intermediate step towards complex nanocrystals.

Au-peryene hybrid crystals. It found that single-crystalline perylene domain can grow around polycrystalline Au seeds, forming core-shell nanocrystals (Figure 1-8a).^[93] This result was unconventional considering the mismatch with the polycrystalline phase. During the growth, the intermediate crystals can be trapped by PANI encapsulation. It was observed that the

perylene crystal initiated from 1~2 locations on the Au seed, and then grew larger, eventually engulfing the entire seed (Figure 1-8b-e). Thus, it appeared that the perylene crystal can accommodate AuNP and small mis-oriented perylene domains as defects. We postulated that it was because of the weak and non-directional van der Waals interactions within the perylene crystal, which can tolerate slight distortion or reorientation of the perylene molecules.

Nanowire double-helices. When metal NWs are used as seeds, coaxial core-shell NWs can be obtained. We reported an interesting case where the core NWs were made of Au-Ag alloy (Figure 1-8f) and had a unique type of twisted structure, namely Boerdijk-Coxeter-Bernal (BCB) helix.^[40] The NW can wound around itself, forming a double helix (Figure 1-8g), as an additional metal layer such as Pd, Au, or Pt is grown on its surface. The product was a mixture of left- and right-handed helices, but within an individual helix the chirality was highly consistent. We proposed that this chirality originated from the initial Au-Ag alloy NWs. Upon metal deposition, the lattice strain in the NWs increased to a critical level, so that they “want” to convert back from the BCB helix to the conventional fcc structure. The NW is forced to unwound, forming a double helix.

Figure 1-8 (a) TEM image of AuNP@perylene hybrid nanocrystal; (b) Schematics showing the growth intermediates of AuNP@perylene without PANI encapsulation; (c-e) TEM images of the PANI-trapped intermediates of AuNP@perylene. (f) TEM image of Au-Ag alloy nanowires; (g) TEM image of (Au-Ag)@Pd double helix; (h-i) TEM images of 2D (h) and 3D (i) Au-PANI dendritic nanostructures. Reprinted with permission from ref 93, 40, 94, and 31, respectively. Copyright 2011 from Wiley-VCH Verlag GmbH & Co; and 2011 from American Chemical Society; 2010 from the Royal Society of Chemistry; 2012 from the Royal Society of Chemistry.

Au/Ag growth in PANI. In the above two cases, the unusual nanocrystals arise from the core-shell structure and the unique combination of materials. In addition, we showed that deposition of Au/Ag on Au seeds in the presence of PANI shells can also give rise to unique crystal growth modes.

When AgNO_3 was used to oxidize aniline in the presence of Au seeds, the partially oxidized PANI domains acted like liquid. They were able to move to one side of the Au seeds, forming Janus NPs.^[38] While Ag can diffuse through the PANI domains and grew epitaxially on the Au seeds, the growth was highly anisotropic because of the eccentric PANI domains (Figure 1-5f). Thus, the partial shells provided a means to break symmetry in the growth of nanocrystals.

In contrast, when HAuCl_4 was used as oxidant, the resulting PANI was highly crosslinked. Once a PANI domain was formed, it cannot move on the Au seed. However, the subsequent Au deposition was dependent on the size and shape of the PANI domain.^[94] As a result, the Au growth was branching and chaotic. Controlled by the diffusion of AuCl_4^- and aniline, the extending branches on the Au seeds eventually gave fractal nanostructures. Thus, 3D dendritic nanostructures (Figure 1-8i) were synthesized in colloidal solutions^[94] and 2D (Figure 1-8h) ones were synthesized on a substrate surface.^[31]

1.5.2 Creating Active Nanostructures

In conformational motion system, simple single-component systems are hard to set nanostructures in predictable motion. Owing to the decrease of the energy, it is not able to trap nanostructures at high-energy states. In two-component systems (e.g., core-shell structure), while the overall system energy decreases, one component can do work to the other, allowing the design of nanostructures with stored potential energy.

We reported the coiling of AuNWs and carbon nanotubes (CNTs) into ring structures by contracting polymer shells.^[41, 42] We observed that PSPAA shells coated on NWs or nanotubes function like liquid droplets. By simply altering the solvent ratio, the interfacial tension of polymer/solvent will be changed, causing the contraction of PSPAA shells and minimizing its surface-to-volume ratio. Thus, the embedded nanofilaments were forced to coil into rings (Figure 1-9). In addition, the racket-, 8- and peanut-shaped rings were also obtained. Most memorably, it is found that the mutual interaction between incorporated nanofilaments and polymer shells can assist the energy transfer between them, realizing conformational motion at nanoscale. When PSPAA shell was swelled or removed, the force exerted on Au ring was relieved, thus, it spontaneously sprang back to form straight wire. Since CNTs are robust, the CNT rings could be reversibly converted between compressed (8- and peanut-shaped rings) and released structures (circular ring) by tuning solvent ratio. The compressed CNT

Figure 1-9 (a) Schematics showing the PSPAA polymer-induced coiling of AuNWs and CNTs. TEM images of (b) coiled AuNW rings encapsulated in PSPAA shells; and (c) coiled CNT rings after removal of the PSPAA shells. Reprinted with permission from ref 41, and 42, respectively. Copyright 2010 from American Chemical Society; and 2011 from American Chemical Society.

rings are at high-energy states, thus, they are able to spontaneously expand to circular rings when the polymer shells are swelled. Conversely, the contraction of shells provides extra energy and thus overcome the elastic force, driving the compression of CNT ring.

In the above case, the mutual interaction of two components induces the conformational changes of the AuNWs or CNTs. Moreover, we observed that the synergistic effects of preferred etching and PTh growth can induce the

coiling of AgNWs.^[95] It is appeared that the defect resulting from angular misfit occurred on the same side of five-fold twinned AgNWs. Upon coating PTh shells, we found that AgNWs were partially etched from the angular misfit defects. This preferred etching of defects broke the AgNW five-fold symmetry and resulted in the *in situ* polymerization of PTh is not symmetrical. Thus, etched AgNWs caused bending along their surface, leading to an imbalance growth of PTh. It results in the coiling of Ag@PTh into planar spirals.

1.5.3 Unconventional Assembly

Polymer displays various morphologies, and these morphologies can transform between each other. Thus, it is believed that the morphological change of polymer shell would assist the assembly of embedded nanoparticle core. For example: grafting linear amphiphilic block copolymer on surface of Au nanoparticles, which self-assembled into tubules and vesicles in selective solvents due to the morphological transformation of polymer shells (Figure 1-10a-c).^[25] Duan et al. group used two types of chemically distinct polymers to modify Au nanoparticles, and the sphere-to-vesicle transformation of polymer via the film rehydration induced the assembly of AuNPs (Figure 1-10d).^[28] In another group, they found that inorganic nanoparticles (CdS) coated with polymer of hydrophobic and hydrophilic chains underwent amphiphilic self-assembly to form multiple colloidal nanostructures with hierarchical

organizations of nanoparticles (Figure 1-10e-i).^[29]

Figure 1-10 (a) Amphiphilic BCP-assisted self-assembly of NPs into vesicles or tubules; (b-c) representative SEM images of vesicles and tubules. (d) SEM image of the plasmonic vesicles assembled from 14 nm gold nanocrystals with mixed poly(ethyleneglycol) and poly(methyl methacrylate) brushes. Tertiary wormlike assemblies produced by adding 75 wt% water dropwise to 1.0 wt% SM-NPs in THF, followed by immediate transfer into excess water. (e and f) and (g) HAADF images showing the segmented structure of the assemblies; (h)

Schematic of lamellar ordering of SM-NPs; TEM image of a microtomed section of a wormlike assembly (h, inset); (i-k) HAADF images highlighting regularly spaced disks containing two-dimensional arrays of CdS nanoparticles with preferred hexagonal ordering; (j) Mechanism of wormlike assembly formation. Reprinted with permission from ref 25, 28 and 29, respectively. Copyright 2012 from American Chemical Society; 2011 from American Chemical Society; and 2011 from American Chemical Society.

1.6 Mechanistic Studies Based on Core-Shell Nanostructures

The core-shell NPs allow versatility in developing new synthetic methodologies and exploring mechanistic details. These abilities not only are explored for fundamental studies, but also for practical applications.^[96-106]

1.6.1 Trapping Intermediates for Mechanistic Study

If NPs can be preserved during their growth or aggregation, the trapped species can be viewed as “reaction” intermediates. As such, they can be readily isolated and characterized, permitting insights in the mechanism of NP aggregation or crystal growth. Shell encapsulation is in general much less disruptive than drying as means of trapping intermediates.

The ability to trap aggregation intermediates is invaluable for studying the spontaneous linear aggregation of AuNPs.^[85] We found that the aggregation was

kinetically controlled: Once the AuNPs made contact, their mutual interactions were usually too strong for them to separate. Hence, the critical point in determining the cluster structure occurred at the “transition state”, before the NPs met each other, at which point the charge repulsion among the NPs was the dominant factor. For an incoming NP attaching to a dimer/chain, it experiences less charge repulsion via end-on than side-on aggregation, thus favoring the formation of chains (Figure 1-11a, b).

The same methodology can be applied to studying the controlled aggregation of two different types of NPs.^[91] A tetra-thiol ligand was used to cover AuNPs with thiol groups (type A); whereas type B was citrate-stabilized AuNPs since their surface ligands can be easily replaced. Using PSPAA shells to preserve the reaction products, we were able to obtain the population of the different-sized clusters and analyze the reaction outcome. When treating A-NPs with excess amount of B-NPs, one would expect the A-NPs to be fully surrounded by B-NPs. Surprisingly, the stoichiometry of the resulting cluster was highly dependent on the ionic strength of the media. We found that the nano-reaction was, again, kinetically controlled and that the charge repulsion among the B-NPs was a key factor in the “activation barrier” at the “transition state” (Figure 1-11c-g).

Figure 1-11 (a) TEM image of linear aggregation of AuNPs trapped by PSPAA shells; (b) Schematics showing the distribution of electric field around linearly aggregated AuNP chain. (c) TEM image of PSPAA encapsulated AB nanoclusters; (d) Schematics showing the fabrication of AB nanoclusters. Dashed circle with arrows indicates the strength of charge repulsion; (e-g) TEM images of different types of Au nanoclusters. Reprinted with permission from ref 85, and 91, respectively. Copyright 2010 from Wiley-VCH Verlag GmbH & Co; and 2010 from Nature Publishing Group.

1.6.2 Use of Core-Shell motif for Diffusion Kinetics

It is well known polymer shells possess porous structure, which is able to facilitate the diffusion of ions. However, it is hard to measure the pore size and quantity of the nanoscale polymer shells, thus, leading to the lack of means to quantify ionic diffusion through these shells. We demonstrated a feasible approach for probing the diffusion rate of ions through a chemical reaction in polymer shells of AuNP@OANI (Figure 1-12).^[83] The OANI shell was partially oxidized to PANI upon contacting HAuCl_4 , and the oxidized shell can be easily

Figure 1-12 (a) Schematics showing the diffusion kinetics of oxidative ion through the polymer shells of AuNP@OANI; (b) Effects of organic solvent on the ionic diffusion. A plot showing the volume of oxidized PANI against the incubation time. Reprinted with permission from ref 83. Copyright 2012 from American Chemical Society.

identified and measured via selectively dissolving OANI layer. This *in situ* polymerization in the absence of organic solvent, gives uniform PANI shells without irregular pores, thus leading to much slower diffusion rate. Meanwhile, it is found that the oxidation of OANI shells is a time-dependent process. All results indicate the oxidation reaction takes place fast and the diffusion of AuCl_4^- in PANI layer is the rate-determining step. Thus, on the basis of the size of PANI layer, we are able to estimate the diffusion coefficient of AuCl_4^- , which comes at 700 times slower than the usual rates of ionic diffusion recorded in PANI. The slow release rate could be improved by swelling of polymer shells with a small amount of organic solvent (e.g. DMF) (Figure 1-12b), which opened up gaps among the polymer chains and facilitated ionic diffusion. Therefore, the AuNP@OANI core-shell nanostructures may have potential application in the wastewater treatment.

Besides understanding of the diffusion kinetics of ions, the polymer encapsulated nanocages (Figure 1-13a) were used as nanocarriers and studied the drug release kinetics in respond to the irradiation of near-infrared laser (Figure 1-13b-d).^[107] Well understanding the underlying mechanism of drug release can help to apply in the biological system.

Figure 1-13 (a) TEM images of Au nanocages for which the surface was covered by a pNIPAAm-co-pAAm copolymer; (b-d) Controlled release of a dye from the Au nanocages covered by a copolymer with an LCST at 39 °C. Reprinted with permission from ref 107. Copyright 2009 from Nature Publishing Group.

1.7 Summary and Outlook

In this chapter, we summarized the principles of fabricating core-shell nanostructures and demonstrated the use of core-shell motives for synthetic developments as well as fundamental studies.

In comparison to single-component systems, core-shell systems are more complex. Such a system requires more understanding but it can offer new

opportunities. As demonstrated above, we can rationally design novel nanostructure and study new fundamental problems. Systematic development of these capabilities is essential for future application of sophisticated nanostructures. In the future study, it is believed that improving the understanding of the core-shell systems can promote to explore more complex systems for new inspirations.

1.8 Bibliography

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Chapter 2 Probing the Kinetics of Short-Distance Drug Release from Nanocarriers to Nanoacceptors

In this work, we have established a model drug-delivery system to study the kinetics of drug release. The core-shell AuNP@PSPAA were used as nanocarriers because of two reasons: first, it provides a location to accommodate hydrophobic molecules; second, the fluorescent molecules embedded in PSPAA shells can be quenched by the Au core. Nanoscale acceptors were used to represent proteins and lipid membranes, and hydrophobic and fluorescent pyrene was chosen as a model drug. Thus, pyrene incorporated in AuNP@PSPAA could be readily distinguished from those released into nanoacceptors or solution. On the basis of this model system, the kinetics of drug release was study in detail, and the critical role of acceptors in facilitating drug release was demonstrated. Moreover, the exact pathway of hydrophobic drugs diffuse from nanocarriers to nanoacceptors was investigated.

2.1 Introduction

Targeted delivery and controlled release of a drug to specific organs or cells would potentially maximize its therapeutic efficacy while minimizing the side

effects. This is of particular importance for insoluble drugs, as there is a lack of means to transport them in a biological system. Many insoluble drug candidates failed clinical trials due to poor pharmacokinetics. With an effective delivery system, they could be re-explored to unleash their potential. So far, a variety of micro- and nano-scale materials have been developed as drug carriers.^[1-7] Of paramount significance for biological application is to understand the pathway and the rate of drug release from these new materials.

A model system for kinetics study entails the delivery of a model drug, typically an organic dye, from a carrier to an acceptor through a solvent. In contrast to the focus on nanocarriers, though, few systems in the literature included *nanoscale* acceptors to model biological acceptors such as proteins and lipid membranes. A practical concern is the lack of means to distinguish the drug molecules in carriers from those in acceptors. Dialysis-based methods separated nanocarriers from water by a semi-permeable membrane, so the drug content in each side could be analyzed by methods such as UV-vis,^[8-11] fluorescence^[12, 13] or chromatography.^[14] Alternatively, a bulk organic phase was used to extract the released drug in water away from the nanocarriers.^[15-18] In these examples, the released drug molecules have to diffuse through a bulk phase (water and/or an organic solvent) before being characterized. In a different approach, paramagnetic ions (Tl^+)^[18] were used to quench the fluorescence of released drug and Au nanoparticles (NPs) were used as

quenchers for the loaded drugs in nanocarriers.^[17] Thus, the fluorescence change of the model drugs in the different media (solvent vs. carriers) allowed real-time monitoring of the drug release without disrupting the delivery system.

For drug release in a cellular environment, the nanocarriers would be intimately mixed with the nanoscale bio-acceptors. Hence, short-distance diffusion ($\sim 1 \mu\text{m}$) would dominate, and use of bulk-phase acceptors cannot fully mimic this process. Recently, an enlightening work by Chen et al. used dual-labelled polymer micelles as nanocarriers,^[19] so that the drug release could be studied in the presence of nanoacceptors.

Herein, we report a new model delivery system, where pyrene was incorporated in the polymer shells of AuNPs and then released to nanoacceptors (Figure 2-1). The fluorescence of pyrene was quenched in the vicinity of the AuNPs but re-emerged upon its release, allowing *in situ* kinetics study by optical measurements. To mimic cell components, bovine serum albumin (BSA), L- α -phosphatidylcholine (a phospholipid) micelles, sodium dodecyl-sulfate (SDS) micelles, and polystyrene-*block*-poly(acrylic acid) (PSPAA) micelles were used as nanoacceptors. The intimate mixing of the nanocarriers with the nanoacceptors creates a realistic model for short-distance drug release. Using pyrene as a limiting model for hydrophobic drugs, the critical role of nanoacceptors in the kinetics of drug release was demonstrated. In addition, one-third of the drugs in development stage are insoluble in water and many

candidate drugs failed in trials because of their poor pharmacokinetics. If an effective release mechanism can be found for the hydrophobic drugs, many of the failed candidates could be re-examined to unleash their potentials.

