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Recent Advances towards Fabrication and Biomedical Applications of Responsive Polymeric Assemblies and Nanoparticle Hybrid Superstructures

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Shiyong Liu has been a professor of Polymer Science and Engineering at the University of Science and Technology of China since 2004. He is recipient of 100 Talents Program (CAS, 2004), Distinguished Young Scholars Award (NSFC, 2004), and Cheung Kong Professor Award (Ministry of Education of China, 2009). He served or has served on the Editorial Advisory Board for Macromolecules (ACS, 2008-2010), Macromolecular Rapid Communications, Macromolecular Chemistry and Physics, Biomacromolecules, and Polymer Chemistry. He has published over 200 peer-reviewed journal papers with a total citations of more than 8000 and an H-index of 50. His current research interests include the design and synthesis of functional polymeric materials, colloids, and stimuli-responsive polymeric assemblies with controlled properties for applications in imaging, sensing, diagnostics, and nanomedicine.

Abstract

Responsive polymeric assemblies and hybrid superstructures fabricated from stimuli-sensitive polymers and inorganic nanoparticles (NPs) have received extensive investigations during the past few decades due to distinct advantages such as improved water solubility, stimuli-responsiveness, excellent biocompatibility, and facile introduction of functional units. In addition, chemical compositions of polymeric assemblies and corresponding hybrid superstructures can be modulated via the initial synthetic design to target desired functions, fabricate smart nanostructures, and explore morphology-dependent functional optimization. Promising applications in the field of imaging, sensing, drug/gene delivery, diagnostics, and nanoreactors are being extensively investigated. This perspective article focuses on recent developments, microstructural control, and biomedical applications of stimuli-responsive polymeric assemblies as well as responsive hybrid superstructures fabricated from responsive polymers and inorganic NP building blocks (gold NPs and magnetic iron oxide NPs), and highlights the current status and future developments with selected literature reports.

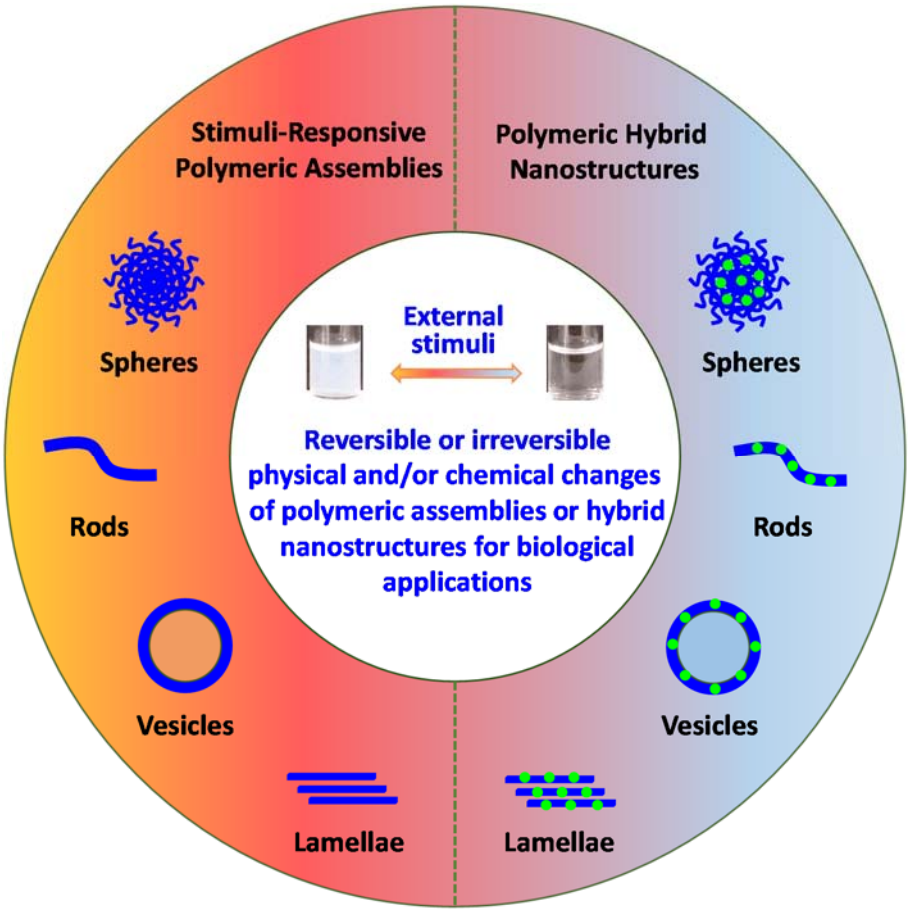
1. Introduction

Self-assembly of stimuli-responsive polymers has attracted considerable attention over the past decade due to their promising applications in a variety of fields ranging from imaging,[1-3] drug/gene carriers,[4-6] diagnostics,[7] smart actuators,[8] adaptive coatings,[9] to self-healing materials.[10] Responsive polymers typically exhibit reversible or irreversible changes in chemical structures and/or physical properties in response to external stimuli such as pH, temperature, CO_2, ionic strength, light irradiation, electric and magnetic fields, mechanical forces, and other bioactive molecules of interest.[1, 2, 10-19] The aqueous self-assembly of responsive block copolymers can lead to spherical micelles (spheres), rod-like micelles (rods), lamellae, vesicles, and other complicated nanostructures.[20-22] Meanwhile, the morphology-modulated biological effects such as biodistribution, cellular internalization, blood circulation, and cytotoxicity of various nanostructures have been increasingly taken into account.[23-26]

On the other hand, self-assembled nanostructures of responsive polymers provide excellent templates or matrix for the spatial organization of inorganic nanoparticles (NPs), affording responsive polymeric hybrid superstructures.[27] The incorporation of inorganic NPs into responsive polymeric assemblies mainly follows two primary pathways.[28] The first one relies on *in situ* formation of inorganic NPs within preformed polymeric nanostructures: metal ions are confined into polymeric aggregates, and then transformed into NPs via appropriate chemical reactions. For the second approach, preformed inorganic NPs are surface functionalized with responsive polymeric building blocks, the subsequent co-assembly of inorganic NPs and polymers leads to the formation of polymeric hybrid superstructures such as hybrid spheres, rods, lamellae, vesicles, and other nanostructures.

Morphological transitions of these self-assembled nanostructures can also occur in the presence of an appropriate triggering motif due to the stimuli-responsive feature of responsive polymeric assemblies and corresponding nanoparticle hybrid superstructures. In the context of structural design of responsive polymers, the tuning of chain topologies, density and spatial distribution of functional and/or responsive moieties, linkages between respective building blocks, hydrophilicity-hydrophobicity balance, and stimuli-triggered disintegration have received extensive investigations.[16, 29-31]

This perspective article aims to discuss recent developments concerning the fabrication and biomedical applications of responsive polymeric assemblies and corresponding hybrid superstructures co-assembled from synthetic block copolymers and inorganic NPs (Scheme 1). Fundamental principles concerning the self-assembly of block copolymers and construction of hybrid polymeric assemblies have been well-documented in previous literature reports and reviews,[28, 32] and we wish that this perspective can stimulate more innovative ideas, concepts, and scientific advances.



Scheme 1. Schematics of the fabrication of a variety of stimuli-responsive polymeric assemblies and nanoparticle hybrid superstructures for biomedical applications.

2. Self-Assembly of Synthetic Stimuli-Responsive Block Copolymers

Self-assembly of block copolymers is a very broad and active research field, including self-assembly both in bulk and in solution. Bulk self-assembly has been well documented by some books and reviews.[33-37] Herein we attempt to focus on the microstructural fabrication of synthetic stimuli-responsive block copolymers in aqueous media. Some recent efforts have

been devoted to develop functional polymeric assemblies which possess broad biological applications in bio-imaging, diagnostics, and drug/gene delivery. Some emerging research directions in the field of polymer self-assembly as well as their biomedical functions are highlighted below.