2.2 Materials and Methods

2.2.1 Materials

All chemical reagents were used without further purification. Hydrogen tetrachloroaurate(III) hydrate, 99.9% (metal basis Au 49%), Sodium dodecylsulfate (SDS, 99%) and pyrene (99%) were purchased from Alfa Aesar; amphiphilic diblock copolymer polystyrene-block-poly(acrylic acid) (PS₁₅₄-*b*-PAA₆₀, $M_n = 16000$ for the polystyrene block and $M_n = 4300$ for the poly(acrylic acid) block, $M_w/M_n = 1.15$) was purchased from Polymer Source, Inc.; 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol (sodium salt) was purchased from Avanti Polar Lipids; L- α -Phosphatidylcholine (Approx. 99%, TLC) and Bovine Serum Albumin (BSA, cell culture test) were purchased from Sigma Aldrich; Copper specimen grids (200 mesh) with formvar/carbon support film were purchased from Electron Microscopy Sciences. Florescence spectra were collected from sample solutions in a cuvette (pathlength = 1.00 cm) on a steady-state spectrofluorometer with the excitation wavelength of 334 nm. Ultraviolet-visible (UV-vis) spectra were collected on a Cary 100 spectrophotometer. TEM images were collected on a JEM-1400 (JEOL)

Transmission Electron Microscopy operated at 100-120 kV. $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ was used as stain (3.4 mM) in all TEM images reported in this study.

2.2.2 Methods

Encapsulation of AuNPs by PSPAA. The method used here was modified from our previous report on the isotropic encapsulation of AuNPs. A AuNPs solution ($d_{\text{av}} = 16 \pm 1.8$ nm, 3 mL) were concentrated to ~ 20 μL by centrifugation at 16000 g for 15 min. The deep red solution collected at the bottom of the eppendorf tubes was diluted with H_2O (162 μL). Then, the mixture was added to 818 μL of PSPAA solution which was prepared by mixing 738 μL DMF with $\text{PS}_{154}\text{-}b\text{-PAA}_{60}$ in DMF (80 μL , 8 mg/mL). Finally, ligand 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol in EtOH (40 μL , 2 mg/mL) was added to the mixture. The final mixture has a volume of 1.04 mL with $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 4.5:1$. It was then heated at 110 $^{\circ}\text{C}$ for 2 h and slowly cooled down till room temperature. The size distributions of AuNPs (16 ± 1.8 nm) and AuNP@PSPAA (43 ± 2.6 nm) are shown in Figure 2-2.

Incorporation of pyrene into AuNP@PSPAA. Pyrene can be directly incorporated into AuNP@PSPAA during the AuNP encapsulation, where a mixture of AuNPs, 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol (40 μL , 2 mg/mL in EtOH) and PSPAA was added pyrene (10 μL , 10 mg/mL in DMF),

making a final solution where DMF/H₂O = 4.5. The solution was then heated to 110 °C for 2 h and cooled to room temperature.

However, this method does not work with strong coordinating dyes that could cause the AuNPs to aggregate. An alternative method was thus adopted here to transfer pyrene from free PSPAA micelles to pre-formed AuNP@PSPAA.

Pyrene was first incorporated in PSPAA micelles. Pyrene (25 µL, 10 mg/mL) in DMF was dissolved in 683 µL of polymer solution which was prepared by mixing 583 µL DMF with PS₁₅₄-*b*-PAA₆₀ in DMF (100 µL, 8 mg/mL), and then 1417 µL H₂O was added dropwise (final V_{DMF}/V_{H₂O} = 1:2). Finally, the mixture was incubated at 60 °C for 2 h and cooled at room temperature. The enhanced solubility of pyrene in this solution indicated its incorporation in the polymer micelles.

The as-synthesized AuNP@PSPAA (800 µL, estimated 3.87 nM) was first diluted with water (15 times in volume) and centrifuged at 16000 *g* for 30 min to separate the AuNP@PSPAA from the supernatant. The collected product was diluted by water and centrifuged again. Then the purified AuNP@PSPAA were added to the pyrene-loaded free PSPAA micelles (1 mL) (final V_{DMF}/V_{H₂O} = 1:2). The mixture was incubated at 80 °C for 4 h and cooled to room temperature. The high DMF content of this solution swelled the polymer micelles, allowing the pyrene to equilibrate in the system.

To isolate the pyrene-loaded AuNP@PSPAA, the product was directly centrifuged from the synthetic solution, and diluted by water to lower the DMF content, trapping pyrene inside of the polymer shells. The products were centrifuged again at 16000 *g* for 30 min, and the collected loaded AuNP@PSPAA were diluted with NaOH (0.1 mM) and purified again by centrifugation. The NaOH solution was used to minimize the aggregation of AuNP@PSPAA during the multiple steps of centrifugation. This purification step was typically repeated 5 times total, where the product was repeatedly diluted by NaOH (0.1 mM) and then centrifuged. The final concentrated solution (~ 10 μ L) contains the desired pyrene-loaded AuNP@PSPAA with no DMF and free PSPAA micelles. The residue NaOH in this product is considered insignificant as the pyrene-loaded AuNP@PSPAA were typically diluted to 2 mL (residue of $\sim 0.5 \times 10^{-6}$ M NaOH) during release experiments.

Quantification of the pyrene loading in AuNP@PSPAA. To quantify the relative loading of pyrene, the pyrene-impregnated AuNP@PSPAA were purified by five cycles of centrifugation to completely remove the free PSPAA micelles. Table 2-2 shows the fluorescence intensity trace at 395 nm after each separation, whereby the purified products were re-suspended in 0.1 mM NaOH and diluted to the original concentration. The fluorescence intensity decreases after the second centrifugation can be ascribed to the elimination of the residual empty micelles. Subsequently, the fluorescence intensity remained mostly

unchanged in the last three centrifugations, indicating that the residue pyrene was associated with AuNP@PSPAA and not with the free PSPAA micelles or the supernatant. The constant absorption peaks of pyrene centered at 323 and 339 nm (Figure 2-4a) further supported this point. While at the same time, little loss of the AuNPs was observed at 530 nm.

To quantify the incorporated pyrene, the purified AuNP@PSPAA (200 μ L) were concentrated to $\sim$ 10 μ L by centrifugation at 16000 g for 30 min. Then it was incubated in a DMF solution (1 mL) at 80 $^{\circ}$ C for 4 h. The PSPAA shells disassembled in the pure DMF solution, releasing the incorporated pyrene. This solution was then centrifuged to remove the AuNPs (to minimize the inner filter effect). The supernatant was characterized by UV-vis and fluorescence (Figure 2-4c); and the pyrene loading was quantified on a standard curve. Parallel experiments were carried out three times, the absorbance at 339 nm was 0.1028 (Figure 2-4c), 0.1024 and 0.0958, respectively.

Preparation of free PSPAA micelles. PS₁₅₄-*b*-PAA₆₀ (100 μ L, 8 mg/ mL) in DMF was added 25 μ L DMF, and then water was added dropwise (562 μ L), making a solution of $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 1:4.5$. The polymer solution was incubated at 60 $^{\circ}$ C for 2 h. After cooling, the mixture was diluted to 2 mL with water (0.4 mg/mL PSPAA), and an aliquot of the free micelle solution was placed into a dialysis tube (25,000 molecular weight cutoff). The tube was then placed into 1 L H₂O with gentle stirring for 24 h to remove the DMF. During the dialysis, the

water was changed four times. The size distribution (24 ± 3.0 nm) is shown in Figure 2-2.

Preparation of L- α -Phosphatidylcholine micelles. The method used was modified from a report in the literature.^[20] L- α -Phosphatidylcholine (50 μ L, 10 mg/ml) in chloroform was evaporated to dryness with nitrogen flow in an 8 mL vial. To this vial was added 3 mL tris buffer (pH 7.4); the mixture was sonicated at 5 °C under nitrogen until an almost clear suspension was obtained.

Preparation of BSA and SDS as nanoacceptors. Bovine serum albumin (BSA, 40, 20 and 10 mg) and micelles of sodium dodecylsulfate (SDS, 46, 23 and 11.5 mg) were dissolved in 2 mL H₂O and used directly. The SDS concentration used is above the critical micelle concentration of SDS.

Fluorescence measurements of the pyrene release. The purified pyrene-loaded AuNP@PSPAA (200 μ L, estimated 0.968 nM) was concentrated to ~10 μ L by centrifugation, and the collected solution was added to a 2 mL release media in a fluorescence cuvette (either H₂O, free PSPAA micelles, BSA, SDS micelles or L- α -phosphatidylcholine micelles). The emission intensity at 395 nm was recorded over time. The excitation wavelength was at 334 nm; all measurements were carried out in a cuvette of 1 cm pathlength; and the excitation and emission slits were kept at 5 nm. Figures 2-6 to Figure 2-11 showed the kinetics release profiles when PSPAA micelles, BSA, SDS micelles and L- α -phosphatidylcholine micelles with different concentrations were used

as nanoacceptors. Figure 2-3 displayed the TEM images of isolated AuNP@PSPAA after the release experiments, where the AuNP@PSPAA appeared identical to those before the release.

Inner filter effect caused by AuNPs. Inner filter effect^[21] is a common factor in fluorescence spectroscopy, referring to the absorption of excitation and/or emission light by absorbers in the sample solution. In our system, the strong absorbance of AuNPs inevitably reduces the fluorescence of pyrene. The observed weak fluorescence intensity of the pyrene-loaded AuNP@PSPAA can be at least partially attributed to the inner filter effect.

Experiments were carried out to verify the presence of this effect. The fluorescence intensity of the pyrene-loaded AuNP@PSPAA nearly doubled when the sample was diluted 8 times by water (Table 2-1). This 16 times increase in fluorescence intensity could be a combined result of (a) the reduction in fluorescence quenching as pyrene was released into water upon dilution; (b) the attenuation of the inner filter effect as a result of the significant reduction in the concentration of the AuNPs; and (c) the reduction in surface-enhanced fluorescence (SEF)^[22] as the incorporated pyrene was decreased. Subsequent purification was carried out to isolate the pyrene-loaded AuNP@PSPAA and re-disperse them in equivalent volume of water (Table 2-1 sample c). The fluorescence intensity only slightly decreased (by 10%), which can be partially attributed to the release of pyrene and also to a slight loss of

AuNP@PSPAA due to the centrifugation. Nevertheless, this result indicated that the pyrene release was not a major contributor of the fluorescence increase in Table 2-1, sample a to b. Hence, both the quenching and SEF effects remained largely unchanged as they depend on the incorporated pyrene, and the decrease in inner filter effect is probably the main reason.

Table 2-1. Emission intensity trace at 395 nm of pyrene-loaded AuNP@PSPAA: (sample a), a purified sample dispersed in 1 mL water; (sample b), sample a diluted 8 times by water; (sample c), sample b after centrifugation to isolate the AuNP@PSPAA and re-dispersion in equivalent volume of water. The excitation wavelength was 334 nm and emission intensities were measured using a cuvette of 1 cm pathlength.

	Sample a	Sample b	Sample c
Emission intensity at 395 nm	37.9 ± 0.9	66.5 ± 0.6	61.2 ± 0.7

In Figure 2-4b, the weak fluorescence signal is due to both inner filter effect and the quenching by AuNPs. In addition, SEF probably also contributed, though it is much weaker than the quenching effect. In the kinetic experiments, the fluorescence was measured *after* the mixing of AuNP@PSPAA and free PSPAA micelles. The concentration of AuNP remained unchanged during the course of pyrene release and the inner filter effect should also remain constant.

Thus, the inner filter effect did not contribute to the increase in fluorescence during the release process.

2.2.3 Calculation

Weight of HAuCl_4 in the as-synthesized 16 nm AuNP solution = 30 mg

Weight of each 16 nm AuNP = $\rho_{\text{Au}} \times V_{\text{AuNP}} = 4.141 \times 10^{-14}$ mg

Number of AuNPs in the 309 mL as-synthesized 16 nm AuNP solution =

$14.999 \text{ mg} / 4.141 \times 10^{-14} \text{ mg} = 3.622 \times 10^{14}$ particles = 0.601×10^{-9} mol

Concentration of the as-synthesized 16 nm AuNP solution = 0.601×10^{-9} mol /

$0.309 \text{ L} = 1.945 \times 10^{-9} \text{ mol/L} = 1.945 \text{ nM}$

1. The Number of pyrene incorporated per AuNP@PSPAA

Concentration of pyrene-impregnated AuNP@PSPAA in 1 mL water (based on

UV-vis absorption at 530 nm) = 0.968 nM (in number of particles)

Concentration of the pyrene in the impregnated AuNP@PSPAA dispersed in 1

mL water (based on UV-vis absorption at 337 nm of the disassembled

pyrene-loaded AuNP@PSPAA in DMF) = $1.969 \times 10^{-6} \text{ M}$

Number of pyrene per AuNP@PSPAA = $1.969 \times 10^{-6} \text{ M} / 0.968 \times 10^{-9} \text{ M} =$

2034 molecules

The equivalent local concentration of pyrene in the AuNP@PSPAA = 2034

molecules / $((\pi/6)(40^3 - 16^3) \text{ nm}^3) = 6.5 \times 10^{-2} \text{ molecules/nm}^3$

Parallel experiments have been carried out for three times: UV-vis

absorption at 337 nm of the disassembled pyrene in DMF was 1.987×10^{-6} M and 1.851×10^{-6} M, which corresponded to 2052 and 1972 molecules per AuNP@PSPAA, respectively. Thus, the average number of pyrene per AuNP@PSPAA is 2019 ± 42 molecules, which is equivalent to about 0.06 ± 0.001 molecules/nm³ in the polymer shell.

2. The ratio of AuNP@PSPAA to free PSPAA micelles in 2 mL release system

It is assumed that the density of polystyrene (PS) is 1.0 g/cm³; that the observed part in TEM of the free micelles are mostly PS; and that poly(acrylic acid) blocks are dissolved in solution (thus, the PAA blocks mixed with the stain solution on a TEM grid, giving no contrast to the background in a TEM image). The free micelles have an average diameter of 24 ± 3.0 nm based on TEM measurements.

Volume of one PS block in the PSPAA micelle core = $(104 \text{ g/mol} \times 154) / 1.0 \text{ g/cm}^3 = 16016 \text{ cm}^3/\text{mol}$

Volume of PS core in each PSPAA micelle = $(4/3)\pi R^3 = 7.235 \times 10^{-18} \text{ cm}^3$

The average number of PSPAA molecules in each micelle = volume of polymer core / volume of one PS block = 272 molecules

Weight of each micelle = $(104 \text{ g/mol} \times 154 + 72 \text{ g/mol} \times 60) \times 272 \text{ molecules} = 5531392 \text{ g/mol}$

The total number of micelles = $((0.4 \text{ mg/mL} \times 2 \text{ mL})/5531392 \text{ g/mol}) \times 6.023 \times 10^{23} \text{ mol}^{-1} = 8.71 \times 10^{13} \text{ particles}$

Number of the AuNP@PSPAA in the release solution = $5.83 \times 10^{11} \text{ particles}$
(based on UV-vis absorption at 530 nm)

The ratio of free micelles to the pyrene-loaded AuNP@PSPAA = $8.71 \times 10^{13} \text{ particles} / 5.83 \times 10^{11} \text{ particles} = 149:1$

3. Accounting for the decrease in the fluorescent intensity at equilibrium when the concentration of free PSPAA micelles is reduced from 0.4 mg/mL to 0.05 mg/mL

When 0.4 mg/mL of free PSPAA micelles were used as nanoacceptors, the ratio of free micelles to pyrene-loaded AuNP@PSPAA is 149:1 (see above).

Volume of polymer shell of one AuNP@PSPAA = $(\pi / 6) (40^3 - 16^3) \text{ nm}^3 = 3.135 \times 10^{-17} \text{ cm}^3$

Volume of PS core in each PSPAA micelle = $7.235 \times 10^{-18} \text{ cm}^3$

The ratio of the total volume of free micelle to the total volume of polymer shell on AuNP = $(7.235 \times 10^{-18} \text{ cm}^3 / 3.135 \times 10^{-17} \text{ cm}^3) \times 149 = 34:1$

The total pyrene in release system = $1.969 \times 10^{-9} \text{ mol}$

Assuming pyrene is uniformly distributed in the free PSPAA micelles and the polymer shells of AuNP@PSPAA, when the release attained equilibrium. Therefore, average amount of pyrene in the free PSPAA micelles = $1.969 \times 10^{-9} \text{ mol} \times (34 / 35) = 1.913 \times 10^{-9} \text{ mol}$.

When 0.05 mg/mL of free PSPAA micelles were used as nanoacceptors, the ratio in the number of free micelles to that of pyrene-loaded AuNP@PSPAA is 18:1, and the ratio in volume is 4.25:1; repeating the above calculation given the average amount of pyrene in free PSPAA micelles of 1.575×10^{-9} mol.

So the ratio of pyrene in 0.4 mg/mL free micelles to pyrene in 0.05 mg/mL free micelles = 1.913×10^{-9} mol / 1.575×10^{-9} mol = 1.21

Since the fluorescence intensity is directly related to the concentration of the released pyrene, at equilibrium, the ratio of fluorescence intensity in these two micelles solutions is 1.21 in theory.

Based on the emission spectra (Figure 2-10b, c), the ratio of fluorescence intensity at equilibrium in the two concentrations solution = $239 / 203 = 1.18$, which consists to the above estimated value.

4. The distance of AuNP@PSPAA from a closest neighboring free PSPAA micelle in the release system

Assuming all AuNP@PSPAA and free PSPAA micelles are distributed uniformly in a solution (2 mL), forming an idealized face-centered-cubic superlattice.

Average volume occupied by each unit cell = $2 \text{ mL} / ((5.83 \times 10^{11} \text{ particles})/4)$
 $= 1.37 \times 10^{-11} \text{ mL}$

So the unit cell dimension $a = 2.39 \times 10^{-4} \text{ cm} = 2.39 \text{ }\mu\text{m}$

That is, the average distance between two closest AuNP@PSPAA is $a \times \sqrt{2} / 2 = 1.69 \mu\text{m}$.

Similarly, assuming all free PSPAA micelles form an idealized face-centered-cubic superlattice

$$\begin{aligned} \text{Average volume occupied by each unit cell} &= 2 \text{ mL} / ((8.71 \times 10^{13} \text{ particles})/4) \\ &= 9.18 \times 10^{-14} \text{ mL} \end{aligned}$$

So the unit cell dimension $a_2 = 4.51 \times 10^{-5} \text{ cm} = 451 \text{ nm}$

That is, the average distance between two closest free PSPAA micelles is $a_2 \times \sqrt{2} / 2 = 319 \text{ nm}$.

Then, in order to achieve the longest distance, d , between the AuNP@PSPAA to its closest neighboring free PSPAA micelle, one has to place the AuNP@PSPAA in the center of the fcc unit cells in this hypothetical lattice: $d = a_2/2 = 160 \text{ nm}$.

Since the nanocarriers (AuNP@PSPAA) and nanoacceptors (free PSPAA micelles) are intimately mixed in the solution. On average, a released pyrene molecule has to diffuse over 160 nm to reach the first layer of acceptors and about 840 nm ($a/2$) to reach most of the acceptors. Obviously, in a real solution both the carriers and the acceptors are fast moving owing to Brownian motion, facilitating the pyrene transfer. The actual diffusion distances should be shorter than the values given above.

When 0.05 mg/mL of free PSPAA micelles were used as nanoacceptors,

repeating the above calculation gives the averaged separation d of 320 nm. Thus, doubling the average separation distance between AuNP@PSPAA and free PSPAA micelles did not change the rate in reaching release equilibrium (Figure 2-12a).

2.3 Results and Discussions

2.3.1 Synthesis of Nanocarriers and Drug Loading

To prepare drug carriers, AuNPs were encapsulated by PSPAA shells (AuNP@PSPAA, Figure 2-1b) following previously reported methods.^[23-25] The uniform micellar shells on AuNPs are similar in nature to empty micelles of PSPAA (Figure 2-1c), which have been shown to incorporate hydrophobic molecules such as pyrene.^[26] Pyrene could be directly loaded in AuNP@PSPAA during the AuNP encapsulation,^[27] but an alternative method was used here which is compatible with other molecular payloads. Pyrene was transferred from "free" PSPAA micelles (with no AuNP) to AuNP@PSPAA: It was first incubated with PSPAA in a DMF-rich solvent ($V_{H_2O}/V_{DMF} = 2:1$); its enhanced solubility in this solution indicated its incorporation in the polymer micelles. Purified AuNP@PSPAA ($d_{Au} = 16 \pm 1.8$ nm; $d_{overall} = 43 \pm 2.6$ nm, Figure 2-2) were then added and the mixture was incubated at 80 °C for 4 h. The concentration of pyrene in the free PSPAA micelles is higher than that in the AuNP@PSPAA, and this concentration gradient drove the transfer of pyrene to

the AuNP@PSPAA. The high DMF content of the solution swelled the polymer micelles, facilitating the equilibration of pyrene in the system.

After the pyrene incorporation, the heavy AuNP@PSPAA were readily isolated by centrifugation, and the product was diluted by water to reduce the

Figure 2-1 A new kinetics model for drug release. (a) Schematics showing the release of pyrene from the polymer shells of AuNPs: in the absence of nanoacceptors, the system quickly reaches equilibrium without significant materials transfer; in the presence of excess free PSPAA micelles, the short-distance transfer of pyrene is fast. (b, c) Transmission electron microscopy (TEM) image of pyrene-loaded AuNP@PSPAA (b) and of free PSPAA micelles (c).

DMF content, de-swelling the PSPAA micelles and trapping pyrene inside. In the collected solution of pyrene-loaded AuNP@PSPAA, there were still a small amount of residue DMF and free PSPAA micelles. To completely remove these impurities, the concentrated samples ($\sim 10 \mu\text{L}$) were diluted with NaOH (0.1 mM, 1.5 mL) and further purified by centrifugation (typically 5 cycles in total). The NaOH solution was used to increase the charge repulsion and minimize the aggregation of AuNP@PSPAA during the multiple steps of centrifugation.^[28-31]

Figure 2-2. Size distributions of (a) Au cores (283 data points), the average diameter is $16 \text{ nm} \pm 1.8 \text{ nm}$; (b) AuNP@PSPAA, the average diameter is $43 \text{ nm} \pm 2.6 \text{ nm}$; and (c) free PSPAA micelles (220 data points), the average diameter is $24 \text{ nm} \pm 3.0 \text{ nm}$. The radii were measured using ImageJ (<http://rsb.info.nih.gov/ij/>, last accessed on May 13th, 2010).

2.3.2 Characterization and Quantification of the Pyrene Loaded in AuNP@PSPAA

TEM characterization of the resulting AuNP@PSPAA (Figure 2-1b, 2-3) showed that they were structurally identical to those before the pyrene incorporation. In particular, there was no apparent change in the thickness of the

polymer shells. UV-vis spectra of the sample after each cycle of purification showed the characteristic absorption peaks of pyrene at 323 and 339 nm, and the plasmon absorption peak of AuNPs at 530 nm (Figure 2-4a). The fact that pyrene was co-separated with the AuNP@PSPAA strongly supported its habitation in the polymer shells.

The fluorescence signal of pyrene is affected by three main factors. AuNPs have strong absorbance at 200 – 600 nm, and it attenuates both the excitation beam and the fluorescence signal (inner filter effect). Though metal NPs quench the fluorescence of pyrene at close proximity, molecules at a distance from Au surface could be enhanced by SEF.^[22] In kinetics experiments, the fluorescence intensity was measured *in situ* with an invariant AuNPs concentration. Thus, the inner filter effect did not contribute to the *increase* in fluorescence, though it reduced all signals by a constant ratio. Furthermore, pyrene probably distributed uniformly inside the polymer shells, and thus the combined effects of quenching and SEF affected fluorescence signals proportionally to the number of incorporated pyrene. Since the signal of pyrene-loaded AuNP@PSPAA (Figure 2-4b) was weaker than that of the same sample after release (63 vs. 215, vide infra), SEF should be less significant than the quenching effect. In addition, the fluorescence excitation and emission wavelengths are far away from the plasmon wavelength of the AuNPs, thus, the plasmon-enhanced fluorescence does not take effect. In our system, the pyrene was confined in the polymer

shells, thus, a nonfluorescent ground complex between AuNP and pyrene cannot be formed, and the collision frequency between AuNP and pyrene is low. It is believed that the fluorescent quenching is due to the Förster resonance energy transfer, which is a dynamic quenching. The short distance between pyrene and AuNP leads to the energy transfer from excited pyrene to AuNP, bringing the quenching of the emission of pyrene.

Nevertheless, these factors make it difficult to quantify the incorporated pyrene. To overcome this problem, the pyrene-loaded AuNP@PSPAA were disassembled in a hot DMF solution: the polymer shells were dissolved and the stripped AuNPs were removed by centrifugation. The resulting solution showed pyrene absorption peaks at 307, 323, and 339 nm, and its emission peaks at 373, 384, 395, and 415 nm (Figure 2-4c). The fluorescence of pyrene was not used as it is different in water and DMF. Based on UV-vis spectra of standard samples, pyrene in this solution was quantified. It was further estimated that there was an average of about 2019 ± 42 pyrene molecules per AuNP@PSPAA, which is equivalent to about $0.06 \text{ molecules/nm}^3$ in the polymer shell.^[27]

Figure 2-3. Large TEM images of the isolated AuNP@PSPAA after the release experiment, where the pyrene-loaded AuNP@PSPAA were mixed with different nanoacceptors: (a) free PSPAA micelles; (b) BSA micelles; (c) SDS micelles; (d) L- α -Phosphatidylcholine micelles solution. The products were purified before the TEM characterization. The AuNP@PSPAA appeared identical to those before release.

Figure 2-4 Evidences for the loading of pyrene in AuNP@PSPAA. (a) UV-vis absorption spectra of the pyrene-impregnated AuNP@PS-PAA in water after five cycles of purification, lines 2-5 are samples after the respective centrifugation cycle; (b) Emission spectrum of pyrene-loaded AuNP@PSPAA in water (* indicates the emission peak at 395 nm that was followed in all kinetic traces); (c) Absorption and emission spectra of the released pyrene, after disassembling the pyrene-loaded AuNP@PSPAA in DMF and removing the AuNPs.

Table 2-2 Fluorescence intensity trace of pyrene-impregnated AuNP@PSPAA dispersed in water after five cycles of centrifugation.

Pyrene-loaded AuNP@PSPAA	Test condition	Emission intensity at 395 nm
1	After the first centrifugation	56.1 ± 0.9
2	After the second centrifugation	39.9 ± 0.4
3	After the third centrifugation	38.0 ± 0.9
4	After the forth centrifugation	37.0 ± 0.5
5	After the fifth centrifugation	35.1 ± 0.7

2.3.3 Studying the Kinetics of Short-Distance Drug Release

In an aqueous solution and in the absence of nanoacceptors, pyrene is stable in AuNP@PSPAA. The fluorescence of the loaded nanocarriers in 2 mL water did not show obvious change in 2 h (Figure 2-5). The minimal pyrene release could be attributed to its very low solubility in water (7×10^{-7} M) and also to the lack of organic solvent (*e.g.* DMF) to swell the polymer shells. While the former limits the pyrene release thermodynamically, the latter controls the rate of release kinetically. The slow release allowed the purification of the

pyrene-loaded AuNP@PSPAA without losing significant amount of the payload.

With the development and characterization of nanocarriers, the next step is to find suitable nanoacceptors. A biological system is abundant in nanoacceptors such as proteins and cell membranes, which could readily take up hydrophobic molecules by recognition, adsorption or simple dissolution. Hence, the drug transfer among the various hosts could be non-specific and difficult to study, particularly with the unknown concentrations of the bio-acceptors. In a model system, we could introduce nanoacceptors in known concentrations, so that the drug release kinetics could be studied in detail. BSA

Figure 2-5 Fluorescence intensity trace (at 395 nm) of pyrene-loaded AuNP@PSPAA upon incubation in water.

is the main plasma protein in bovine blood circulation, and it is known to absorb hydrophobic molecules such as pyrene.^[32, 33] Thus, it represents a general protein that could recognize or absorb a specific drug. In a kinetic experiment, BSA was added to a solution of pyrene-loaded AuNP@PSPAA in water, so that the nanoacceptors were homogenously mixed with the nanocarriers. As pyrene dissociated from the AuNP@PSPAA, its fluorescence intensity increased, due to the increased distance from pyrene to a Au surface. Therefore, the concentration of the released pyrene is directly related to the fluorescence intensity, allowing *in situ* and real-time monitoring of the drug

Figure 2-6 Fluorescence intensity trace (at 395 nm) showing the kinetics of pyrene release when different concentrations of BSA were used as nanoacceptors; the red lines were fitted to the Fickian diffusion model.

release by fluorescence measurement. Fast increase of the pyrene emission at 395 nm was observed (Figure 2-6). Based on the fluorescence intensity, over 60 % of the incorporated pyrene was released within 2 h.

In addition to BSA, we also used micelles of small molecules to mimic cell membranes, which are primarily made of proteins and lipids. L- α -phosphatidylcholine is a type of naturally occurring lipid. We prepared micelles of this molecule following literature procedures,^[20, 34] and then added them to an aqueous solution of pyrene-loaded AuNP@PSPAA. As shown in Figure 2-7, the release of pyrene was also fast. Similar result was obtained when SDS micelles were used as the nanoacceptors (Figure 2-8). The similarity in the kinetic behaviour of the drug release when three different nanoacceptors were used (Figure 2-6, 2-7, and 2-8) is a strong indication for a common mechanism.

Figure 2-7 Fluorescence intensity trace (at 395 nm) showing the kinetics of pyrene release when different concentrations of L- α -phosphatidylcholine micelles were used as nanoacceptors; the red lines were fitted to the Fickian diffusion model.

Figure 2-8 Fluorescence intensity trace (at 395 nm) showing the kinetics of pyrene release when different concentrations of SDS micelles were used as nanoacceptors; the red lines were fitted to the Fickian diffusion model.

However, how exactly pyrene was transferred from the nanocarriers to the nanoacceptors is still unknown. There exist at least three different pathways. Pyrene could be first released to the aqueous phase and then diffuse into the nanoacceptors, or it could be directly transferred when a transient bridge formed between a nanocarrier and a nanoacceptor during their collision. The latter scenario was previously suggested to explain the fast drug transfer in the presence of nanoacceptors.^[19] Alternatively, the micelles of nanocarriers and nanoacceptors could dynamically disassemble and reform in solution, facilitating the materials transfer among them. This is particularly likely when

the nanoacceptors were made of small molecules such as BSA, SDS and lipid, which are known to rapidly dissolve and reform in an aqueous solution.

In contrast to the dynamic behaviour of small-molecule micelles, PSPAA micelles have been shown to be kinetically frozen with low liquidity.^[20, 34] A vivid demonstration can be found in our recent report, where hollow PSPAA micelles were shown to maintain their structure in an aqueous solution, until they were heated beyond the glass transition temperature of polystyrene.^[30] Thus, under the conditions of our experiments, the PSPAA molecules in the micelles could not exchange and the micelles could not split or fuse with each other. Moreover, it is known that the PSPAA micelles are highly charged and do not aggregate even in saturated CsCl solutions.^[28, 29, 31] Given the long PAA chains (~60 acrylic acid monomers) on the surface of the micelles, it is unlikely that hydrophobic channels could form between two micelles during a collision. Therefore, use of PSPAA in both nanocarriers and nanoacceptors minimizes the mechanistic ambiguities.

To prepare empty PSPAA micelles as nanoacceptors, PSPAA was heated in a H₂O/DMF (v/v = 4.5:1) mixture to induce its self-assembly; the resulting free micelles (Figure 2-1, and 2-2c, $d = 24 \pm 3.0$ nm) were diluted by water and further dialyzed against water to completely remove DMF. Then, the pyrene-loaded AuNP@PSPAA were added into this solution to initiate the drug transfer. Fast increase of emission intensity at 395 nm was observed

Figure 2-9 Fluorescence intensity trace (at 395 nm) showing the kinetics of pyrene release when different concentrations of free PSPAA micelles were used as nanoacceptors; the red lines were fitted to the Fickian diffusion model.

(Figure 2-9), indicating the efficient release of pyrene. In this system, the free PSPAA micelles were in large excess to the AuNP@PSPAA and the ratio was estimated to be 149:1 in number of particles ($[\text{free micelle}] = 0.4 \text{ mg/mL}$). On average, each AuNP@PSPAA occupies a cube of $1.5 \text{ }\mu\text{m}$ wide with these acceptors. If assuming that the nanoacceptors form an idealized face-centred-cubic superlattice, the distance of any AuNP@PSPAA from a closest neighbouring free PSPAA micelle was estimated to be 160 nm. Hence, the distance of pyrene diffusion in water should be in the range of a few hundred nanometers, and the fast Brownian motion of both the nanocarriers and

the nanoacceptors is expected to facilitate the pyrene transfer.

Since the average diffusion distance between nanocarriers and nanoacceptors is determined by their concentrations, the kinetic experiments were carried out at varying concentrations of the nanoacceptors. In Figure 2-9, the rate of pyrene release barely changed when the concentration of free PSPAA micelles changed from 0.4 to 0.2 (Figure 2-10a) and 0.1 mg/mL, indicating that the “perfect sink” condition was attained for the pyrene released from nanocarriers. The “perfect sink” refers to the condition where excess acceptors are present for absorbing the released drug. In cases where no acceptor is used, it could also mean that the solubilizing capacity of the solvent is significantly greater than the released drug. However, further reducing the concentration of nanoacceptors (to 0.05 mg/mL) led to a lower plateau of emission intensity (Figure 2-9). It can be attributed to the reduced amount of pyrene released at equilibrium (10 h, Figure 2-10b, c, and 2-11) owing to the lack of acceptors. The amount of decrease in the fluorescence intensity at plateau is also consistent with the decrease in the estimated volume ratio of nanocarriers to nanoacceptors, supporting the attainment of equilibrium at the end of pyrene release.

Importantly, after normalization, the four traces with different nanoacceptor concentrations exactly overlapped with each other (Figure 2-12a). Since the average distance from AuNP@PSPAA to free PSPAA micelles changed in these

Figure 2-10 Release kinetics when free PSPAA micelles were used as nanoacceptors: (a) 0.2 mg/mL; (b) 0.4 mg/mL free micelles for 10 h; (c) 0.05 mg/mL free micelles for 10 h.