2.1 Polyprodrug-Based Self-Assembly and Therapeutic Applications

For conventional drug nanocarriers, drug molecules are usually physically embedded within or covalently conjugated onto polymeric scaffolds. In these drug delivery nanomedicine systems, drug molecules in nanocarriers or polymeric assemblies are recognized as transported cargos for controlled release at the lesion site to achieve therapeutic efficacy. However, drug molecules do not typically participate in the polymeric self-assembly process. By taking advantage of the physiochemical features of drug molecules, a variety of prodrug-based polymeric nanomedicine systems have been constructed from drug conjugates.[38, 39]

In an earlier example, Shen *et al*.[40] utilized camptothecin (CPT) anticancer drugs as the hydrophobic moiety in combination with a very short oligomer chain of ethylene glycol (OEG) as the hydrophilic part, affording amphiphilic phospholipid-mimicking prodrug molecules, OEG-CPT or OEG-DiCPT (Figure 1). The aqueous self-assembly of two types of prodrug molecules afforded stable liposome-like nanocapsules, exhibiting a CPT loading content as high as 40 or 58 wt% with no burst release in water. Self-assembled nanocapsules loaded with hydrophilic doxorubicin salt (DOX·HCl) can synergistically deliver two types of anticancer drugs to achieve combined chemotherapy. In another intriguing report, Cui *et al*.[41] developed a prodrug-aided platform by functionalizing CPT drugs with a β-sheet-forming Tau peptide through a reducible disulfylbutyrate linker, possessing CPT loading contents ranging from 23% to 38%. The peptide-functionalized prodrugs can self-assemble into filaments or tubular nanostructures in aqueous solution. Interestingly, they found that the number of CPT molecules in the terminal of Tau peptide had serious influence on the self-assembled morphologies. In another report, Shen and coworkers[42] further explored amphiphilic linear-dendritic drug conjugates by modulating the number of conjugated CPT drugs with dendritic polylysine to adjust the ratio between hydrophobic and hydrophilic

segments. The subsequent controlled self-assembly process afforded nanosized spheres or rods, which displayed differences in the cellular uptake behavior and *in vivo* blood circulation.

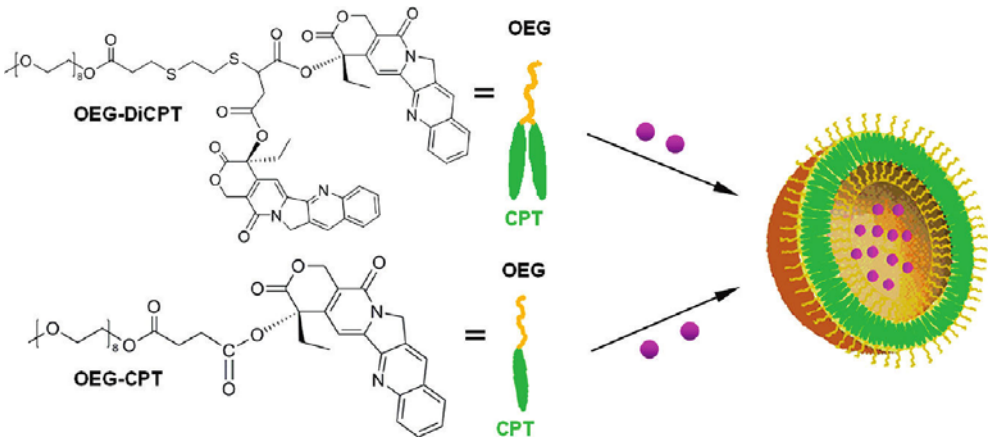


Figure 1. Amphiphilic camptothecin (CPT) prodrug molecules (OEG-CPT and OEG-DiCPT) and their self-assembly into nanocapsules to load other hydrophilic drugs (the hydrophobic bilayer membrane is solely made of CPT). Reproduced from ref. 40, Copyright 2010, American Chemical Society.

Just recently, Hu *et al.* [43] developed a novel reduction-responsive drug delivery system with high drug loading content, termed as *polyprodrug amphiphiles*. Polyprodrug amphiphiles possessed two typical features including amphiphilicity and repeating prodrug units through stimuli-cleavable linkages between drug molecules and polymer backbones, which highlighted the potency of polymer chemistry into the design of polymeric drug nanocarriers. PEG-*b*-PCPTM polyprodrug amphiphiles with >50 wt% CPT loading content were prepared via direct reversible addition-fragmentation transfer (RAFT) polymerization of reduction-cleavable CPT prodrug monomer, CPTM, starting from poly(ethylene glycol) (PEG) based macroRAFT agent (Figure 2). Unexpectedly, PEG-*b*-PCPTM can self-assemble in a controlled manner into four types of uniform nanostructures including spheres, large compound vesicles (LCVs), smooth disks, and unprecedented staggered lamellae with spiked periphery. Among these, staggered lamellae exhibited extended blood circulation duration, the fastest cellular internalization, and unique endocytic pathways, as compared to other three types of nanostructures. In addition, shape-optimized CPT release kinetics, nanostructure degradation, and *in vitro* cytotoxicities were also investigated, highlighting the importance

of nanostructure morphologies towards drug delivery efficiency. This work represents the first example of polyprodrug amphiphiles which are amenable to the fabrication of multiple hierarchical nanostructures for investigations of shape-tunable biological performance.

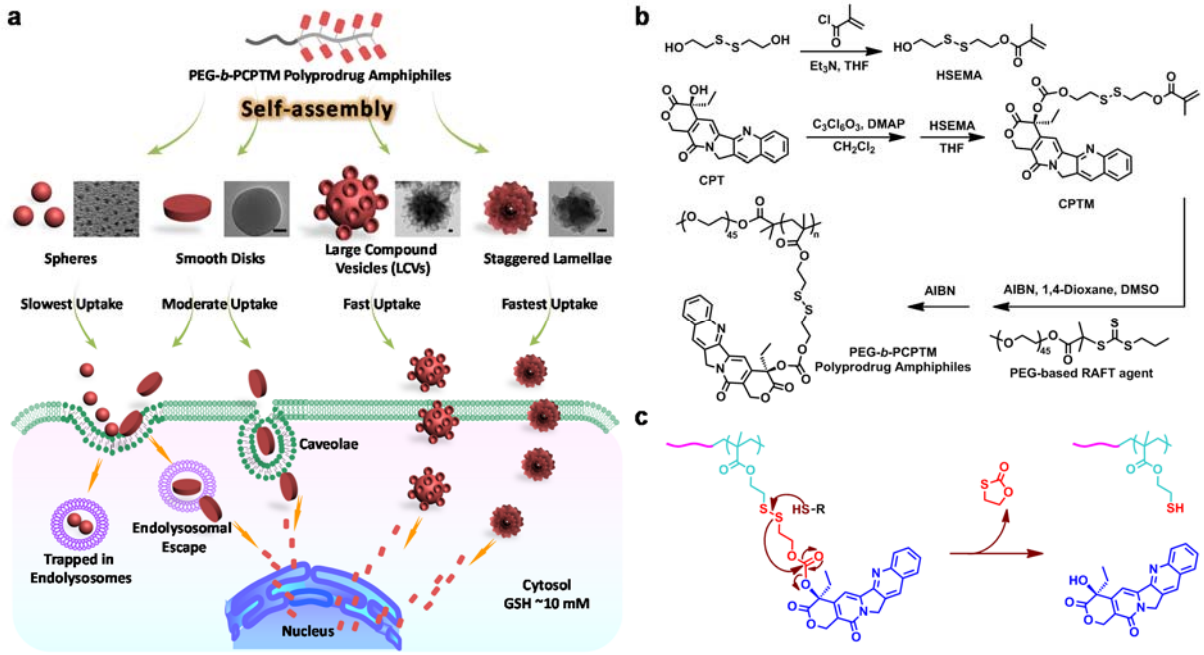


Figure 2. Multiple hierarchical assemblies of PEG-*b*-PCPTM polyprodrug amphiphiles for intracellular reduction-responsive drug delivery. (a) Schematic illustration for the self-assembly of polyprodrug amphiphiles into four types of uniform nanostructures (including spheres, smooth disks, flower-like large compound vesicles, and unprecedented staggered lamellae with spiked periphery), which exhibit shape-dependent performance in the context of blood circulation, cellular internalization and transport, subcellular distribution, and degradation kinetics. (b) Synthesis of reduction-responsive CPT prodrug monomer, CPTM, and polyprodrug amphiphiles, PEG-*b*-PCPTM. (c) Proposed mechanism of reduction-responsive CPT parent drug release from PEG-*b*-PCPTM polyprodrug amphiphiles. Reproduced from ref. 43, Copyright 2013, American Chemical Society.