Figure 2-11 Release kinetics for 10 h when (a) 40 mg/mL BSA solution, (b) 23 mg/mL SDS solution, and (c) 0.167 mg/mL L- α -Phosphatidylcholine solution were used as the nanoacceptor.

experiments, this observation indicated that the diffusion of pyrene through water was not the rate-determining step. Same conclusions can be reached when other types of nanoacceptors were used (Figure 2-12b-d). Therefore, the kinetic model of our system is *virtually* equivalent to a direct diffusion of pyrene from AuNP@PSPAA to the surrounding nanoacceptors. Indeed, the fluorescence intensity traces of pyrene in Figure 2-6, 2-7, 2-8 and 2-9 fit well to the Fickian diffusion model^[35] (correlation coefficient $R > 0.98$):

$$Y = P_1 + P_2 \times t^{1/2} - P_3 \times t \quad [\text{Eq. (1)}]$$

where Y is the fluorescence intensity, which is proportional to the concentration of the released pyrene; t is the time; and P_1 , P_2 , and P_3 are coefficients (values are given in Table 2-3). The Fickian diffusion model was previously used to describe the diffusion-controlled release of a substance from a sphere, assuming a constant diffusion coefficient. It best describes purely radial diffusion in the initial (< 70%) release stage. The fact that all of our data fit well to this simple diffusion model supports a common mechanism when the diffusion of pyrene through water is efficient.

Therefore, the use of *nanoscale* acceptors has an important consequence on the kinetics of drug release. By removing the released pyrene from the aqueous phase, the presence of nanoacceptors greatly enhanced the rate of release from the nanocarriers. Similar results have been observed in a recent report using dual-labelled polymer micelles as nanocarriers.^[19] Importantly, the

Figure 2-12 Normalized traces in panel Figure 2-9, Figure 2-6, Figure 2-7, and Figure 2-8, respectively. (a) Free micelles solution, (b) BSA solution, (c) L- α -Phosphatidylcholine lipid solution, (d) SDS solution.

short-distance diffusion of a hydrophobic molecule like pyrene through an aqueous phase is actually *not* the rate-determining step. Thus, the release kinetic can be solely described by the radial diffusion through the hydrophobic phases of both the carriers and acceptors, which reduces mechanistic complexity. This feature is independent on the nature of the nanoacceptors, so long as there is excess of them around. But it is distinctively different from other model systems that involved drug diffusion through a bulk phase. Most of hydrophobic drugs are conceivably more soluble than pyrene in an aqueous

Table 2-3 Coefficients from fitting the spherical diffusion model, $Y = P_1 + P_2 \times t^{1/2} - P_3 \times t$, to the release data of pyrene in the presence of nanoacceptors (PSPAA, BSA, SDS and L- α -Phosphatidylcholine micelles) with different concentrations.

Nanoacceptors	P ₁	P ₂	P ₃	Correlation Coefficient	Reference
PSPAA (0.4 mg/mL)	114 ± 1	17 ± 0.4	0.79 ± 0.03	0.992	Figure 2-9
PSPAA (0.2 mg/mL)	117 ± 1	15 ± 0.4	0.62 ± 0.03	0.992	Figure 2-10
PSPAA (0.1 mg/mL)	110 ± 1	17 ± 0.4	0.81 ± 0.02	0.992	Figure 2-9
PSPAA (0.05 mg/mL)	90 ± 1	13 ± 0.5	0.59 ± 0.04	0.984	Figure 2-9
BSA (20 mg/mL)	107 ± 1	16 ± 0.4	0.70 ± 0.03	0.991	Figure 2-6
BSA (10 mg/mL)	106 ± 1	14 ± 0.3	0.50 ± 0.02	0.997	Figure 2-6
BSA (5mg/mL)	108 ± 1	14 ± 0.3	0.45 ± 0.03	0.995	Figure 2-6
Lipid (0.333 mg/mL)	125 ± 2	12 ± 0.6	0.63 ± 0.04	0.954	Figure 2-7
Lipid (0.167 mg/mL)	127 ± 1	12 ± 0.5	0.65 ± 0.04	0.965	Figure 2-7
Lipid (0.083 mg/mL)	101 ± 1	8.9 ± 0.25	0.45 ± 0.02	0.984	Figure 2-7
SDS (23 mg/mL)	140 ± 2	14 ± 0.6	0.71 ± 0.05	0.966	Figure 2-8
SDS (11.5 mg/mL)	130 ± 1	13 ± 0.4	0.56 ± 0.04	0.982	Figure 2-8
SDS (5.6 mg/mL)	130 ± 1	12 ± 0.47	0.51 ± 0.04	0.981	Figure 2-8

phase; and hence their diffusion through water is expected to be faster. The abundance of nanoscale bio-acceptors in a cellular environment makes it imperative to include nanoacceptors in model delivery systems. Otherwise, the rate of drug release or diffusion could be misinterpreted. For example, drug molecules incorporated in a delivery vehicle could be quickly and non-specifically lost to the bio-acceptors in a cell, even though they may be stable in the vehicle in the absence of nanoacceptors.

2.4 Conclusion

In conclusion, we have developed a new kinetic system which allows the use of both nanocarriers and nanoacceptors. The intimate mixing of the two at nanoscale realistically mimics drug delivery in a cellular environment. In the model system, the carrier/acceptor concentrations could be readily controlled and the drug transfer could be monitored in real-time. Using this unique system, pyrene was shown to quickly transfer (~ 2 h) from the nanocarriers to the nanoacceptors. This nanoacceptor-induced fast release of pyrene follows the Fickian spherical diffusion model, and it could be explained by the short-distance diffusion of pyrene through water. For its low solubility in water, pyrene was used as a limiting model for hydrophobic drugs with higher solubility. Biologically relevant nanoacceptors, such as BSA and lipid micelles, were used in our delivery system to model bio-acceptors. But the use of PSPAA

micelles as both nanocarriers and nanoacceptors provided unique advantages in understanding the pathway of pyrene release. We presented evidences to show that pyrene probably did transfer through water and that the transfer was not assisted by dynamic re-assembly or fusion-splitting of the polymer micelles.

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Chapter 3 Unconventional Chain Growth Mode in the Assembly of Colloidal Au Nanoparticles

Core-shell nanoparticles possess two distinct components, both of which can be used for nanoparticle assembly. Polystyrene-*b*-poly(acrylic acid) was used in this research since it has many types of morphologies such as sphere, cylinder, toroid and vesicle. Detailed understanding on how the PSPAA shells respond to environmental stimuli will allow the development of new synthetic methodologies. At present, we have shown that PSPAA shell could respond to changes in solvent and drive the coiling of the embedded nanofilament such as gold nanowires and carbon nanotubes. The question is what will happen if core-shell AuNP@PSPAA are suffered from environmental stimuli? To answer this question, in this chapter, we focus on investigating the sphere-to-cylinder transformation of PSPAA shells of AuNP@PSPAA in response to pH. It resulted in that the rearrangement of the PSPAA shells promoted the assembly of AuNPs into 1D nanoparticle chains.

3.1 Introduction

Assembly of nano-objects is a first and fundamental step towards functional

nanodevices. For optimizing properties and functions, it is always important to achieve structural precision. Electronic and plasmonic coupling among metallic nanoparticles (NPs) has been known to give novel electronic and optical properties.^[1-4] Such coupling is critically dependent on structural parameters such as interparticle spacing^[5] and the spatial organization of individual NPs. In comparison to higher order nanostructures, one-dimensional (1D) NP chains are more convenient building blocks for circuits in nanoelectronics, optoelectronics,^[6-10] and biosensors.^[11, 12] Minimizing structural irregularity is essential: large gap may break the coupling along a chain; and branching may cause short circuit.

A popular approach for assembling NPs is to utilize templates such as biomolecules,^[13] polymers,^[14] and lithographically created patterns.^[15] A general problem of these methods is the lack of means to achieve close packing of the NPs in a chain and avoid irregular gaps. On the other hand, simple colloidal aggregation can bring NPs closely together, with short and regular gaps dictated by their ligand layers.^[16-18] However, colloidal assembly methods based on random aggregation are generally difficult to control. There is thus great interest in the recent literature to devise controlling methods. Linear assembly of NPs has been achieved by exploiting the anisotropic ligand organization on Au nanorods,^[19-23] or the phase separation of immiscible ligands on nanospheres.^[24-26] In addition, intrinsic interactions of NPs such as

dipole-dipole interactions^[27-34] or electrostatic charge repulsion^[35-37] have been proposed to explain the 1D aggregation of NPs. Despite these advances, it remains a great challenge to program the behavior of NPs for a longer range order, for example, in terms of preventing branching and controlling cross-sectional width.

The aggregation of colloidal NPs in a 3D space can be likened to the polymerization of organic monomers.^[22, 38] The general characteristic of step-growth polymerization is that all monomers and oligomers are reactive (Figure 3-1). Their random combination quickly consumes monomers, so that the later stage reaction mostly involves the coupling of oligomers (i.e., *m-to-n* coupling; m and n are the numbers of structural units in the chain). If this reaction is inefficient, few long chains will be obtained. In contrast, in chain-growth polymerization the active species (e.g., radicals or initiators) are limited in concentration, making the coupling between monomers (*I-to-I* coupling) unfavorable. The excess monomers can continuously react with the active species and feed the chain growth via *I-to-n* coupling (Figure 3-1).

Because all NPs in a colloid are capable of aggregating, their “polymerization” tends to follow the step-growth mode. Once monomers are depleted, the *m-to-n* coupling of larger clusters becomes more difficult and less selective, leading to branches and irregularities. Hence, obtaining long chains (> 30 NPs in length) is very difficult. Here, we report the use of polymer shells to

Step Growth

Dimer

Trimer

Tetramer

Pentamer

Hexamer

Chain Growth

Dimer

Trimer

Tetramer

Pentamer

Hexamer

★ initiator ● monomer

Figure 3-1 Schematics showing the general characteristic of step-growth polymerization and chain-growth polymerization.

assist the 1D assembly of colloidal AuNPs (Figure 3-3). The system is unique in that the aggregation of NPs follows a mode that is similar to chain-growth polymerization, giving long chains up to 300 NPs in length. The monomers used are polystyrene-*block*-poly(acrylic acid) encapsulated AuNPs (AuNP@PSPAA, Figure 3-3b). Induced by the structural transformation of the polymer micelles from spheres to cylinders, the embedded AuNPs are forced to form chains. We provide evidences that the end-on aggregation (*1-to-n* coupling) is more favorable than the monomer aggregation (*1-to-1* coupling), setting the stage for continued chain growth.

3.2 Materials and Methods

3.2.1 Materials

Refer section 2.3.1.

3.2.2 Methods

Encapsulation of AuNPs with PSPAA. The method used here was modified from our previous report on the isotropic encapsulation of AuNPs. A solution of citrate-stabilized AuNPs ($d_{Au} = 16$ nm, 3 mL) was concentrated to ~ 20 μ L by centrifugation at 16000 g for 15 min. The deep red solution collected at the bottom of the eppendorf tubes was diluted with H₂O (162 μ L). Then, the mixture was added to 818 μ L of PSPAA solution which was prepared by mixing 738 μ L DMF with PS₁₅₄-*b*-PAA₄₉ in DMF (80 μ L, 8 mg/mL). Finally,

1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol in EtOH (40 μ L, 2 mg/mL) was added to the mixture. The final mixture has a volume of 1.04 mL with $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 4.5:1$. It was then heated at 110 $^{\circ}\text{C}$ for 2 h and slowly cooled down till room temperature.

The same procedure was used to encapsulate Ag nanocube (45 nm) and AuNPs ($d_{\text{Au}} = 30$ and 40 nm) with PSPAA.

Self-assembly of AuNP@PSPAA into double-line chains. The as-synthesized AuNP@PSPAA monomers were diluted (800 μ L diluted by 11.2 mL water), divided into individual eppendorf tubes, and then centrifuged at 16000 g for 30 min to remove the supernatant. The red solution at the bottom of eppendorf tubes was diluted with NaOH (0.1 mM) and purified again. The final concentrated solution (~ 20 μ L) contained the desired AuNP@PSPAA monomers free of DMF and empty PSPAA micelles. The concentrated AuNP@PSPAA were dispersed into a DMF/H₂O solution (1 mL, $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 7:3$). HCl (5 μ L, 1 M) was then added into the solution, and the final concentration is $[\text{HCl}]_{\text{final}} = 5$ mM. The mixture was incubated at 70 $^{\circ}\text{C}$ for 2 h to facilitate the shape transformation of PSPAA polymer shell.

Self-assembly of AuNP@PSPAA into single-line chains. The purified AuNP@PSPAA monomers were dispersed into a DMF/H₂O solution (1 mL, $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 6:1$). HCl (5 μ L, 1 M) was then added into the solution, and the final concentration is $[\text{HCl}]_{\text{final}} = 5$ mM. The mixture was incubated at 70 $^{\circ}\text{C}$ for

2 h to facilitate the shape transformation of PSPAA polymer shell.

Purification and Enrichment of AuNP chains. The resulting solution contained AuNP chains, small clusters of AuNPs, monomers, empty PSPAA micelles, DMF and acid. To isolate the nanoparticle chains from the excess reactants, the product solution was diluted (800 μ L diluted by 11.2 mL NaOH (1M)), divided into individual eppendorf tubes, and then centrifuged at 16000 g for 30 min to remove the supernatant. The red solution at the bottom of eppendorf tube was diluted by NaOH (0.1 mM), and further purified by repeating the above purification steps. The collected product was again diluted by NaOH (0.1 mM), giving a stable solution that contains NP chains, excess monomers and clusters of AuNP aggregates. The NaOH solution was used to neutralize the acid and minimize the aggregation of AuNP@PSPAA during the multiple steps of centrifugation.

To enrich the AuNPs chains, the resulting solution was diluted by NaOH (0.1 mM) and then centrifuged at various speeds. After each step of centrifugation, a supernatant (top) and a red solution (bottom) can be obtained; NaOH was used to dilute these separated solutions for the subsequent steps. After the AuNP aggregate solution was centrifuged at 300 g for 25 min to remove the largest aggregates, the supernatant was collected and centrifuged at 2000 g for 30 min. The bottom solution was enriched with chains. The chains could be further enriched in a sample by centrifugation of the bottom solution at 2000 g twice 20

min. The excess monomers were removed after these processes, leaving only long NP chains.

Transformation from single-line chains to double-line chains. The purified single-line chains (800 μL) were concentrated to ~ 20 μL , then dispersed into a $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 7:3$ solution with HCl (2.5 μL , 1 M). The final mixture has a volume of 1 mL with $[\text{HCl}]_{\text{final}} = 5$ mM. The mixture was incubated at 70 $^{\circ}\text{C}$ for 1 h to facilitate the contraction of PSPAA polymer shell, inducing the transformation from single-line chains to double-line chains.

Similar procedure was used to synthesize triple-line chains (Figure 3-13), where the DMF/water ratio was decreased to 3:2.

Preparation of empty PSPAA micelles. $\text{PS}_{154}\text{-}b\text{-PAA}_{49}$ (80 μL , 8 mg/ mL) in DMF was added 738 μL DMF, and then water was added (182 μL), making a solution of $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 4.5:1$. The polymer solution was incubated at 110 $^{\circ}\text{C}$ for 2 h and slowly cooled down till room temperature.

Self-assembly of empty PSPAA micelles on the end of AuNP chains. The empty PSPAA micelle solution (400 μL) was added into the above unpurified double-line chain solution (800 μL), then added 67 μL H_2O , making a solution of $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 7:3$. The mixture solution was added HCl (2 μL , 1M) and incubated at 70 $^{\circ}\text{C}$ for 2 h to facilitate the aggregation and shape transformation of empty PSPAA micelles. The final mixture has a volume of 1.27 mL with $[\text{HCl}]_{\text{final}} = 5$ mM.

Self-assembly of AuNP@PSPAA via seeded growth approach. The as-synthesized AuNP@PSPAA ($d_{\text{Au}} = 30$ or 40 nm) was diluted (50 μL diluted by 1.45 mL water), and then centrifuged at 8100 g for 20 min to remove the supernatant. The red solution at the bottom of centrifuged tube was diluted with NaOH (0.1 mM) and purified again. The final concentrated solution ($\sim 10\text{ }\mu\text{L}$) was dispersed into a DMF/ H_2O solution (200 μL , $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 7:3$) containing 4 μL of HCl (0.1 M). The solution was shaken at room temperature for 30 min, and then added into the AuNP@PSPAA ($d_{\text{Au}} = 16$ nm) monomer solution containing 557 μL DMF, 234 μL H_2O , and 4.6 μL HCl (1M). The mixture was incubated at $70\text{ }^\circ\text{C}$ for 2 h to facilitate the chain growth. The final mixture has a volume of 1 mL with $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 7:3$, and $[\text{HCl}]_{\text{final}} = 5\text{ mM}$.

Same assembly procedure was used when Ag nanocubes (45 nm) were employed as seeds.

3.2.3 Calculation

1. Estimating the volume of the “missing” polymer *when core-shell nanoparticles aggregated into 1D chains via the sphere-to-cylinder transformation of polymer shell.*

$$\begin{aligned} \text{Volume of polymer shell of one spherical AuNP@PSPAA} &= (\pi / 6) (40^3 - 16^3) \\ &= 3.135 \times 10^{-17} \text{ cm}^3 \end{aligned}$$

Assuming AuNP@PSPAA assembled into double-line chain with the length is L (L treated as infinity here); the average diameter is 63 nm. Therefore, the volume of the double-line chain = $\pi \times (63 / 2)^2 \times L = 3115.67 L \text{ nm}^3$, and total number of AuNPs in one double-line chain = $(L / 16) \times 2 = L / 8$.

Total volume of AuNPs in one double-line chain = $(L / 8) (\pi / 6) \times 16^3 = 267.95 L \text{ nm}^3$

Volume of polymer shell of one double-line chain = $3115.67 L - 267.95 L = 2847.72 L \text{ nm}^3$

In the double-line chain, the average volume of polymer micelle occupied by one AuNP = $(2847.72 L) / (L / 8) = 22781.72 \text{ nm}^3 = 2.278 \times 10^{-17} \text{ cm}^3$

The reduced volume fraction of polymer shell of one AuNP@PSPAA = $(3.135 \times 10^{-17} - 2.278 \times 10^{-17}) / 3.135 \times 10^{-17} = 27\%$

2. Calculation of surface to volume (S/V) ratio of the above six types of aggregate.

(A) core-shell nanoparticle: the radius is r, and the interparticle spacing $d_1 > 2r$;

$$\left(\frac{S}{V}\right)_A = \frac{4\pi r^2}{\left(\frac{4}{3}\right)\pi r^3} = \frac{3}{r}$$

(B-D) core-shell nanoparticles (r) aggregate into one-dimensional chains, assuming the overlapped part is a spherical crown with the height of h ($h < r$, h is limited by radius of core NP, in our system, the radius of core is 8 nm and the

radius of core-shell is 20 nm, thus, $h_{\max} = 12$ nm) (Figure 3-2G). Make $X = h/r$ ($0 < X \leq 0.6$) and the number of aggregated nanoparticles is treated as infinity; thus the interparticle spacing $d = 2(r-h)$, and the relation between S/V and h/r fits to the following equation.

$$\frac{S}{V} = \frac{\sqrt{3}}{r} \left(\frac{1}{X - 1 + \sqrt{3}} + \frac{1}{1 - X + \sqrt{3}} \right)$$

Figure 3-2 (A-F) Schematics showing different aggregates of core-shell nanoparticles with varied interparticle spacing; (G) Schematic showing the relation between the interparticle spacing d and the height h of the overlapped polymer section (special crown), and the relation between the radius of core-shell nanoparticles r and h ; (G) Curve showing the relation between S/V and h/r in cases B-D.

Assuming in case B, $X = 0.01$, $\left(\frac{S}{V}\right)_B = \frac{2.97}{r}$

Assuming in case C, $X = 0.1$, $\left(\frac{S}{V}\right)_C = \frac{2.74}{r}$

Assuming in case D, $X = 0.5$, $\left(\frac{S}{V}\right)_D = \frac{2.18}{r}$

As shown in Figure 3-2H, compared with the S/V of monomers ($3/r$), the S/V of the aggregates was only slightly reduced when the monomers aggregate via simple surface contact (small h/r).

(E) core-shell nanoparticles aggregated into single-line chain, where the shape of polymer shell is cylinder, the radius is r and the length is l_1 , assuming l_1 is infinite;

$$\left(\frac{S}{V}\right)_E = \frac{2\pi l_1 r}{\pi r^2 l_1} = \frac{2}{r}$$

(F) core-shell nanoparticles aggregated into double-line chain (two parallel chains for simplicity), where the shape of polymer shell is cylinder, the radius is r_2 ($r_2 > r$) and the length is l_2 , assuming l_2 is infinite;

$$\left(\frac{S}{V}\right)_F = \frac{2\pi l_2 r_2}{\pi r_2^2 l_2} = \frac{2}{r_2}$$

Because of $r_2 > r$, $\frac{2}{r_2} < \frac{2}{r}$

Thus, the S/V of double-line chains is smaller than that of single-line chains.

3.3 Results and Discussion

3.3.1 Fabrication of Double-Line Chains

The AuNP@PSPAA monomers were synthesized as previously reported.^{[39,}
^{40]} Citrate-stabilized AuNPs ($d = 16$ nm) were first functionalized with a hydrophobic ligand, 1,2-dipalmitoyl-*sn*-glycero-3-phosphothioethanol. The amphiphilic polymer PSPAA was then self-assembled on the NP surface, forming a uniform shell with poly(acrylic acid) (PAA) blocks dissolved in water facing outwards (Figure 3-3a). The core-shell NPs were isolated by centrifugation to completely remove the excess PSPAA. To induce 1D assembly, the purified monomer NPs were then re-dispersed in a DMF-water mixture ($V/V = 7:3$).^[41] After adding HCl (5 mM), the mixture was incubated at 70 °C to facilitate the shape transformation of the polymer shells. Though the glass transition temperature of neat polystyrene (PS) is above 100 °C, under our conditions, the PS domains were swollen by DMF. Thus, they are quite plastic even at lower temperatures (50-70 °C). After 2 h, the solution was diluted with a large amount of aq. NaOH to neutralize the acid and remove DMF from the polymer shells. The PSPAA shells solidified after deswelling,^[41] and the products were isolated by centrifugation and characterized by transmission electron microscopy (TEM).

Figure 3-3c and Figure 3-4 showed the resulting product, where most of the

AuNPs were in the form of double-line chains and the excess unreacted monomers. All of them still have polymer shell on their surface. Given their large difference in size and weight, the long chains were easily enriched by differential centrifugation (Figure 3-3d, 3-5).^[38, 42] Most of the chains were very long with typical length of 30-200 NPs, but a few short chains were also observed. Nevertheless, their width was remarkably uniform of almost exactly two NPs. Unlike the typical 1D aggregates of NPs,^[27, 37, 43] branching was rarely observed in our products. The long and wavy chains often contain kinks, probably resulted from their Brownian motion or the collapsing of their 3D conformation upon drying.

Figure 3-4 Large TEM images showing the unpurified double-line chains obtained in $V_{\text{DMF}}: V_{\text{H}_2\text{O}} = 7:3$ and HCl (5 mM), heated at 70 °C for 2 h.

Figure 3-5 Large TEM images showing the purified double-line chains obtained in $V_{\text{DMF}}: V_{\text{H}_2\text{O}} = 7:3$ and HCl (5 mM), heated at 70 °C for 2 h.

The ultralong chains must have been formed by extensive aggregation, which is counterintuitive considering that a lot of monomers still remained. Random and equivalent coupling of monomers would have followed the step-growth mode, but obviously all of the monomers did not participate equally in the aggregation. Previously, we studied the random aggregation of ligand-coated AuNPs, using polymer encapsulation only to preserve the

resulting aggregates after their formation.^[37, 43] In the current study, the monomers already have polymer shells before participating in the aggregation. Because the resulting ultralong chains were unique, it appeared that the polymer shells played important roles. In particular, the conversion of core-shell NPs to long cylindrical aggregates is reminiscent of the transformation of spherical PSPAA micelles to cylindrical ones upon addition of acid.^[44-46] Most importantly, the uniform width of exactly two NPs can hardly be explained by any other mechanism except the transverse confinement provided by the polymer shells.

A prominent feature of the chains was that the AuNPs were in direct contact with each other (Figure 3-3d, inset), which cannot be achieved by simple aggregation of the core-shell NPs. Because the polymer layers between the two cores have to move away, the assembly process must have involved the reorganization of the polymer shells. It should be noted that the volume of the polymer shell on an aggregate is always less than the total volume of the initial shells on the constituent AuNPs (by about 20% for the double-line chains). This excess polymer was found in the form of free (without AuNP) polymer spheres, cylinders and vesicles, which have been removed during the centrifugation step. Figure 3-6 shows the samples before isolation. Thus, the assembly involved two distinct processes: the aggregation of the core-shell NPs and the reorganization of the PSPAA shells.

Figure 3-6 TEM images showing the excess polymer was found in the form of spheres, cylinders and vesicles. These samples were obtained by directly drying the reaction mixture on TEM grid without purification to remove the supernatant.

Actually, the conditions of our experiments have been optimized to facilitate both of the processes. The PAA blocks were protonated at low pH, reducing the surface charges on the monomer NPs. With less charge repulsion among the PSPAA micelles, their aggregation was a favourable process driven by van der

Figure 3-7 TEM image of the AuNP@PSPAA after being treated in the absence of acid, $V_{\text{DMF}}: V_{\text{H}_2\text{O}} = 7:3$, heated at 70 °C for 2 h (a control experiment).

Figure 3-8 TEM image of the AuNP@PSPAA after being treated in $V_{\text{DMF}}: V_{\text{H}_2\text{O}} = 7:3$ and HCl (5 mM), heated at 30 °C for 2 h. The circles show the partially fused small clusters.

Waals and hydrophobic interactions among the PS domains.^[45] The kinetic motion of the NPs at high temperature helped to overcome the mutual charge repulsion and thus, promoted aggregation.^[44, 45, 47] On the other hand, the mobility of the polymer domains was improved at high temperature and also by the swelling of the PS blocks in the DMF-rich solvent. As shown by control

experiments, in the absence of HCl and at 70 °C, the core-shell NPs did not aggregate; their polymer shells mostly remained unchanged (Figure 3-7). Similarly, at 30 °C, the acid only caused slight aggregation of the core-shell NPs (Figure 3-8). In the few aggregates that we observed, the monomers did not fully merge and roughly retained their spherical shape.

3.3.2 Effects of Solvent Quality

Because it was known that solvent quality can greatly influence the mobility as well as the final morphologies of PSPAA micelles,^[44, 45, 48-51] we studied the influence of solvent on the assembly. When the DMF/water ratio was increased from 7:3 to 6:1, the obtained product was mostly ultralong single-line chains (up to 300 NPs in length) in addition to the excess monomers (Figure 3-9a-c). The decrease in chain width was clearly associated with the polymer shells: Because DMF was still the dominant component in the solvent, direct aggregation of NPs cannot explain the dramatic change in products. After the excess monomers were removed, it can be seen in Figure 3-9d-f that the purified product was highly uniform with only few defects in terms of branches and additional AuNPs attached on the sides. Though the long chains stacked across each other in the dried sample, their shells did not fuse together. With further increase of the DMF/water ratio to 9:1, however, the resulting product was messier (Figure 3-10). There was more frequent occurrence of branches

Figure 3-9 TEM images showing the single-line chains obtained in $V_{\text{DMF}}: V_{\text{H}_2\text{O}} = 6:1$ and HCl (5 mM), heated at 70 °C for 2 h: (a-c) before purification and (d-f) after purification.

Figure 3-10 TEM images showing the typical products obtained in $V_{\text{DMF}}: V_{\text{H}_2\text{O}} = 9:1$ and HCl (5 mM), heated at 70 °C for 2 h: (a-c) before purification; (d-f) after purification.

Figure 3-11 TEM images showing the typical products obtained in $V_{\text{DMF}}: V_{\text{H}_2\text{O}} = 2:3$ and HCl (5 mM), heated at 70 °C for 2 h. Very few monomers were found even though the sample has not been purified.

and misplaced AuNPs at the sides. It was also apparent that the crossed chains were able to fuse together, causing the chains to entangle (Figure 3-10). Such entanglement can be more clearly seen in the purified products (Figure 3-10d-f). On the other hand, when the DMF/water ratio was decreased to 2:3, the aggregation changed dramatically to the step-growth mode, as indicated by the

disappearance of monomers. Figure 3-11 was a sample before purification. The products were still chain-like with irregular width of 1-3 NPs, but long chains (> 30 NPs in length) were not found.

3.3.3 Post-Synthetic Transformation

The formation of single-line chains can be understood from the sequential *1-to-n* coupling between a chain and monomers, but the formation of double-line chains is more complex. In particular, the question is whether the transverse confinement provided by polymer shell is a kinetic or thermodynamic factor. That is, does the polymer shell facilitate the end-on aggregation, or does it simply make the double-line chains more stable? To answer this question, we carried out the following set of experiments. Single-line chains were first prepared in $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 6:1$ solution and purified (Figure 3-9). They were then dispersed in $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 7:3$ solution with HCl to favor the double-line chains. After incubation at 70 °C for 1 h, the isolated product showed mostly double-line chains, though sometimes they still had a few remaining single-line sections (Figure 3-12). Thus, the double-line chains were indeed more stable in the $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 7:3$ solution. Even if single-line chains were initially formed during the aggregation, they could be converted to the double-line chains. Further decreasing the $V_{\text{DMF}}/V_{\text{H}_2\text{O}}$ to 3:2, triple-line chains can be also obtained by this method, though they often contained more

Figure 3-12 TEM images showing the double-line chains transformed from the single-line chains (as shown in Figure 3-9) in $V_{\text{DMF}}: V_{\text{H}_2\text{O}} = 7:3$ and HCl (2.5 mM) solution.

Figure 3-13 TEM images showing the triple-line chains transformed from the single-line chains (as shown in Figure 3-9), in $V_{\text{DMF}}: V_{\text{H}_2\text{O}} = 3:2$ and HCl (2.5 mM) solution.

defects, for example, remaining single- and double-line sections (Figure 3-13). Conversely, however, when the double-line chains were first synthesized and then introduced into the conditions favoring single-line chains ($V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 6:1$ with HCl), the latter were not obtained. It appeared that the compression of the chains was easier than their elongation. This result highlighted the

importance of the selectivity in the initial end-on aggregation: If somehow monomers were attached wrongly to the side of the chains, such error cannot be easily fixed due to the difficulty in reorganizing the NPs in the chains.

3.3.4 Surface Ligands

We postulated that the encapsulated ligands would exert influence on the width control. To confirm this assumption, AuNPs functionalized with different hydrophobic ligands (e.g. 2-naphthalenethiol (**2**) and thiol-ended polystyrene (**3**)) were employed as assembly units. Unlike (AuNP-**1**)@PSPAA, mostly of which formed double-line chains in $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 7:3$ solution, whereas (AuNP-**2**)@PSPAA generated single-line chains extended over 2-3 μm in length (Figure 3-14a, b). To our surprise, triple- or multiple-line chains were fabricated when (AuNP-**3**)@PSPAA were used (Figure 3-14c, d). These ligands were encapsulated in PSPAA polymer shell, which provides a dielectric environment. Thus, we concluded that the charge repulsions were not the critical factor. One possible reason is the different swelling abilities of these molecules. Thiol-ended polystyrene is similar in structure to PS block; hence, it has strongest ability in swelling PSPAA under same conditions, compared to another two molecules. As a result, the stretching of the PS chains is greatest in the direction of radius, increasing the effective volume fraction of PS block.^{[52,}

^{53]} This result gave rise to the highest polymer strain, which facilitated

Figure 3-14 TEM images showing (a-b) the single-line chains synthesized using 2-naphthalenethiol-modified AuNPs, (c-d) the triple-line chains synthesized using thiol-ended polystyrene-modified AuNPs, in $V_{\text{DMF}}: V_{\text{H}_2\text{O}} = 7:3$ and HCl (5 mM) solution.

the formation of multiple chains to reduce the stretching penalty.

3.3.5 End-on Growth Mode

Under the typical temperature used (70 °C), the aggregation went too fast for us to isolate the intermediates to confirm the end-on aggregation. Fortunately,

Figure 3-15 TEM images showing the intermediate species trapped by addition of water after the sample was treated at 50 °C in $V_{\text{DMF}}: V_{\text{H}_2\text{O}} = 6:1$ solution.

as we lowered the temperature to 50 °C ($V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 6:1$), we were able to identify quite a number of examples where a monomer just started in fusing with a chain (Figure 3-15). Apparently, the polymer at some newly formed junctions was solidified by deswelling before the monomers had enough time to fully merge with the chains. Among the 323 chains we have surveyed, 146 of them contained gapped intermediates, including 121 cases of end-on *1-to-n*

coupling, 19 cases of end-on *m-to-n* coupling, and 6 cases of side-on *l-to-n* coupling. The assembly errors were obviously more frequent than the typical results obtained at 70 °C (Figure 3-9). Nevertheless, these results indicated that end-on aggregation was a favored process.

We were not able to characterize the initial chain growth by UV-vis absorption due to the following reasons. The chains constituted only a small population in the mixture and long chains only absorb in near-infrared region.^[19] Moreover, with the current ligand, the plasmonic coupling between AuNPs was not strong enough to give prominent absorption peaks.^[38]

Control experiments were carried out to further understand the assembly process. In one set of experiments, we first synthesized double-line chains; without stopping the reaction, excess amount of empty PSPAA micelles were added into the reaction mixture, so that the subsequent aggregation were dominated by the assembly of empty micelles. We found a number of examples where empty polymer cylinders grew on one end of the double-line chains (Figure 3-16). We did not find any example where polymer was grown on both ends of a chain. In another set of experiments, we used large NP@PSPAA (30 or 40 nm AuNPs or 45 nm Ag nanocubes) as seeds to grow chains, so that the starting points could be identified. A small amount of seeds were first dispersed in $V_{\text{DMF}}/V_{\text{H}_2\text{O}} = 7:3$ solution and treated with 2 mM HCl. Under the acidic condition, the polymer shells of the seeds were destabilized but they cannot

Figure 3-16 In a control experiment, empty PSPAA micelles were added to double-line chains after their assembly. The empty PSPAA cylinder grew on only one end of the double-line chains. In a different experiment, when single-line chains were used, the polymer cylinder only grew on one end of the single-line chains.