Polyprodrug-based drug delivery systems possess combined advantages such as high drug loading content, easy preparation, facile multifunctional integration and nanostructural fabrication.[44-53] In other relevant examples, Hubbell *et al*.[54] utilized RAFT technique to polymerize the prodrug monomer of ibuprofen (IBU), a widely applied anti-inflammatory drug, affording PEG-*b*-PIBU amphiphilic diblock copolymers (Figure 3). Spherical and

worm-like micelles were obtained through the aqueous self-assembly of PEG-*b*-PIBU with varying hydrophobic PIBU block lengths. Furthermore, IBU release profiles and antiproliferative effect of IBU-containing polymeric assemblies in human cervical carcinoma (HeLa) and murine melanoma (B16-F10) cells were also investigated, exhibiting nanostructure shape-dependent degradation and IBU release kinetics.

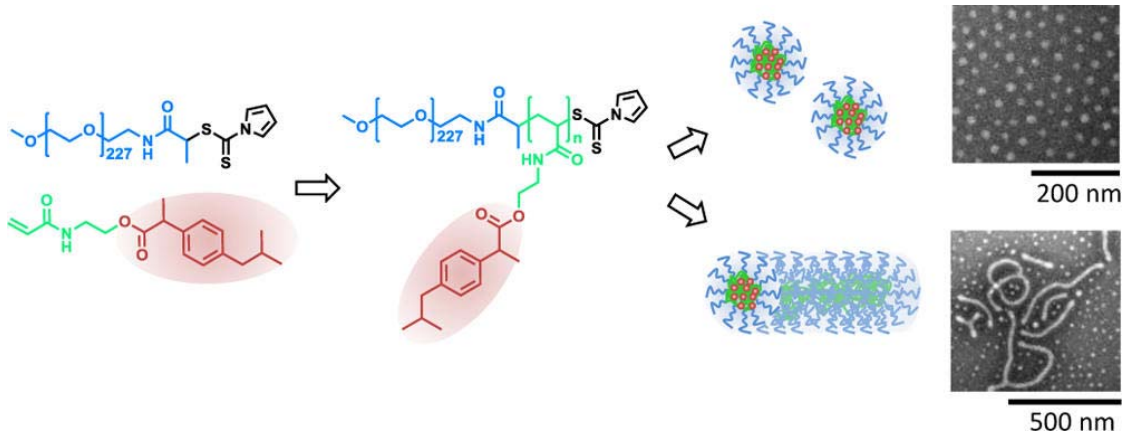


Figure 3. Schematic illustration of the synthesis and nanostructure fabrication of PEG-*b*-PIBU polyprodrug amphiphiles. Ibuprofen (IBU) is a widely applied anti-inflammatory drug. Reproduced from ref. 54, Copyright 2013, American Chemical Society.

Representing an emerging research direction, the field of polyprodrug-based drug delivery systems as well as the nanostructure fabrication needs to be further explored. Further efforts might consider the introduction of targeting ligands, more potent prodrug types, extra polymer chain topologies, and other stimuli-cleavable linkages to enrich the choices for biomedical applications. Moreover, the self-assembling mechanism of polyprodrug amphiphiles and intracellular trafficking as well as the *in vivo* fate and stimuli-triggered degradation of self-assembled nanostructures deserve more detailed investigations in the future.

2.2 Stimuli-Responsive Microstructural Modulation for Polymersomes

Self-assembly of synthetic block copolymers, one of the bottom-up approach in nanoscience, can afford a variety of nanostructures such as spheres, rods, lamellae, and vesicles. The further modulation of self-assembled polymeric nanostructures via external stimuli is vital for optimizing their functional applications. Among these, polymeric vesicles

(polymersomes) are quite unique due to their microstructural characteristics, consisting of an aqueous interior enclosed by a hydrophobic bilayer membrane. Polymersomes have been extensively utilized as drug nanocarriers,[29, 55, 56] nanoreactors,[57, 58] and artificial organelles.[59-61] However, the functional applications of polymersomes are severely limited by membrane permeability issues, i.e., they are almost impermeable to small organic molecules, ions and even water molecules.[62, 63] The polymersome permeability can be modulated via chemical reactions or changes in physiochemical properties of building components, including the incorporation of channel proteins into membranes,[64] incoporation of stimuli-responsive moieties (e.g., pH, sugar, light),[16, 30, 31, 65-67] co-assembly with oppositely charged polymers,[68] and self-assembly of helical rod-coil block copolymers.[58]

In a recent study, Yuan *et al*.[69] designed a series of amphiphilic diblock copolymers consisting of hydrophilic PEG and CO_2-sensitive poly(*N*-amidino)dodecyl acrylamide (PAD) as the hydrophobic portion. Target diblock copolymers, PEG-*b*-PAD, were prepared by atom transfer radical polymerization (ATRP) techinique (Figure 4). PEG-*b*-PAD amphiphilic diblock copolymers can self-assemble into vesicular nanostructures in aqueous solution. Most importantly, polymersomes can continuously swell upon CO_2 bubbing, accompanied with changes in the membrane structure and bilayer permeability. Based on this concept, CO_2/N_2 can be employed as external stimuli to tune polymersome membrane permeability reversibly over a broad range and renders it "breathing".[70] In another example, Zhou, Zhu and coworkers[71] reported the fabrication of pH-modulated reversible "breathing" vesicles. They employed hydrophilic PEG-based macroinitiator to initiate the polymerization of azobenzene-containing monomer (DMA-Azo), 6-(4-(4-dimethylamino phenylazo)-phenoxy)hexyl methacrylate, affording PEG-*b*-PDMA-Azo diblock copolymers. Self-assembled polymersomes of PEG-*b*-PDMA-Azo in aqueous media exhibited pH-induced reversible "breathing" behavior accompanied with concomitant fluorescence quenching or activation due to protonation or deprotonation of tertiary amine residues.

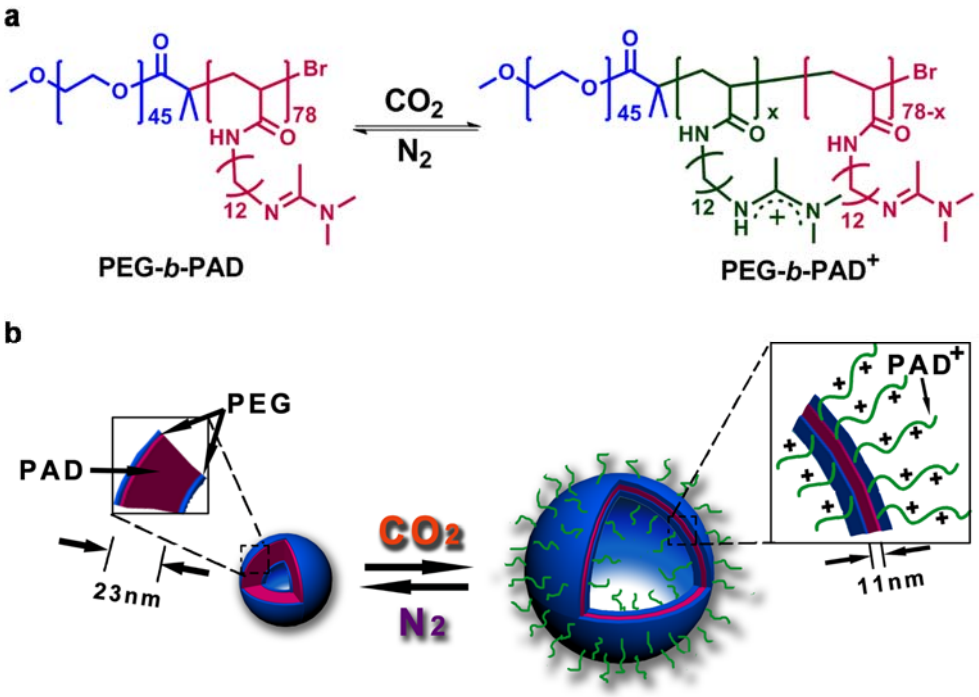


Figure 4. (a) Schematic illustration of gas-switchable chemical structural changes of PEG-*b*-PAD diblock copolymers. (b) The self-assembly of PEG-*b*-PAD amphiphilic diblock copolymer into polymersomes and reversible gas-controlled "breathing" behavior in aqueous media. Reproduced from ref. 69. Copyright 2013, Wiely-VCH.