Figure 3-17 TEM images showing the resulting nanostructures when large NP@PSPAA (30 or 40 nm AuNPs or 45 nm Ag nanocubes) were used as seeds to grow chains. Arrows indicate the large seed NPs.

aggregate effectively due to their low concentration. After 30 min, a large amount of AuNP@PSPAA ($d_{Au} = 16$ nm) monomers were added to initiate the aggregation. The mixture was then heated at 70 °C for 2 h. As shown in Figure 3-17, the large seed NPs often occurred at the end of the resulting double-line chains. It was much rarer for chains to grow on two sides of a seed (Figure 3-17). But even in this case, one side grew much longer than the other, suggesting unequal growth. Some seeds had a few monomers merged to their surface (Figure 3-17), but they did not develop into chains.

3.3.6 Mechanism Analysis

The 1D assembly of AuNP@PSPAA is both thermodynamically and kinetically favored. From the thermodynamic perspective, the morphologies of PSPAA micelles are governed by three major factors: (a) the entropy of the coiled polymer in the PS domain limits the domain depth;^[49, 53] (b) the charge and steric repulsion among PAA blocks favor high surface to volume ratio (S/V),^[44-46] i.e., spheres; and (c) the polymer-solvent interfacial tension favors low S/V ,^[51] i.e., cylinders. Protonating PAA blocks reduces their mutual charge repulsion, favoring cylinders. However, the overall S/V can only be slightly reduced if the aggregated core-shell NPs simply contact each other^[54] (by 0.55-11.5% in Figure 3-15). A more significant driving force can be achieved by fully merging the shells, removing their spherical outline and bringing the

cores closely together. Thus, the thermodynamic stability of polymer cylinders makes it hard to form branches. In forming the double-line chains, the higher water content in the solvent reduces swelling and thus leads to higher polymer-solvent interfacial tension, driving to reduce the S/V of the chains. This small and yet controllable driving force leads to expansion of the chain width.

From the kinetic perspective, the ends of polymer cylinders are more active due to their high curvature,^[55, 56] favoring end-on aggregation. Moreover, both the chain and the monomers are negatively charged because of the PAA blocks on their surface. It is known that, for an incoming monomer, end-on aggregation to a chain is favored because the charge repulsion from the end is smaller than that from the side.^[37] Most importantly, in chain growth mode the *l-to-n* coupling is the dominant process, which is more selective than the *m-to-n* coupling between chains during the later stage of step-growth “polymerization”.

In our system, the fact that a large amount of monomers remained after forming long chains is a strong indication for chain growth mode. The main difference from the step-growth mode is that not all monomers were equally active towards aggregation. There are clear evidences for the unequal activation of the polymer shells: (a) not all monomers participated in the aggregation; (b) the ends have higher activity than the sides, as shown by the intermediates in

Figure 3-15; (c) the empty polymer cylinder only grew on one end of the double-line chain in Figure 3-16; and (d) the chains preferentially grew on one end of the seeds (Figure 3-17).

It is found that unequal activity of the polymer shells was the key to the end-on aggregation. We postulated that the active site consists of irregularly mingled PS and PAA blocks, leading to lower surface charge and higher interfacial energy. After incorporating a monomer in a chain, the polymer blocks on the freshly generated surface are not fully reorganized and thus, the active site is passed on to the newly created end. By this process, the favored end-on aggregation keeps an active end, whereas the rest of the chain surface is deactivated because it does not participate in aggregation and the polymer blocks thereon have fully reorganized.

3.4 Conclusion

In summary, we observed an unprecedented mode of “polymerization” in nanoparticle aggregation. This mode was essential for achieving high selectivity in the assembly of chains, reducing branching and other errors. We were able to obtain single-line chains of 300 nanoparticles in length and double-line chains of 200 nanoparticles. These ultralong chains, with uniform width and interpartical spacing, could be potentially used as plasmonic waveguide and device component.

Before applying in the plasmonic waveguide, we may study the optical resonance depended on different nanoparticle arrangements via single particle spectroscopy.^[57] The double-line chain and single-line chain have different repeat unit, thus, they may display different plasmonic resonance properties. In addition, the ultra-long chains can be used as templates to catalyze the growth of metal oxide or metal arrays.^[58] Alternatively, the magnetic chain can be synthesized via the same chain growth polymerization method, and used as the nanostirbar in microdroplet system. The detail research will be carried out in the future work.

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Chapter 4 Ultrasound-Assisted Synthesis of Nanoparticle Chains and Nanoclusters

Rational control of the size and structure of nanoparticle assemblies would be helpful to the generation of new properties through the coupling of adjacent nanoparticles. Colloidal assembly of nanoparticles presents a great challenge because of the difficulty in programming the behavior of nanoparticles in 3D space. Lithography-based assembly methods have been well developed, but they are time consuming and costly. To solve this dilemma, in this chapter, we developed a nanoscale "top-down" method to fabricate 1D nanoparticle chains and 3D nanoclusters. Specifically, the polymer encapsulated AuNP chains synthesized via a "bottom-up" route were used as basic building blocks. Under the ultrasound irradiation, the scission of PSPAA shells led to the breakage of embedded AuNP chains, as well as the cylinder-to-sphere transformation of PSPAA shells induced the reorganization of AuNP chains into spherical nanoclusters. The influences of ultrasonic energy and time on the final length of the chains were studied comparatively. Moreover, we found that the transformation from nanoparticle chains to nanoclusters is related to the mobility of surface ligands.

4.1 Introduction

Plasmonic properties of metal nanoassemblies offer the advantages in practical applications such as: chemical/biological sensors, plasmonic wavelength, optical fibers, magnetic resonators, surface-enhanced Raman scattering.^[1-5] These properties are highly dependent on the size and structure of nanoassemblies. Thus, precise controlling the size and structure of nanoassemblies is critical for achieving desired properties and functions in nanodevices.

Exploration of assembly strategies has been a hot topic in the recent literature. In principle, colloidal assembly of nanoparticles (NPs) is more scalable and can achieve smaller structures than photolithography-based methods.^[6] One-dimensional (1D) NP chains have been synthesized via organizing the anisotropic ligands on the NPs,^[7-11] or by dipole interactions,^[12-15] or charge repulsions.^[16, 17] However, a general problem is the lack of means to control the random collision of the suspended NPs. Thus, it places challenges in avoiding the formation of high branches which may bring short circuit. Alternatively, NP chains were obtained via the structural transformation of polymer shell from spheres to cylinders.^[18] However, it was difficult to control the length of the chains. Recently, three-dimensional (3D) nanoclusters of close packed NPs as a kind of promising building block have been extensively studied.^[19-23] In most cases, the spherical supercrystals of hundred NPs have

been synthesized. In contrast, the fabrication of small clusters of NPs is particularly difficult, owing to the challenge in balancing the random aggregation and the structural equilibration/stability of nanoclusters.

Ultrasound is a powerful tool in the nanomaterial synthesis,^[24, 25] polymer degradation,^[26-28] cutting carbon nanotube,^[29-31] molecular crystal breakage^[32] and solid modification. The effects of ultrasound arise from the extreme transient conditions, which promotes the formation of "hot spots" storing high temperature and high pressure. Obviously, ultrasound provides unique synthetic conditions compared with conventional wet chemistry. Thus, exploring the interaction between ultrasound and colloidal assembly would offer the advantages in developing new synthetic methodologies.

Random aggregation usually occurs in direct assembly of NPs in a colloid, giving rise to branches and irregulars. Hence, precise controlling the size and structure of nanoassemblies is difficult. In this chapter, we report the use of ultrasound to assist the synthesis of NP chains and nanoclusters (Figure 4-1a). The polystyrene-*b*-poly(acrylic acid) (PSPAA) encapsulated ultralong NP chains were firstly prepared and used as the building blocks, which thus were suffered from shear stress arising from ultrasound. Induced by the scission or the cylinder-to-sphere transformation of polymer shells, the embedded ultralong NP chains were forced to break into short ones or form nanoclusters. We found that the intensity of ultrasound and surface ligands play important roles in

controlling the size and structure of nanoassemblies.

4.2 Materials and Methods

4.2.1 Materials

All chemical reagents were used as purchased without further purification. Hydrogen tetrachloroaurate(III) hydrate, 99.9% (metal basis Au 49%) was purchased from Alfa Aesar; Amphiphilic diblock copolymer polystyrene-*block*-poly(acrylic acid) (PS₁₅₄-*b*-PAA₄₉, Mn = 16000 for the polystyrene block and Mn = 3500 for the poly(acrylic acid) block, Mw/Mn = 1.15) and thiol-terminated polystyrene (PS-SH, Mn = 2000) were purchased from Polymer Source, Inc. and SRI Biomaterials; 2-naphthalenethiol was purchased from Sigma Aldrich. Hydrochloric acid (HCl) was purchased from P. P. Chemicals Sdn Bhd. Sodium hydroxide was purchased from Sinopharm Chemical Reagent Co. Ltd; Deionized water (resistance > 18.2 MΩ/cm) was used in all reactions. All other chemicals were purchased from Sigma Aldrich. Copper specimen grids (300 mesh) with formvar/carbon support film were purchased from Beijing XXBR Technology Co. (NH₄)₆Mo₇O₂₄ was used as negative stain (3.4 mM) in all TEM images reported in this study.

4.2.2 Methods

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

collected from a Cary 100 UV-vis spectrophotometer.

The methods of encapsulation of AuNPs with PSPAA shells and assembly of single-line chains refer 3.2.2.^[33, 34]

Sonofragmentation of single-line chains. All sonication processes were carried out using Sonics Vibra CellTM Ultrasonic processor (130 W, 20 KHz). The purified single-line chains were dispersed into NaOH (4 mL, 0.1 mM) solution, and then the mixture was sonicated with the tip sonicator in an ice bath. The diameter of the tip is 3 mm. Various ultrasound amplitudes of 20%, 40%, 60%, 80% and 100% as well as different durations of 10, 20, 30, 40, 60, 90, and 120 min were applied. The use of NaOH can supply sufficiently strong charge repulsion to avoid the aggregation of nanoassemblies.

4.3 Results and Discussion

The single-line chains were synthesized as previously reported.^[18] The as-synthesized AuNP@PSPAA (surface ligand: PS-SH) were redispersed in a DMF-water mixture (V/V = 6:1). By introducing HCl (5 mM) and elevating the temperature, single-line chains were obtained via the structural transformation of PSPAA shells from spheres to cylinders. This propensity of polymer shells guided the aggregation of NPs follow a chain-growth polymerization mode, thus, reducing the branches and irregulars (Figure 4-1b). To investigate the respond of NP chains to the ultrasound irradiation, the purified single-line

chains were dispersed into NaOH (0.1 mM) solution, and then sonicated under a series of sonication amplitudes in an ice bath. The NaOH solution was used to increase the charge repulsion and minimize the aggregation of nanoassemblies during the sonication process. Finally, the products were readily purified by centrifugation and further characterized by transmission electron microscopy (TEM).

Figure 4-1c showed the resulting products, where the ultralong chains were cut into short ones, upon suffering from the shear stress induced by ultrasound. All of them still have polymer shell on their surface. Apparently, the elongate polymer at some breaks was retained by withdrawing the ultrasound before it had enough time to fully break off. A prominent feature of the breaks was that the interspacing between two AuNPs was increased. Thus, the scission process must have involved the stretch of polymer shells. At the end of some chains, the broken polymer was solidified before reassembling. These results indicate the scission of polymer shell induced the formation of short chains. Though the PSPAA micelles are kinetically frozen with low liquidity in the aqueous solution, the ultrasound provides sufficient drag force to section the PSPAA shells into short pieces.

4.3.1 Synthesis of Nanostructures via Varying Ultrasonic Energy

Because the scission of polymer shells is essentially governed by the

ultrasonic intensity, we studied the influence of ultrasonic power and time on the control of the size of NP chains. When the amplitude of ultrasound is 20%, the obtained product was mostly short single-line chains (Figure 4-3a, c, and e). The decrease in chain length was clearly with increasing sonication time. As shown in Figure 4-2, the as-synthesized single-line chain has a wide size distribution from 10 to 300 NPs in length. After sonication, the size distribution clearly narrows down (Figure 4-3b, d, and f). It is found that the length of chains sharply decreased in the initial stage, and the change became smooth, where the average length of chains decreased only from 11 NPs at 10 min to 7 NPs after 120 min. It is because the scission of short chains is more difficult, unless stronger shear force is applied. Doubling the amplitude, the same changes were observed (Figure 4-4). The average length of NP chains decreased from 8 NPs at 10 min to 5 NPs after 120 min. It is found that the decrease in length was associated with the ultrasonic energy (Table 4-1), that is a higher energy leads to a higher breakage rate. With further increase of the amplitude to 60%, however, the resulting products were complex. After 10 min, most products were in the form of short chains, and the average length was about 7 NPs (Figure 4-5a, b). Though the NP chains could further fracture with extending the ultrasound irradiation (30 and 60 min), there was more frequent occurrence of nanoclusters (Figure 4-5c-e). It was apparent that the straight chains were bended and the coiled chains were able to fuse together. After 120

min, the polymer shells could not maintain the cylindrical structure and tended to transform into spherical structure (Figure 4-5f), leading the rearrangement of embedded AuNPs. It seems that the high ultrasonic energy induced both the scission of the PSPAA shells and the reassembly of the PSPAA shells.

Figure 4-1 (a) Schematic illustrating the ultrasound-assisted scission of 1D nanoparticle chains and formation of 3D nanoclusters; TEM images of (b, c) single-line chains before (b) and after (c) ultrasonic treatment.

Figure 4-2 Size distribution of the single-line chains before ultrasonic treatment.

As discussed above, the polymer shells of NP chains exhibited eccentric behavior when suffering from high ultrasonic energy. To further understand this process, we increased the amplitude to 80%. Interestingly, the 1D NP chains changed dramatically to 3D nanoclusters (Figure 4-6c) after 30 min. Obviously, induced by the structural transformation of polymer shells from cylinders to spheres, the AuNPs chains were forced to organize into nanoclusters with close packing. The average thickness of the PSPAA shells is about 25 nm, which is larger than the normal thickness in single-encapsulated AuNP (10-16 nm). As

Figure 4-3 (a, c, and e) TEM images of the short chains synthesized via ultrasonic treatment for 10 min, 30 min, and 120 min, respectively. The ultrasonic amplitude is 20%. (b, d, and f) Corresponding size distributions of the short chains in a, c, e, respectively.

Figure 4-4 (a, c, e, and g) TEM images of the short chains synthesized via ultrasonic irradiation for 10 min, 30 min, 60 min and 120 min, respectively. The ultrasonic amplitude is 40%. (b, d, f, and h) Corresponding size distributions of the short chains in a, c, e, and g, respectively.

Figure 4-5 (a, c, e, and f) TEM images of the short chains synthesized via ultrasonic irradiation for 10 min, 30 min, 60 min and 120 min, respectively. The ultrasonic amplitude is 60%. (b, d) Corresponding size distributions of the short chains in a and c, respectively.

Table 4-1. Summary of the average length of the short chains synthesized under different conditions.

	average length 10 min	average length 30 min	average length 120 min
amplitude 20%	11 NPs	9 NPs	7 NPs
amplitude 40%	8.5 NPs	7 NPs	5 NPs
amplitude 60%	7 NPs	6 NPs	--

shown in Figure 4-6d, no obvious changes of the nanoclusters could be discerned from 30 to 120 min.

The question is whether the formation of nanoclusters originates from the cylinder-to-sphere transformation of the ultralong chains, or the short chains. That is, does the formation of nanoclusters involve one process or two processes? To answer this question, we trapped the intermediates at 5 min and 20 min, respectively. Initially, short chains and clusters were found (Figure 4-6a). Upon further ultrasound irradiation, most of the short chains were changed into nanoclusters (Figure 4-6b). On the basis of the observed intermediates, it appears that the ultralong chains were sectioned into short ones before completely transform into nanoclusters. Thus, the formation of spherical nanoclusters involves two distinct processes: the scission and the cylinder-to-sphere transformation of the PSPAA shells. A higher ultrasound

Figure 4-6 TEM images of the resulting products synthesized via ultrasonic irradiation for (a) 5 min, (b) 20 min, (c) 30 min, and (d) 120 min, respectively. The ultrasonic amplitude is 80%.

energy (amplitude: 80%) offers stronger drag force and higher temperature. The former promotes the rapid cleavage of the cylindrical polymer shells into short pieces. The latter improves the mobility of the polymer domain, facilitating the structural transformation.

4.3.2 The Influence of Surface Ligands on the Formation of Nanoclusters

As discussed in our previous work, the surface ligands played an important role in swelling of the PSPAA shells. It is found that the 2-naphthalenethiol-modified AuNPs tend to form rigid aggregation,^[16, 35] while the PS-stabilized AuNPs can move pass each other. To further understand the effects of ligand mobility, the ligand 2-naphthalenethiol was used to functionalize the AuNPs, and then the PSPAA self-assembly around the AuNPs, forming core-shell structures. To induce 1D assembly, the purified AuNP@PSPAA incubated in a DMF: H₂O = 4:1 solution. The addition of HCl promoted the formation of single-line chains (Figure 4-7a). Figure 4-7b shows the UV-vis spectrum of the single-line chains. A new absorption peak at 635 nm was observed, corresponding to the longitudinal plasmon absorption of linear AuNP aggregates. Finally, the responds of the single-line chains to the shear stress were carried out via imposing different ultrasonic amplitudes.

Figure 4-8a, c and e show the resulting products when 20% amplitude was imposed. The single-line chains were sectioned into short ones, the length of which is decreased with time, from 8 NPs at 20 min to 7.5 NPs after 60 min (Figure 4-8b, d, and f). When the amplitude is 40% or 60%, the long NP chains were still cut into short chains after 60 min (Figure 4-9a, b). Interestingly, when the amplitude was adjusted to 80%, most of the resulting products were in the

Figure 4-7 (a) TEM images of the single-line chains synthesized via the cylinder-to-sphere transformation of PSPAA shells, where the AuNPs were functionalized with 2-naphthalenethiol. (b) UV-vis spectra of the AuNP@PSPAA and single-line chain, respectively.

form of short chains even after 60 min. This result is significantly different from the above case, where NP chains fully transformed into 3D nanoclusters in 30 min (Figure 4-6). The short chains could be retained even when the amplitude increased to 100% (Figure 4-9d). The only difference between the two cases is the surface ligand, thus, it is believed the surface ligand is a critical factor on the structural transformation of PSPAA shells from cylinders to spheres.

4.3.3 Discussion

The scission of the ultralong chains into short pieces is a consequence of the ultrasound.^[24, 25] In the liquid solution, the ultrasonic waves can induce the nucleation and growth of the bubble. The collapse of the bubble leads to two

Figure 4-8 (a, c, e) TEM images of the short chains synthesized via ultrasonic irradiation for 20 min, 40 min, and 60 min, respectively. The ultrasonic amplitude is 20% and the AuNPs were functionalized with 2-naphthalenethiol. (b, d, f) Corresponding size distributions of the short chains in a, c, and e, respectively.

Figure 4-9 TEM images of the short chains synthesized via ultrasonic irradiation for 60 min. The ultrasonic amplitude is (a) 40%, (b) 60%, (c) 80% and (d) 100%, respectively.

results: creating high pressure and temperature, and generating a strong hydrodynamic flow field in its vicinity. The high temperature enhances the mobility of polymer domain, and the strong flow results in the occurrence of drag forces,^[27, 36] which exert on the NP chains. When the drag force exceeds the tensile strength of the PSPAA shells, the breakage occurs. As a result, the

ultralong AuNP chains were sectioned into short ones.

In single-component system, such as carbon nanotube or pure cylindrical polymer, increasing ultrasonic power can accelerate their scission.^[36] However, in our core-shell system, the scission appeared to be more complex under a higher ultrasonic power (80% amplitude). It is found that the PS-stabilized AuNP chains transformed into spherical nanoclusters, while the 2-naphthalenethiol-stabilized AuNP chains were only cut into short chains. We postulated it is due to the mobility of the surface ligand. Thiol-terminated polystyrene has the similar structure as the polystyrene block, thus, it can distort or stretch or move in the PSPAA shells. Under a higher ultrasonic power, the mobility of both the surface ligand and the polymer increased, promoting the structural transformation of PSPAA shells as well as the reassembly of AuNPs. Thus, 1D NP chains could readily be converted into 3D nanoclusters. In contrast, 2-naphthalenethiol is more rigid because of the short chain, which results in a low mobility in PSPAA shells. It is conceivable the AuNPs of the chains are tightly locked with each other via the 2-naphthalenethiol, and the strong interaction is sufficient to balance the compressive force arising from the structural transformation of PSPAA shell. As a result, the short chains were obtained.

4.4 Conclusion

We developed a novel method employing the ultrasound to synthesize 1D nanoparticles and 3D nanoclusters. Different from the conventional "bottom-up" method via the assembly of AuNPs, the PSPAA encapsulated single-line chains were used as the building blocks; it can be regarded as nanoscale "top-down" fabrication procedure. Under the ultrasonic irradiation, the scission of polymer shells resulted in the formation of short chains. The size distribution could be controlled by the ultrasonic energy. Increasing the ultrasonic power realized the 1D nanoparticle chains transformed into 3D nanoclusters. We postulated that the ability of surface ligand to move or stretch in the PSPAA shells allows the 1D-to-3D transformation. Exploration in the ultrasound-assisted synthetic methods could create more complex and diverse nanostructures for use in nanodevices.

4.5 Bibliography

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Chapter 5 Controlling Nanostructures via Emulsion Polymerization in the Polystyrene-*b*-Poly(acrylic acid) Micelles

In this chapter, we developed a method to synthesize anisotropic nanocomposites by breaking the symmetry of polymer shells of AuNP@PSPAA. In an emulsion solution, by controlling the swelling of PSPAA shells, the spherical AuNP@PSPAA evolved into anisotropic ones as a consequence of the phase separation. The role of shear force arising from stirring was studied in detail.

5.1 Introduction

Anisotropic nanoparticles (NPs) possess peculiar electronic and optical properties, due to their unusual shapes or interaction patchiness. Assembly of anisotropic NPs into superlattice or hierarchical structures can generate unique size- and shape-dependent properties, which in turn makes them promising building blocks for photonic nanodevices and molecular recognition sensors.^[1-6] Thus, rational and controllable synthesis of anisotropic NPs is a fundamental and essential step for developing sophisticated nanodevices.

Great efforts have been devoted to synthesize different types of anisotropic NPs. In the colloidal solution, by controlling the surface facet-specific growth, a variety of nanocrystals such as cube, rods, icosahedra, and tetrahedra were obtained, which can be categorized as single-component anisotropic NPs.^[7-13] For two-component nanocomposites, anisotropic NPs could be synthesized by tuning the interfacial energy between two materials. For example, Janus particles of metal-metal, or metal-polymer, non-centrosymmetric particles with two dissimilar sides,^[14-17] were readily synthesized by adjusting the ligand density,^[18] the hydrophilicity/hydrophobicity of surface ligands,^[19, 20] or manipulating the reduction kinetic of one component.^[21, 22] In the polymer NP system, the seeded emulsion polymerization method was employed to create diverse NP shapes such as dumbbell, triangle, cone, snowman and diamond.^[23-28] Typically, this method involves two processes: swelling of seed particles and phase separation. The latter process is the determining factor for the structural diversity of NPs. However, it remains a challenge to apply this method in structural controls of small NPs (< 100 nm). The key is to understand the phase separation and polymerization occurred at nanoscale.

As discussed in the Chapter 2 and Chapter 3, core-shell NPs possess unique ability in designing complex nanostructures and studying the kinetics of molecular diffusion through the polymer shell.^[29-33] Herein, we demonstrate a facile and scalable route to fabricate nanoscale fish-shaped NPs in a colloid

using AuNP@PSPAA (PSPAA: polystyrene-*b*-poly(acrylic acid) as basic reaction unit. The multi-generation polymerization of divinylbenzene (DVB) confined inside PSPAA shells plays an important role in promoting the anisotropic growth of core-shell NPs. By controlling the shear condition, and varying the size and internal packing structure of core NPs, we are able to synthesize "cape-shaped", "pinecone fish-shaped", "cuttlefish-shaped", and "octopus-shaped" NPs (Figure 5-1), which thus enrich the structural complexity and diversity of anisotropic NPs. In addition, the PSPAA shells could tolerate saturated salt solution in a NaOH (pH = 10) solution, avoiding prior surface modification with surfactants as well as preventing the aggregation of colloidal NPs.

5.2 Materials and Methods

5.2.1 Materials

Refer 2.3.1.

5.2.2 Methods

Synthesis of AuNP@PSPAA. The method used here was based on our previous report on single-encapsulation of gold nanoparticles with minor modifications.^[34] Two 1.5 mL tubes of AuNPs solution ($d_{\text{Au}} = 15$ nm) were concentrated to ~20 μL by centrifugation at 16000 g for 15 min. The deep red solution collected at the bottom of the eppendorf tubes was diluted with H_2O

(172 μL). Then the mixture was added into 808 μL of polymer solution which was prepared by mixing 728 μL DMF with PS₁₅₄-b-PAA₆₀ in DMF (80 μL , 8 mg/mL). Finally, ligand 1, 2-dipalmitoyl-sn-glycero-3-phosphothioethanol in EtOH (40 μL , 2 mg/mL) was added to the mixture. The mixture was heated at 110 °C for 2 h and then allowed to slowly cool down till room temperature.

The same method was used to encapsulate the 40 nm and 80 nm AuNPs.

Using 2-naphthalenethiol as ligand, the deep red suspension collected at the bottom of eppendorf tube was dispersed into 718 μL DMF, and the ligand 2-naphthalenethiol in DMF (20 μL , 3 mg/mL) was added. The mixture was then incubated at 60 °C for 2 h before 80 μL PS₁₅₄-b-PAA₆₀ (8 mg/mL in DMF) and 172 μL H₂O were added in sequence. The mixture was further heated to 110 °C for 2 h and then allowed to slowly cool down in the oil bath till room temperature.^[35]

Synthesis of fish-shaped AuNP@PSPAA via a seed polymerization

method. In the first growth cycle, 400 μL purified AuNP@PSPAA were concentrated to ~15 μL by centrifugation at 16000 g for 25 min, and dispersed into NaOH (0.1 mM) solution. The solution degassed with nitrogen for 10 min, and then 10 μL divinylbenzene (DVB) was added and the mixture was stirred for 30 min at room temperature. Finally, the temperature of solution was raised to 70 °C, and 5 μL ammonium persulfate (APS) (1 mg/10 μL) was injected to initiate the polymerization. Following a time of 1 h, the solution was allowed to

cool down till room temperature. Similar procedures were used for larger seed particles (40 nm and 80 nm AuNPs).

The products of 1st growth cycle, after being isolated and concentrated by centrifugation, could be used as seeds for multi-generation growth. Following the same seeded polymerization process, different shapes of anisotropic AuNP@PSPAA were synthesized.

Stability of the fish-shaped AuNP@PSPAA. To investigate the degree of the cross-link of anisotropic nanoparticles via multi-generation growth, 200 μL fish-shaped AuNP@PSPAA were purified in NaOH (0.1 mM) twice to completely remove the DVB droplets, and dispersed into 1000 μL DMF solution. The mixture was then heated at 80 $^{\circ}\text{C}$ for 4 h and then cooled to room temperature.

5.3 Results and Discussion

5.3.1 Synthesis of Fish-Shaped AuNP@PSPAA

The seeds AuNP@PSPAA ($d_{\text{Au}} = 15 \text{ nm}$) were prepared by previously reported method.^[34] To initiate polymer assembly, citrate-stabilized AuNPs were modified with a hydrophobic ligand, 1,2-dipalmitoyl-sn-glycero-3-phosphothioethanol. The polystyrene (PS) blocks self-assembled around the NP surface and formed uniform shell, leaving the poly(acrylic acid) (PAA) blocks dissolve in water. The AuNP@PSPAA seeds

have highly spherical symmetry (Figure 5-2a), and the uncross-linked polymer shells on AuNPs are similar in nature to empty micelles of PSPAA, the hydrophobic domain (PS) of which have been demonstrated to incorporate hydrophobic molecules such as pyrene, and 1,2-dichlorobenzene.^[30, 31] Thus, it is believed that the DVB molecules

Figure 5-1 (a) Schematics showing the fabrication of "nanofish-shaped" AuNP@polymer composites through a seeded emulsion polymerization under different shear conditions; (b-e) TEM images of the typical morphologies: "carp-shaped", "pinecone fish-shaped", "octopus-shaped", and "cuttlefish-shaped" NPs, respectively.

could be trapped inside in the PSPAA shells. The core-shell NPs were purified by centrifugation to remove the excess reagents (e.g., empty micelle and DMF). To engineer the core-shell NPs into anisotropic ones, the purified AuNP@PSPAA were dispersed into NaOH (0.1 mM), where PAA blocks were fully deprotonated that provides sufficient surface charge to minimize aggregation. After adding DVB, the mixture was stirred at room temperature for 30 min, where the swollen PSPAA shells facilitated the absorbing of DVB in the PS domain. In the presence of APS, the swollen particles were then heated to initiate the polymerization inside the polymer network under different shear conditions; it generated anisotropic particles with different morphologies (Figure 5-1). Interestingly, each Au core is eccentrically positioned inside the polymer shell. The final product were solidified by NaOH (0.1 mM) solution, and isolated by centrifugation for characterization.

By introducing core-shell NP seeds, different NPs with uniform size were easily synthesized by mixing DVB and APS under different polymerization conditions. If stirring took place in the polymerization process, the AuNP@PSPAA perfectly retained their spherical shape (generation 1, G1), while the Au cores moved away from the center of each particle (Figure 5-2). The formation of the eccentric cores is due to the phase separation occurred in linear polymer: the polymer chains were stretched when swelled by DVB, and

then the polymer chains relaxed upon raising the temperature, causing the seed particle shrink and leaving a bulge attach to the seed particles. It is found that the thickness of the polymer shell gradually increased with increasing the amount of DVB, ranging from 13 nm to 28 nm (Figure 5-2a-d, and 5-3a). If polymerization occurred in absence of stirring, the phase separation led to the spherical symmetry of PSPAA being broken with a tail attached to the seed NPs. Therefore, Au cores were eccentrically positioned at the opposite direction ("carp-shaped" NPs; Figure 5-2f-h, and 5-3c). Increasing the amount of the DVB, the size of the seeds and the length of the tails were increased. At low concentration of DVB (5 μ L), the phase separation was negligible so that the "carp-shaped" NPs did not appear (Figure 5-2e).

Increasing the concentration of DVB to high levels (30 and 40 μ L) led to the intense phase separation; the number of tails per NP significantly increased, but one tail grew longer than the other, indicating uneven growth (Figure 5-2g, h). A notable difference between the stirring-involved and the stirring-absent polymerization processes is the appearance of polymeric tails. This observation indicates that the stirring induced shearing force plays an important role in controlling the anisotropic growth of AuNP@PSPAA. The growth mechanism is further investigated in the following parts.

5.3.2 Multi-Generation Growth under Different Shear Conditions

To confirm this hypothesis on shearing force induced morphological evolution, the G1 NPs (Figure 5-2) were purified and used as seeds for 2nd- and 3rd-generation growth under identical conditions. Under stirring, the spherical G1 NPs (Figure 5-3a) significantly grew into "pinecone fish-shaped" NPs (G2; Figure 5-3b). The polymer shells increased in size but maintained a smooth surface, and the Au cores were still eccentrically located inside the polymer shell. This disruption of spherical symmetry of polymer shell further indicates the occurrence of phase separation. After the 3rd generation growth, the G2 NPs continually grew up to form "giant salamander-shaped" NPs, possessing fat bodies and smooth surfaces (Figure 5-3c). As shown in Figure 5-3c, the G3 NPs consist of two polymeric parts, where the new generated polymer often occurs at the end of the resulting G2 NPs. It indicates the anisotropic growth of polymer started only from one direction of a seed and the multi-generation growth brought intense phase separation. Without stirring, the G2 and G3 NPs kept the "carp-shaped" morphology and showed elongated tails (from 78 nm to 134 nm, and 156 nm; Figure 5-3d-f). Obviously, the tail of G2 NPs contains two segments and that of G3 NPs with three segments. Interestingly, the thickness of each segment roughly decreased in order. This unconventional growth indicates the end of the tail is more active than other

Figure 5-2 TEM images of (a) AuNP@PSPAA seeds; (b-d) G1 NP composites synthesized under stirring, by changing DVB 15 μL , 30 μL , and 40 μL , respectively; (e-h) G1 anisotropic composites synthesized without stirring, by changing DVB 5 μL , 15 μL , 30 μL , and 40 μL , respectively.

parts. Even though two tails simultaneously on a seed appeared in the 2nd and 3rd growth step, one tails grew much longer than the other, suggesting unequal activation and one direction growth. Meanwhile, the heads of G2 and G3 NPs increased in size while the surfaces of them are rougher than those synthesized via stirring.

In both the two cases, the anisotropic growth initiated at one direction of the seeds, and the active sites could propagate towards newly formed rear parts of the "nanofishs" during the multi-generation growth, keeping the elongation of the tails. The shear conditions control the swelling degree of polymer shell, thus affecting the size of the new generated polymer shells and leading to various morphologies. Under stirring, large and uniform polymer shells were generated, while small and non-uniform ones were always obtained in absence of stirring.

To further understand the role of the shear conditions, we carried out another set of control experiments. When using "pinecone fish-shaped" NPs (G2, Figure 5-3b) as seeds and shielding the stirring force in the 3rd generation growth, the G2 NPs grew larger in size but retained the "pinecone fish" shape (Figure 5-4a). However, owing to the phase separation, a small tail was formed on some NPs, locating at the opposite direction of the Au core. The results provided evidence that unidirectional phase separation occurred. Compared to G3 NPs (Figure 5-3c) synthesized under stirring, the "pinecone fish-shaped" NPs have rough surface, which looks like the scale structure of fish. Conversely, when using

Figure 5-3 (a-c) TEM images of G1, G2 and G3 "nanofish-shaped" NPs synthesized under stirring and using 10 μ L DVB, respectively; (d-f) TEM images of G1, G2 and G3 "carp-shaped" NPs synthesized without stirring and using 10 μ L DVB, respectively.