In addition, Spulber *et al.*[72] proposed another post-functionalizing strategy to modulate vesicular permeability, employing a facile and adaptable method to modulate preformed vesicle bilayer membranes and introduce hydrophilic moieties into bilayer membranes (Figure 5). In this system, a hydrophilic α-hydroxyalkylphenone photoinitiator, 2-hydroxy-4′-2-(hydroxyethoxy)-2-methylpropiophenone (PP-OH), was added into the aqueous dispersion of polymeric vesicles and allowed to react with vesicular membranes under UV irradiation. The photoinitiator decomposed into two primary radicals (ketyl and alcohol-types) upon UV irradiation, the generated radicals then reacted with hydrophobic bilayer membranes. Thus, the chemical modification of bilayer membranes with fragments of hydrophilic PP-OH increased the permeability of bilayers. The reported permeability regulation strategy was successfully demonstated with three different types of block copolymers and proved to be unaffected by the presence of specific functional groups. Furthermore, photoreaction with radicals fragmented from PP-OH can also transform

enzyme-filled polymersomes into nanoreactors without the need of membrane proteins or specially designed polymer building blocks. Meanwhile, the modification process did not affect the ability of enzymatic nanoreactors in protecting physically encapsulated biocatalyst against externally added denaturating agents.

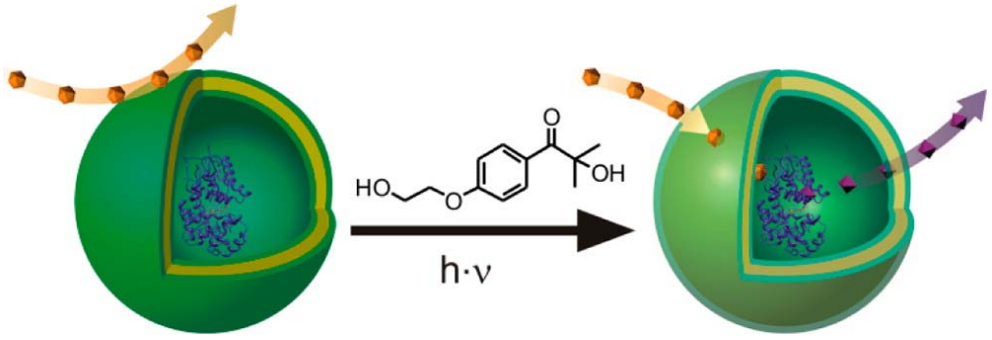


Figure 5. Schematic illustration for photo-actuated post-functionalization of vesicular bilayer membranes to modulate the permeability of vesicle membranes. Reproduced from ref. 72, Copyright 2013, American Chemical Society.

For the modulation of vesicular permeability, there exists an unavoidable contradiction between polymersome stability and bilayer permeability. Interestingly, Wang *et al*.[73] recently reported a photo-regulated "traceless" crosslinking strategy to solve the dilemma of concurrent polymersome crosslinking and permeability switching of vesicular bilayer membranes. PEO-*b*-PNBOC amphiphilic block copolymers with photolabile carbamate-caged primary amine moieties in the hydrophobic block were synthesized via RAFT polymerization of 2-nitrobenzyloxycarbonylaminoethyl methacrylate (NBOC) using poly(ethylene oxide) (PEO)-based macroRAFT agent (Figure 6). Aqueous self-assembly of PEO-*b*-PNBOC formed polymersomes, upon UV-triggered decaging of 2-nitrobenzyl groups, primary amine moieties were released and then prominent amidation reactions occurred. This leads to unexpected vesicle crosslinking reactions in the bilayer membrane instead of vesicle-to-unimer transition. Moreover, the in-situ "traceless" crosslinking process is accompanied with the generation of hydrophilic network channels and hydrophobicity-to-hydrophilicity transition within bilayer membranes, resulting in enhanced bilayer permeabilization. In addition, this feature has been successfully utilized to achieve light-modulated codelivery of both hydrophobic and hydrophilic cargos and UV-switchable biocatalysis of enzyme-

encapsulated vesicular nanoreactors. The reported strategy should also be feasible to other synthetic polymeric hierarchical nanostructures and even 'top-down' fabricated assemblies. Although UV irradiation was used as an external stimulus to trigger synchronized vesicle crosslinking and membrane permeability enhancement in the the work, other triggering motifs such as visible and infrared light and more biologically relevant stimuli (e.g., thiols, hydrogen peroxide, and enzymes) deserve further investigation with rational structural design of synthetic block copolymers.

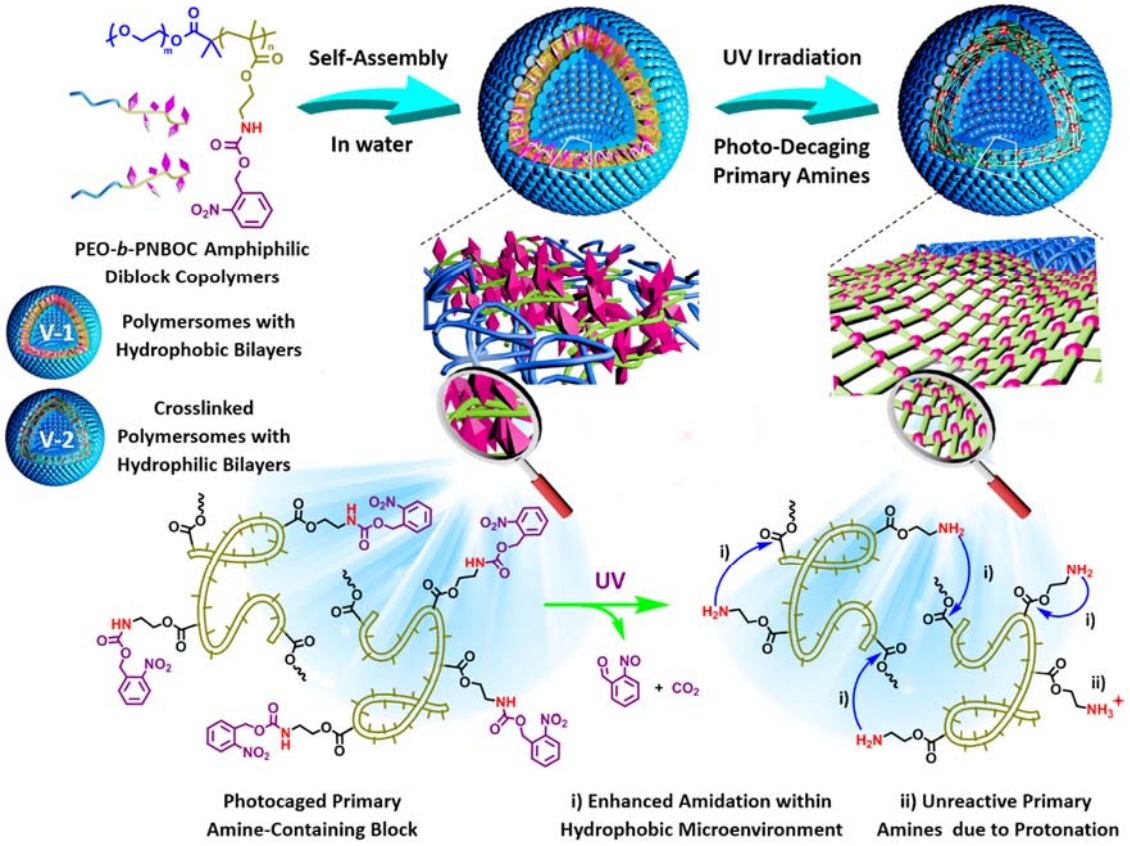


Figure 6. Design of block copolymer vesicles exhibiting concurrent photo-triggered "traceless" crosslinking and vesicle membrane permeabilization. Reproduced from ref. 73. Copyright 2014, Wiely-VCH.

Besides chemical crosslinking reactions for tuning the permeability of polymersomes, stimuli-responsive cascade degradation of vesicle bilayer membranes can also be utilized to sensitively disintegrate polymersomes and release encapsulated agents. Very recently, Liu *et al.*[74] developed a novel type of self-immolative polymersomes (termed as SIPsomes) via self-assembly of amphiphilic block copolymers, consisting of a triggered degradable poly(benzyl

carbamate) (PBC) block and a hydrophilic poly(*N,N*-dimethylacrylamide) (PDMA) block (Figure 7). The terminal of self-immolative PBC block was functionalized with three types of caging groups, perylen-3-yl, 2-nitrobenzyl, or disulfide moieties, which were responsive to visible light (420 nm), UV light (365 nm) or reductive milieu, respectively. Upon stimulus-triggered decaging, PDMA-*b*-PBC diblock copolymers depolymerized into water-soluble residues, 4-aminobenzyl alcohol, CO_2, and PDMA chains. Protons, oxygen, and enzymatic substrates were examined towards the trigger-stimulated drug delivery and controllable approaching potency for cargo-loaded SIPsomes. In-depth applications in programmed (OR, AND, and XOR-type logic) enzymatic catalysis were also demonstrated for SIPsomes in this work. The systematic design of responsive selectivity/specificity of SIPsomes lay a solid foundation for the exploration of next generation drug delivery systems and intelligent devices via the integration with more biocompatible triggering motifs, targeting ligands, and therapeutic/imaging agents.

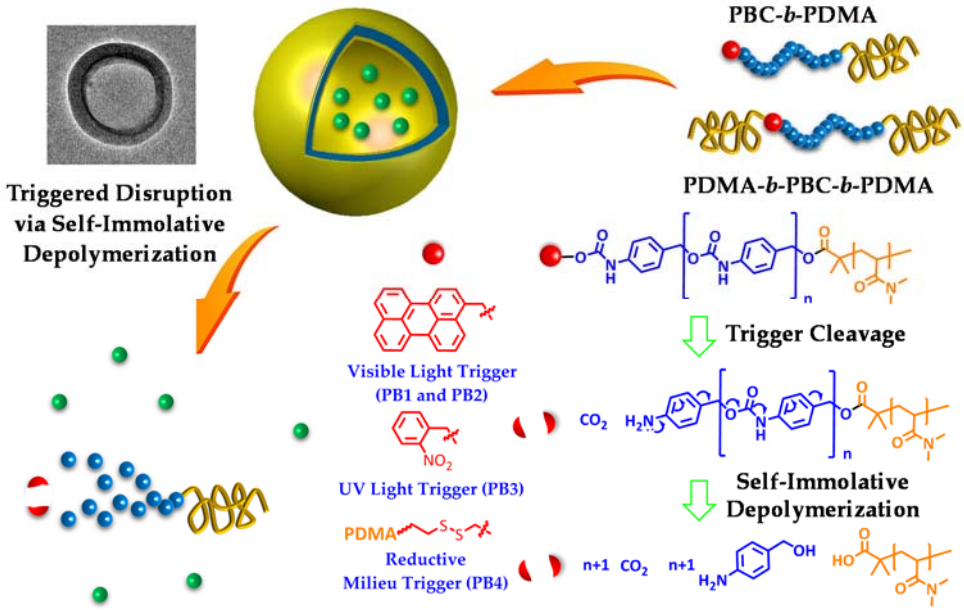


Figure 7. Schematic illustration of SIPsomes self-assembled from poly(benzyl carbamate)-b-poly(*N,N*-dimethylacrylamide), PBC-*b*-PDMA, amphiphilic block copolymers containing hydrophobic PBC blocks, which are subjected to self-immolative depolymerization into small molecules upon cleavage of "capping" moieties (i.e., perylen-3-yl, 2-nitrobenzyl or disulfide for stimuli of visible Light, UV light, and reductive milieu, respectively). Reproduced

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A notable common feature of above examples is that all modulation processes were based on the stimuli-responsiveness of polymeric building blocks, and the nanostructure evolution process can be actuated in a highly selective and controlled manner. Facilely controllable triggering moieties endowed these systems with excellent biocompatibility and augured well for their biomedical applications as delivery platforms for drugs, genes, and other bioactive agents. Future work concerning molecule design, appropriate incorporation of much more biologically practical triggers, and *in vivo* evaluation deserves further examinations.

2.3 Host-Guest Recognition Assisted Fabrication of Synthetic Polymeric Assemblies

In contrast to conventional molecular chemistry based on covalent bonds, host-guest recognition has emerged to be a powerful and versatile strategy for nanostructure fabrication due to its accessibility, extraordinary reversibility and flexibility, and potent applications in diverse fields. The introduction of host-guest chemistry into polymeric self-assembly not only enriches the realm of stimuli-responsive polymers but also opens up new horizons for their promising applications.[75] Most importantly, the integration of stimuli-responsive polymeric assemblies with host-guest recognition motifs will endow self-assembled nanostructures with multiple stimuli-responsiveness and accordingly more sophisticated functions. Typical examples of host-guest recognition promoted fabrication of synthetic polymeric assemblies in recent years are highlighted below.

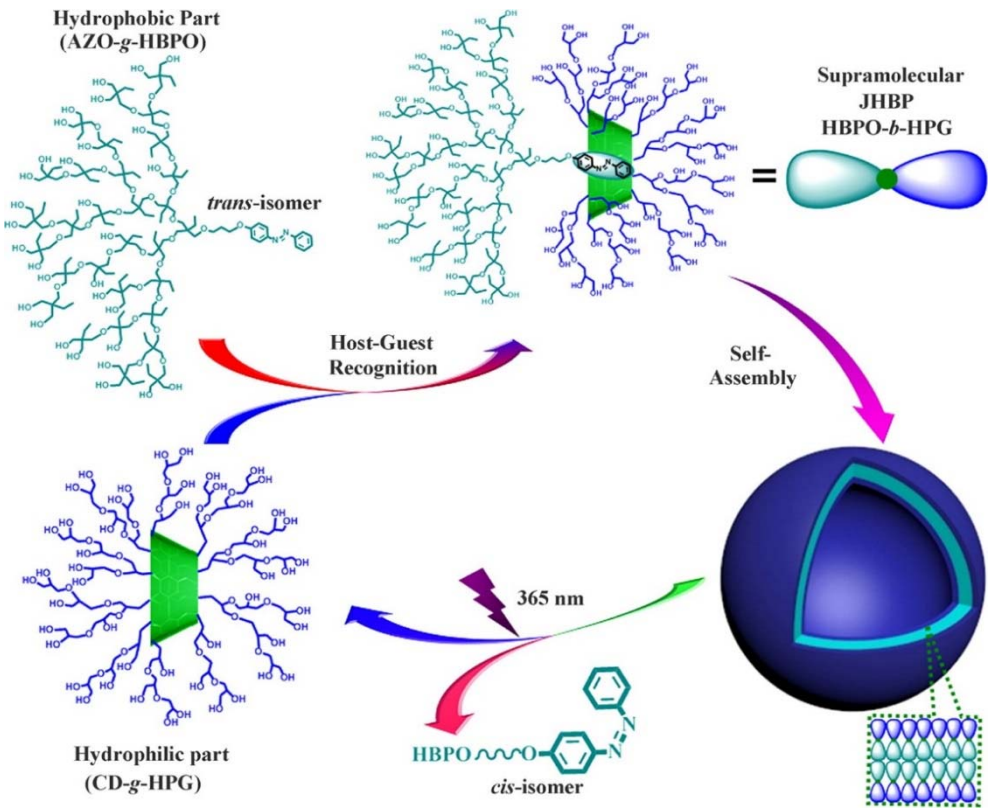

Figure 8. Schematic illustrations for the reversible self-assembly and disassembly of vesicles constructed from supramolecular Janus hyperbranched polymers of HBPO-*b*-HPG. Reproduced from ref. 76, Copyright 2013, American Chemical Society.