Figure 5-4 (a) TEM image of G3 "pinecone fish-shaped" NPs with scale structure, which was synthesized without stirring and using G2 "pinecone fish-shaped" NPs (Figure 5-3b) as seeds. The ring indicates the small tails. (b) TEM image of G3 anisotropic NPs synthesized under stirring and using G2 "carp-shaped" NPs (Figure 5-3e) as seeds.

“carp-shaped” NPs (G2, Figure 5-3e) as seeds and imposing a stirring force in the 3rd generation growth, we found an additional and large elliptical bulge attached to the original G2 NPs (Figure 5-4b). In this case, some of the G2 NPs did not form the polymer bulges.

5.3.3 Effects of the Core Nanoparticles

To study the effect of core NPs on the anisotropic growth, we changed the internal packing of cores, where dimers of AuNP@PSPAA ($d_{Au} = 15$ nm) were used as seeds. Figure 5-5 illustrates the final structures of colloidal composites after the 2nd generation growth. Stirring the system during the polymerization process, the structure of the dimers changed from sphere to "pinecone fish" shape, and the two "eyes" lied in eccentric positions (Figure 5-5a); while the colloidal composites presented "carp" shape with tails when synthesis conducted without stirring, and the length of tails could be controlled by multi-generation growth (Figure 5-5b). The results are similar to those of the AuNP@PSPAA monomers as seeds, indicating that the packing structures of core particles have no significant effect in controlling the anisotropic growth of colloidal composites, in particular, when sizes of AuNPs are same.

Because the size of the core NPs is critical for the phase separation confined inside the PSPAA shell, we used large NP@PSPAA (40 nm, or 80 nm AuNPs) as seeds to gain insight into the growth mechanism. Under stirring condition,

the behavior of the phase separation shows no significant difference, following one direction growth as above. After the 2nd generation growth, it is observed that the isotropic NPs transformed into large anisotropic one. In this case, the phase separation is conspicuous and stirring promotes the diffusion of free radical and DVB. Thus large bulges were attached on the original nanoparticles and generated "cuttlefish-shape" NPs (Figure 5-6a, b, 5-7a, b). Surprisingly, withdrawing the stirring force, the phase separation occurred in large seed of AuNP@PSAA did not only follow one direction. As shown in Figure 5-6c, more than one small polymeric tails were found attached to some AuNP@PSAA (40 nm) after the 1st generation growth. Upon further growth, the attached polymeric tails grew larger and longer (Figure 5-6d). Further increasing the size of the seeds (80 nm), the spherical NPs finally evolved into "octopus-shaped" NPs with three or four tails (Figure 5-7c, d). On the basis of the observed products, it appears that the phase separation was initiated at a few sites on a large seed. The fact is that the interfacial energy between particles and aqueous solution decreases when the sizes of particles increase; it results in the increase of the degree of phase separation.

Figure 5-5 (a) TEM image of G2 "pinecone fish-shaped" NPs with two eyes, which were synthesized under stirring; (b) TEM image of G2 "carp-shaped" NPs with two eyes, which were synthesized without stirring. The two cases used dimers of AuNP@PSPAA as seeds.

Figure 5-6 (a-b) TEM images of G2 eccentric NPs and G3 "cuttlefish-shaped" NPs, respectively, which were synthesized under stirring; (c-d) TEM images of G2 and G3 "nanofish-shaped" NPs, respectively, which were synthesized without stirring. The two cases used 40 nm AuNP@PSPAA as seeds.

Figure 5-7 (a-b) TEM images of G2 eccentric NPs and G3 "cuttlefish-shaped" NPs, respectively, which were synthesized under stirring; (c-d) TEM images of G2 and G3 "octopus-shaped" NPs, respectively, which were synthesized without stirring. The two cases used 80 nm AuNP@PSPAA as seeds.

5.3.4 Effects of the Surface Ligands

It is clear that shear condition and core NPs play important roles in the formation of anisotropic NPs, but in addition to, the surface ligands could affect

the phase separation inside the polymer shells. To confirm this assumption, we chose 2-naphthalenethiol-stabilized AuNPs as seeds and studied the growth

Figure 5-8 To investigate the effect of surface ligands in anisotropic growth, 2-naphthalenethiol was employed to modify AuNPs. (a-b) TEM images of G1 and G2 NPs synthesized without stirring, respectively, where 2-naphthalenethiol-stabilized AuNP@PSPAA were directly used as seeds; (c-d) TEM images of G1 and G2 NPs synthesized without stirring, respectively, where the 2-naphthalenethiol-stabilized AuNP@PSPAA were incubated in empty PSPAA micelle solution for 24 h and then used as seeds.

without stirring. All the seeds increased in size but few have the tails after the 1st generation growth (Figure 5-8a). Even after further growth cycle, most NPs maintained the spherical structures, and a few NPs form "dumbbell" shape (Figure 5-8b), while the "carp-shaped" NPs did not appear. It indicates the surface ligands play an important role in the phase separation. To eliminate the influence of the unbonded ligands locating in the PSPAA shells, the AuNP@PSPAA seeds were dispersed into empty PSPAA micelle solution, and incubated at room temperature for 24 h to remove the unbonded ligands. The AuNP@PSPAA were then purified and used as seeds for multi-generation growth. More eccentric AuNP@PSPAA morphologies took place: some seeds had a large polymer bulge attached to their surface, forming the "giant salamander-shaped" NPs (Figure 5-8c, d). Some still maintained their spherical structures. In this case, the behavior of phase separation is similar to that occurred under stirring. The underlying mechanism of this eccentric behavior is still under investigation.

5.3.5 Mechanism Study

The fabrication of anisotropic NPs involves two steps: seeds swelling and polymerization. Withdrawing and imposing the stirring fore, the Au core is eccentrically positioned in the polymer shells, which is ascribed to phase separation between the seed and DVB monomers. From the thermodynamic

perspective, the phase separation favors minimizing the free energy of the whole system.^[27, 36] By introducing excessive DVB molecules, the PSPAA shells were swollen toward an equilibrium size. Owing to the interfacial tension, the swollen shells tend to contract and reduce the surface area, inducing eccentric cores. Upon elevating the polymerization temperature, the phase separation intensively occurred as a result of increased elastic stress.

From the kinetic perspective, the diffusion rates of monomers and free radicals play an important role in controlling the formation of the polymeric bulge. In our system, we found that the stirring force affects the diffusion rates of materials and gives different morphologies. In the emulsion polymerization, the polymerization mainly occurs inside of the polymer shells (e.g., PSPAA). However, the hydrophilic free radical (e.g., the thermal decomposition of water-soluble APS) cannot directly diffuse into the hydrophobic domain. Therefore, the hydrophilic free radicals grow to hydrophobic oligomeric ones, and then diffuse and initiate the polymerization.^[37] In the allocated time, stirring provides sufficient force to promote DVB and oligomeric free radicals diffuse into the PSPAA shell. Thus, the large polymer bulge formed attached to the seeds. However, more free radicals generate the lower-polymerized shells, which are easily dissociated in organic solvent. Control experiments showed that the polymer shells of the "pinecone fish-shaped" and "giant salamander-shaped" NPs could be dissolved away in a hot DMF solution,

Figure 5-9 TEM images of AuNP@polymer composites after dissolving in a hot DMF solution: (a) the polymer shell of the "pinecone fish-shaped" NPs were dissolved in the DMF, causing the aggregation of AuNPs; (b) the "carp-shaped" NPs were stable in the DMF and remained intact.

inducing the aggregation of AuNPs (Figure 5-9a). Conversely, shielding the stirring force limits the transportation of the free radicals and monomers through aqueous solution, thus, small polymer bulges generated. It is found that the polymer shells have high degree of cross-linking. For example: the "carp-shaped" NPs were stable in a hot DMF solution and remained intact (Figure 5-9b). The DVB molecule contains two C=C double bonds which could copolymerize with its monomers. As a result, cross-linked polymer network forms and intersects with original PSPAA polymer. This net-like structure enhances the stability of polymer shell when exposing to organic solvent.

5.4 Conclusion

In summary, we have developed a versatile polymerization process to synthesize anisotropic particles using core-shell nanoparticles as the basic build blocks. By controlling the diffusion rate of free radical into a confined space to tuning the swelling of PSPAA shells, various "nanofish-shaped" colloidal composites were readily fabricated. The proposed method, namely multi-generation growth, offers a new platform for the formation of anisotropic nanocomposites. The present method could be applied to fabricate more complex and anisotropic particles containing various inorganic cores, which thus enriches the diversity of nanocomposite for potential applications.

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Chapter 6 Summary and Outlook

6.1 Summary

Building tailored nanostructures with multi-functional properties is of great importance. Therefore, effective and rational synthesis strategies are required to meet the ever-increasing demands in nanoscience and nanotechnology. Core-shell nanoparticles as a basic multi-component have been well developed and investigated. The two components possess distinctive properties, thus, both the speciality of individual component and the synergistic effects or the mutual interaction of the two components can be exploited for creating more complex and fundamental study.

In this thesis, two strategies were designed: (1) using core-shell motif for diffusion kinetics; and (2) amphiphilic block copolymer assisted self-assembly. On the basis of these methods, short-distance drug release mechanism in the presence of nanoacceptors was studied. Then we synthesized anisotropic colloidal composites by a seeded emulsion method: from spherical core-shell nanoparticles to "fish-shaped" nanocomposites. By exploiting the structural transformation of polymer shell from sphere to cylinder, a chain-growth mode was developed to fabricate one-dimensional nanoparticle chains. Finally,

ultrasound was used to assist the synthesis of 1D nanoparticle chains and 3D nanoclusters.

To fully mimic the short-distance drug release in cell, and well understand the release kinetics, we proposed a new model delivery system, in which core-shell AuNP@PSPAA were used as nanocarriers and PSPAA micelles were employed as nanoacceptors. Simultaneously introducing both the drug nanocarriers and the nanoacceptors not only creates short-distance drug release but also allows monitoring the kinetics in real time. Moreover, by simply adjusting the concentration of the nanoacceptors, we could effectively control the release distance and understand the release pathway. On the basis of the model system, we clearly found: (a) the short-distance diffusion of even hydrophobic molecules like pyrene through water is efficient, however, the diffusion in water is not the rate-determining step; (b) the release of pyrene from the glassy PSPAA micelles is fast and not assisted by direct transfer between the micelles; (c) the nanoacceptor-induced fast release follows a Fickian spherical model; and most importantly, (d) the stability of the impregnated drug in the absence of nanoacceptors does not guarantee the retaining of the drug in biological systems. Thus, it is believed that this model system provides unique advantages in understanding the pathway of drug release, minimizing the mechanistic ambiguities.

Core-shell nanoparticles have been demonstrated a kind of promising

building blocks in creating more complex nanostructures. In this work, we developed two methods to construct anisotropic nanostructures using core-shell nanoparticles as basic synthetic units. First, we describe the rational synthesis of "nanofish-shaped" colloidal composites in an emulsion system, where the polymerization of oil droplets (i.e., DVB) was confined inside of the core-shell AuNP@PSPAA. Simply mixing the DVB, AuNP@PSPAA and APS, and raising the temperature induced the phase separation occurred at nanoscale, resulting in anisotropic nanoparticles. Multi-generation growth has been demonstrated, which plays an important role in controlling the anisotropy of nanoparticles. Most importantly, the shear conditions and the size of cores are of critical significance in controlling the phase separation and swelling, which create various morphologies and enrich structural diversity. The application of the emulsion method at nanoscale may offer new opportunities for designing complex architectures.

Then, we employed the structural diversity of polymer to assist the assembly of Au nanoparticles into one-dimensional nanoparticle chains. Different from the traditional "step-growth polymerization mode", in our system, the linear aggregation of core-shell AuNP@PSPAA follows an unconventional "chain-growth polymerization mode". Under acid conditions, the spherical PSPAA shells converted into cylindrical micelles in order to decrease the surface/volume ratio, thus, it drove the fusion of the polymer shells and

promoted the assembly of embedded AuNPs into double-line chains (up to 200 NPs in length) or single-line chains (up to 300 NPs in length). Impressively, the single-line chains can be transformed into double- or triple-line chains by simply tuning the solvent ratio. Thus, it is believed that the PSPAA shell plays critical roles during the "polymerization": It guided the linear aggregation and controlled the width of the chains. Moreover, the coexistence of AuNP@PSPAA monomers and long chains strongly supported the unconventional chain-growth mechanism, which is essential for achieving ultralong chains with minimal defects. Such chains with uniform width and interparticle spacing are of importance for fabricating devices and exploring new physical properties. The combination of nanoparticles with polymers will offer new opportunities in designing new structures.

Finally, we developed a new method to synthesize 1D nanoparticle chains and 3D nanoclusters assisted by the ultrasound. The length of the single-line chains fabricated via the sphere-to-cylinder transformation of PSPAA shells was not uniform (10-300 NPs in length). Under ultrasonic irradiation, the cavitation resulted in the scission of PSPAA shells, thus, the ultralong AuNP chains were sectioned into short ones. The average length of the chains and the breakage rate were dependent on the ultrasonic energy. It is found the single-line chains could transform into spherical nanoclusters under a higher ultrasonic energy.

The formation of 3D nanoclusters involved the scission and the

cylinder-to-sphere transformation of PSPAA shells. We hypothesized the mobility of surface ligands on the AuNPs plays a critical role in realizing the transformation from chains to clusters. Exploiting the structural transformation of polymer shells assisted by the ultrasound provides a tool to design advanced nanostructures.

6.2 Outlook

On the basis of the understanding of the distinctive ability of core-shell motif in developing new synthetic methodologies, as well as the strategies developed in this thesis, several future works were proposed in the following:

(1) Study the porosity of the PSAPP micelles. The PSPAA micelles facilitate the diffusion of hydrophobic molecules between nanocarriers and nanoacceptores. The fact is that there exist channels or pores inside the interior of PSPAA micelles. However, the structure, the formation mechanism, and position of the pores are still unknown. Thus, we would exploit a rational approach to study the porosity of the PSPAA micelles, for example, the ionic diffusion through the polymer shells. Based on the understanding of the pores of the PSPAA micelles, we could further adjust the size of the pore to control the release rate. For example: cholesterol (Figure 6-1) provides essential building blocks for cell membrane and modulates the function of cell membrane. When the hydrophobic molecules insert into the pores of the PSPAA

micelles, the structural change of the pores would affect the release kinetics of the drugs.

Figure 6-1 The structure of cholesterol.

(2) Fabrication of nanoclusters via an emulsion method. In chapter 5, we demonstrated that "nanofish-shaped" colloidal composites were synthesized in the emulsion solution. However, by introducing organic solvent (e.g., DMF) into the above system, the core-shell AuNP@PSPAA assembled into nanoclusters (Figure 6-2a). What's the role of DMF? What's the formation mechanism of the nanoclusters? Resolving these two questions would help us well understand the emulsion system, guiding us to control the assembly of the core-shell NPs. For example: synthesizing the dimers of AuNPs with high yield; or constructing hetero-nanoclusters (metal and magnetic particles; Figure 6-2b) to exploit promising properties. In addition, the roles of the surface ligands, especially, the unbonded ligands locating in the polymer shells, in affecting the behavior of polymer shells should be further studied.

Figure 6-2 TEM images of (a) nanoclusters synthesized via adding DMF into an emulsion solution containing DVB, APS and NaOH; (b) hetero-dimers (AuNP and FePt NP).

(3) Construction of complicated hybrid nanostructures by polymer-assisted assembly. Using core-shell AuNP@PSPAA as assembly units, the "polymerization" of the NPs follows a "chain-growth mode". This unconventional growth mode favors end-on aggregation, inducing ultralong chains. Thus, it could be expanded to synthesize block chains of hetero-nanoparticles, which may have potential application in nanodevices. By introducing different core-shell nanoparticles (Fe_3O_4 NPs, AgNPs, Au nanorods), varying the polymer shells (changing the length of PS or PAA blocks), or adjusting the solvent ratio, a series of block chains may be fabricated (Figure 6-3). For example: If the block chain is composed of magnetic nanoparticles and Au nanoparticles, it would exhibit special movement in the presence of

electric field or magnetic field. Fusing the metal nanoparticles would generate block nanowires in the colloidal solution, which gives rise to unique optical or electric properties. Moreover, by employing the structural transformation of PSPAA from sphere to toroid, nanoparticle rings could be synthesized, which could be further used as template to catalyze the growth of oxide or metal arrays.

Figure 6-3 Schematic illustrating of the fabrication of different hybrid nanostructures.

(4) Synthesis of the three-dimensional (3D) network of nanoparticles chains (gel). As discussed in chapter 3, the AuNP@PSPAA assembled into single-line chain with high branches in the high solvent ratio (DMF: H₂O = 9:1), importantly, the crossed chains could be fused together. On the basis of this phenomenon, the 3D network of nanoparticle chains may be synthesized by the assembly of single-line chains in the same condition. Alternatively, the surface of the single-line chain is covered with PAA blocks, thus, simply cross-linking

the PAA blocks via introducing some crosslinking agents (e.g., N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride), the gels of nanoparticle chains may be obtained. The gels of nanoparticle chains have high surface/volume ratio, which endows the gel system with potential application in catalysis, and drug delivery. For this study the scalable synthesis of single-line chains may be a challenge.

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