Liu *et al*.[76] recently designed supramolecular Janus copolymers consisting of two chemically distinct hyperbranched polymers, i.e., hyperbranched polyglycerol functionalized *β*-cyclodextrins (*β*-CD-*g*-HPG) and azobenzene (AZO)-functionalized hyperbranched poly(3-ethy-3-oxetane methanol) (AZO-*g*-HBPO) (Figure 8). Aqueous self-assembly of the amphiphilic Janus hyperbranched copolymer led to unilamellar vesicles, which disassembled under UV light irradiation due to trans-to-cis isomerization of AZO moieties and dissociation of supramolecular linkages. Actually, the assembly/disassembly process can be reversibly modulated via UV and visible light irradiation. This work demonstrated that the incorporation of host-guest recognition motif into polymeric self-assembling systems can significantly broaden the research horizon of polymer self-assembly and enrich the modules of stimuli-responsiveness. In another notable example, Jiang *et al*.[77] demonstrated that multivalent host-guest recognition of inclusion complexation between *β*-CD homopolymers and adamantly groups-decorated poly(*t*-butyl acrylate) promoted the formation of micelles

and hollow spherical nanostructures.

Host-guest recognition has also been applied to fabricate hybrid polymeric assemblies. In a recent report, Zhang *et al.*[78] examined a facile one-step approach to generate porous hybrid microcapsules using the microfluidic technique (Figure 9). Four types of components including cucurbit [8] uril (CB[8]), naphthol-functionalized poly(2-hydroxyethyl acrylamide) copolymer, methylviologen (MV^{2+})-modified gold NPs, and fluorous oil were utilized. CB host is capable of accommodating both electron-deficient MV^{2+} guest and electron-rich naphthol guest simultaneously, affording porous microcapsules with easily tailorable functionalities. Polymer-gold NP composites were held together by CB[8] ternary complexes to fabricate dynamic yet highly stable microcapsules. In this excellent example, the host-guest recognition-promoted microcapsules were responsive to realize on-demand cargo release. Furthermore, surface enhanced Raman spectroscopy was employed to probe the internal chemical microenvironments.

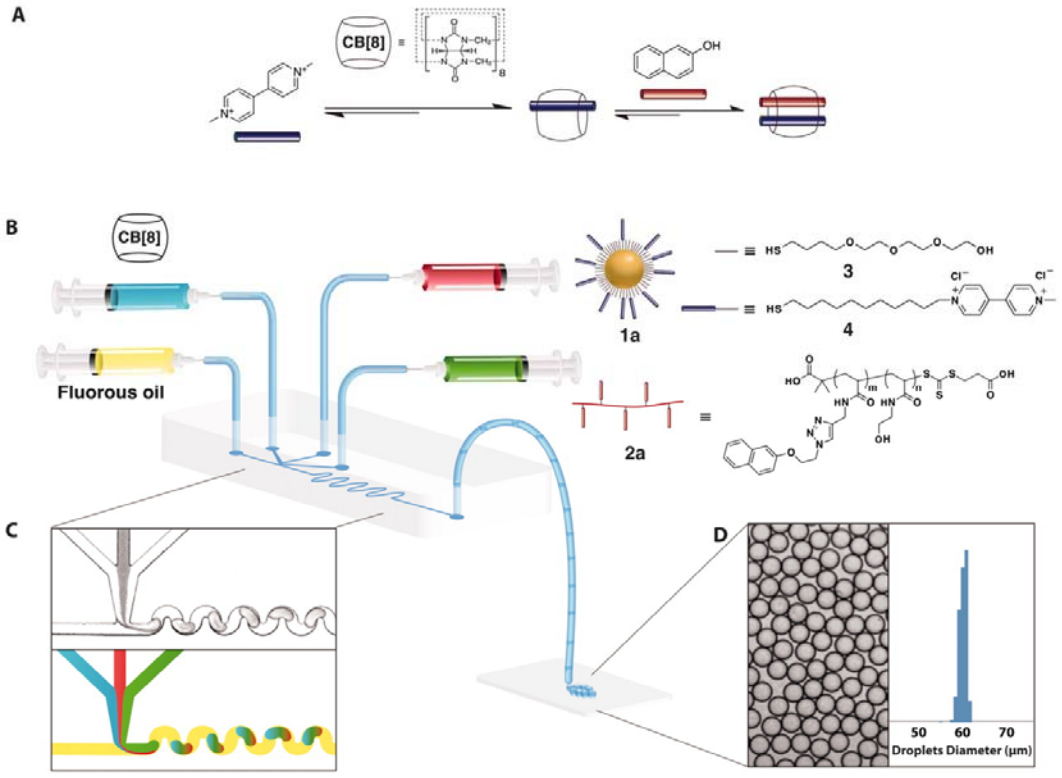

Figure 9. (A) Schematic illustration for the three-component formation of CB[8] ternary complex in aqueous solution with MV^{2+} (blue) and NP (red). (B) Schematic representation of the microdroplet generation process using a microfluidic T-junction device, consisting of a continuous oil phase perpendicular to a combination of three aqueous solutions of CB[8],

1a (Au NP functionalized with a mixture of neutral and viologen-containing ligands 3 and 4), and 2a (copolymer functionalized with NP) as the dispersed phase. (C) Microscopic image and schematics of the T-junction and a wiggled channel for rapid mixing of reagents online. (D) The monodisperse nature of microfluidic droplets is demonstrated by the narrow size distribution (diameter 59.6 ± 0.8 μm). Reproduced with permission from ref. 78. Copyright 2012 AAAS.

Synthetic polymeric assemblies incorporating host-guest recognition motifs possess intrinsic responsive modes originating from the dynamic and reversible nature of supramolecular recognition linkages. In addition, this feature can be further explored to design highly specific and selective responsive systems for targeted functional applications.

3. Fabrication and Biomedical Applications of Polymeric Hybrid Superstructures

Considering physicochemical characteristics and advantages of inorganic NPs, co-assembly of synthetic block copolymers with colloidal inorganic NPs can afford a variety of hybrid superstructures with intriguing properties and functions. The co-organization of inorganic NPs with block copolymers can afford one-, two-, and three-dimensional hierarchical hybrid superstructures with enhanced electronic, magnetic, and optical properties relative to those of individual NPs and corresponding bulk polymers.[28, 79, 80] In the current perspective, we will mainly discuss polymer-directed organization of inorganic NPs such as gold NPs (Au NPs) and magnetic iron oxide NPs.

3.1 Hybrid Superstructures Fabricated from Block Copolymers with Au NPs

In this section, we discuss the fabrication of polymeric hybrid superstructures from synthetic block copolymers and Au NPs by focusing on recent advances. These hybrid assemblies exhibit strong surface plasmon resonances in the visible light range and attract much attention due to their spectrally tunable optical and photothermal conversion features, endowing them with appealing applications in optoelectronics, biosensing, and nanomedicine.[81-85]

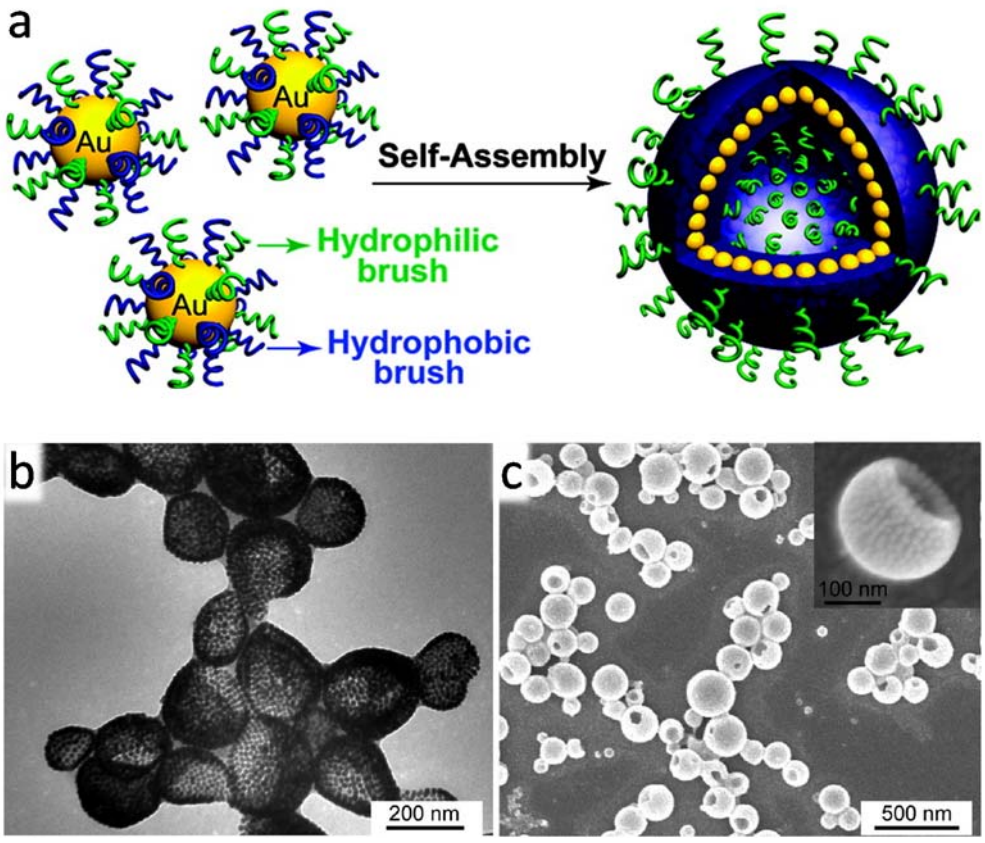

Figure 10. (a) Schematic illustration of the self-assembly of amphiphilic nanocrystals with mixed surface polymer brushes into vesicular structures. TEM (b) and SEM (c) images of plasmonic vesicles assembled from 14 nm gold nanocrystals with mixed poly(ethylene glycol) and poly(methyl methacrylate) brushes. Reproduced from ref. 86, Copyright 2011, American Chemical Society.

In an earlier example, Duan and coworkers[86] fabricated a novel type of plasmonic hybrid vesicles via the self-assembly of amphiphilic Au NPs coated with mixed polymer brushes (Figure 10). They functionalized the surface of Au NPs (spheres or rods) with two types of chemically distinct polymer chains, hydrophilic PEG and hydrophobic poly(methyl methacrylate) (PMMA), which are analogous to block copolymers as a whole. Hybrid vesicular nanostructures were fabricated from hybrid NPs due to the promotion of their amphiphilic nature. The self-assembling system consisting of inorganic nanocrystal cores and organic polymeric shells provides another angle of view to construct hybrid assemblies and the modulation of comprehensive properties of hybrid vesicles as well as their stimuli-triggered disruption.

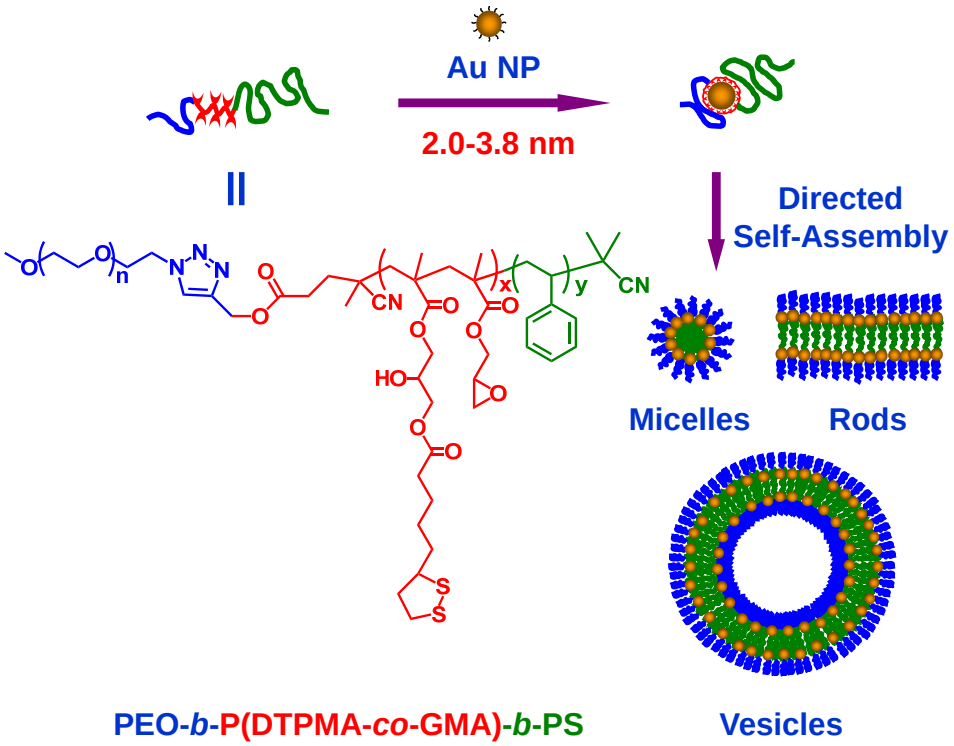

Figure 11. Schematic illustration of the template-free, size-selective fabrication of ultrasmall Au NPs (2.0–3.8 nm) anchored with a single triblock copolymer chain and the hierarchical self-assembly of the resulting amphiphilic hybrid Au NP triblock copolymers PEO–AuNP–PS in aqueous media. Reproduced from ref. 87, Copyright 2012, American Chemical Society.

Other examples based on amphiphilic Au NPs functionalized with both hydrophilic and hydrophobic polymers or small molecule ligands have also been reported and all of them can self-assemble into desired hybrid nanostructures.[88-91] In a typical example, Jiang *et al*.[92] developed a novel type of hybrid inclusion complex (HIC) with an Au NP core covered with α-cyclodextrins (α-CDs). Au NPs coated with α-CDs were further modified with poly(*N*-isopropyl acrylamide-*b*-*N*,*N*-dimethyl acrylamide) diblock terminated with azobenzene moieties, *Azo*-PNIPAM-*b*-PDMA, via host-guest recognition. As-prepared hybrid Au NPs can serve as building blocks to form hybrid vesicles exhibiting both photo- and thermo-responsiveness. Hu *et al*.[87] proposed another strategy for highly efficient, template-free and size-selective fabrication of ultrasmall anisotropic Au NPs (< 4 nm) anchored with a single amphiphilic triblock copolymer chain, which exploited the multidentate AuNP-binding

nature exhibited by the middle block and additional steric hindrance exerted by the two outer blocks (Figure 11). The triblock copolymer precursor was composed of hydrophilic PEO and hydrophobic polystyrene (PS) outer blocks and 1,2-dithiolane-functionalized Au NP-binding middle block. Aqueous self-assembly of preformed amphiphilic Au NP-functionalized hybrid triblock copolymers can afford various hybrid nanostructures, including spherical micelles, branching rods, vesicles, and large compound micelles. The strategy described in this work represents a general methodology for the highly efficient fabrication of monofunctionalized ultrasmall anisotropic inorganic NPs and their polymer-directed self-assembly into polymeric hybrid superstructures. In another valuable example, Nie and coworkers[93] reported that amphiphilic hybrid colloidal NPs fabricated from Au NPs and amphiphilic diblock copolymers can self-assemble into hybrid vesicles and tubular nanostructures consisting of a monolayer of hexagonally packed Au NPs. Most importantly, plasmonic properties of these hybrid nanostructures can be further modulated by the size of Au NPs and molecular weight of block copolymers.

Biomedical applications of Au NP-based plasmonic superstructures have also been extensively investigated. In a typical example, Duan and coworkers[94] fabricated hybrid plasmonic vesicles assembled from surface-enhanced Raman scattering (SERS)-active amphiphilic Au NPs for cancer-targeted intracellular drug delivery, and the drug release process can be dually monitored via plasmonic imaging and Raman spectroscopy (Figure 12). The pH-responsive disintegration nature of plasmonic vesicles, which were triggered by the hydrophobic-to-hydrophilic transition of initially hydrophobic brushes within acidic intracellular compartments, allowed for intracellular triggered drug release. Self-assembled plasmonic vesicles exhibited drastically different plasmonic properties and enhanced SERS intensity in comparison with single Au NPs due to strong interparticle plasmonic coupling. Thus, triggered disassembly of hybrid vesicles within specific endocytic compartments led to considerable changes in scattering properties and SERS signals, which can serve as independent feedback signals to monitor cargo release from hybrid vesicles.

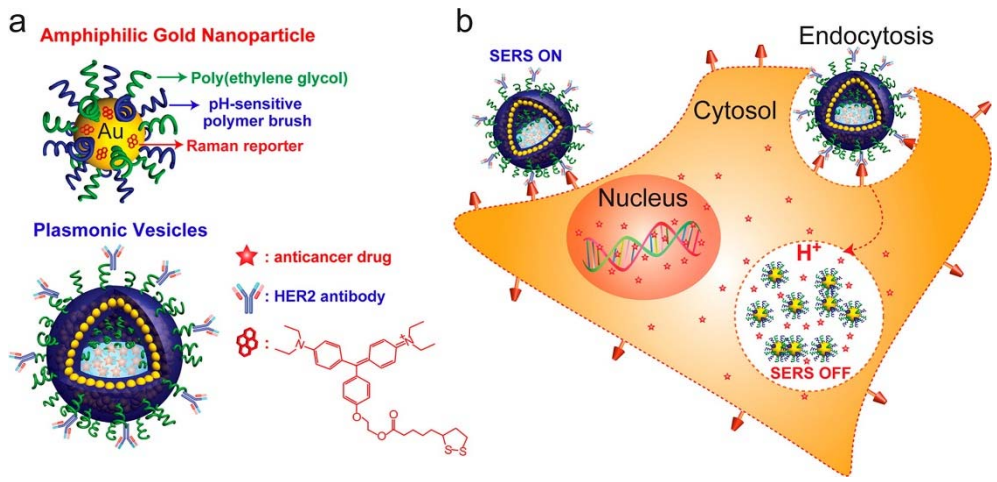

Figure 12. (a) Schematic illustration of amphiphilic Au NPs coated with Raman reporter BGLA and mixed polymer brushes of hydrophilic PEG and pH-sensitive PMMAVP grafts and drug-loaded plasmonic vesicles surface tagged with HER2 antibody for cancer cell targeting. (b) The cellular binding, uptake, and intra-organelle disruption of SERS-encoded pH-sensitive plasmonic vesicles. Reproduced from ref. 94, Copyright 2012, American Chemical Society.

Just recently, Nie, Lu and coworkers[95] reported another unique example of polymer-directed Au NP assembly by concurrent self-assembly of organic molecular amphiphiles (MAMs) and inorganic nanoparticle amphiphiles (NPAMs). This strategy combined inherent properties of both building blocks, affording novel Au hybrid superstructures with increasing complexity and functionality (Figure 13). Typical self-assembled nanostructures included patchy vesicles with multiple NPAM domains surrounded by MAM phases, Janus-like vesicles with distinctive MAM and NPAM halves, and heterogeneous vesicles with uniform distribution of NPAMs. They proposed that the formation of multiple hierarchical nanostructures resulted from the delicate interplay between the dimension mismatch of two types of amphiphiles, the entanglement of polymer chains, and the mobility of NPAMs. In this study, it was notable to fabricate Janus hybrid vesicles with non-spherical shapes such as hemispherical and disk-like shapes. The synergistic assembly strategy serves as a solid foundation for the fabrication of structurally complex functional hybrid superstructures. Meanwhile, this example significantly broadens the library of parameters for controlled self-assembly, as compared to the self-assembly of pure organic amphiphiles or hybrid

nanoparticle amphiphiles.

In this section, representative developments concerning the fabrication and biomedical explorations of polymeric Au NP hybrid superstructures were discussed. It should be noted that in future studies, it is highly desirable to integrate novel molecular design with increased systematic complexity and further elucidate the underlying self-assembling mechanism. Meanwhile, concerning their biomedical applications, in-depth biological evaluations such as *in vitro* cellular analysis and *in vivo* study of self-assembling Au NP hybrid superstructures are also critical.

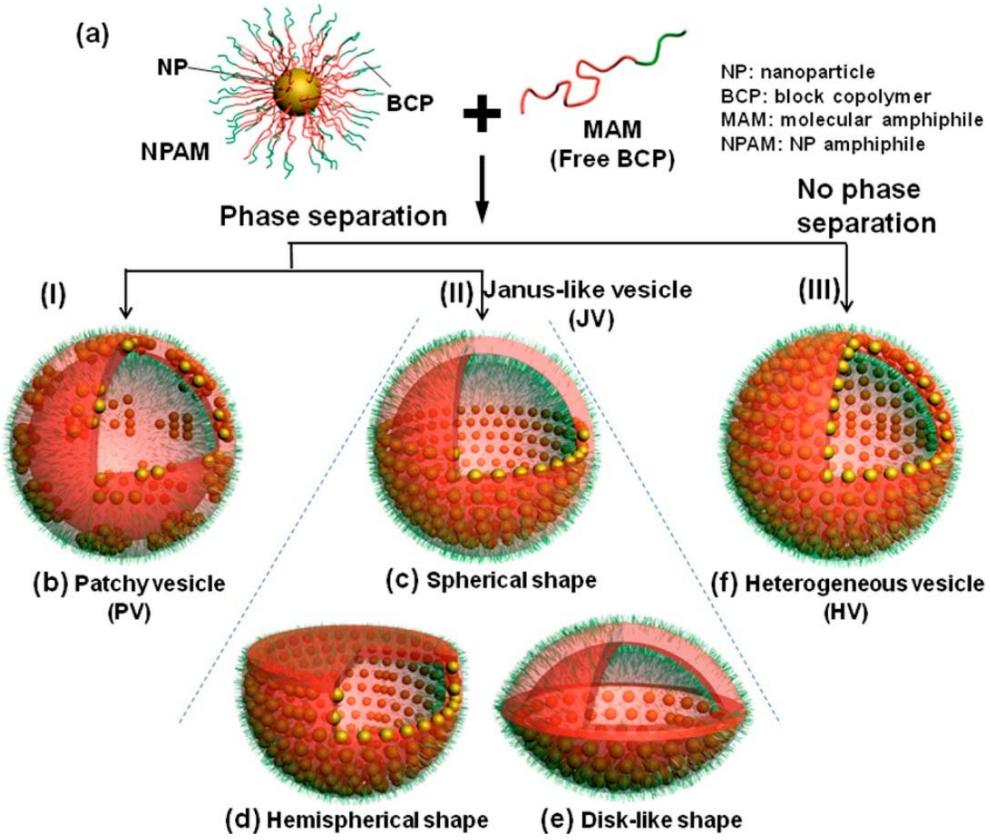

Figure 13. Schematic illustration of the co-assembly of binary mixtures of molecular amphiphiles (MAMs) and inorganic nanoparticle amphiphiles (NPAMs) into hybrid vesicles with defined shape, morphology, and surface pattern. The NPAM consists of an inorganic NP surface tethered with amphiphilic linear block copolymers, whereas free amphiphilic block copolymers are used as MAM (a). When phase separation occurs (I and II), the concurrent assembly affords spherical hybrid PVs with multiple NPAM domains (b) and hybrid JVs with distinguished MAM and NPAM halves (c–e). The JVs acquire a spherical (c),

hemispherical (d), and disk-like (e) shapes depending on self-assembling parameters. When no phase separation occurs (III), the self-assembly generates HVs with uniform distribution of NPAMs in the membrane (f). Reproduced from ref. 95. Copyright 2014, American Chemical Society.

3.2 Superstructures Fabricated from Block Copolymers with Magnetic Iron Oxide NPs

Magnetic NPs including superparamagnetic iron oxide (SPIO) NPs possess combined advantages such as T_2-type magnetic resonance (MR) imaging contrast enhancement, imaging-directed *in vivo* delivery under external magnetic field, and hyperthermia cancer therapy under alternating magnetic field.[96-98] Among these, much progress has been made in the fabrication of polymeric hybrid assemblies involving SPIO NPs and exploration of their biomedical applications.[99-110] In a typical study, Hu *et al*.[111] fabricated hybrid micelles with micellar cores loaded with hydrophobic drugs and micellar coronas stably anchored with hydrophilic SPIO NPs (Figure 14). The theranostic platform possesses combined functions for anticancer drug delivery and MR imaging contrast enhancement. Poly(ε-caprolactone)-*b*-poly(glycerol monomethacrylate), PCL-*b*-PGMA, and PCL-*b*-P(OEGMA-*co*-FA) diblock copolymers were synthesized at first by combining ring-opening polymerization (ROP) and ATRP, where OEGMA and FA are oligo(ethylene glycol) monomethyl ether methacrylate and folic acid-bearing moieties, respectively. A model hydrophobic chemotherapeutic drug, paclitaxel (PTX), was loaded into the hydrophobic micellar cores, and 4 nm SPIO NPs were simultaneously incorporated into the hydrophilic coronas of mixed micelles via strong binding affinity between 1,2-diol moieties in PGMA and Fe atoms at the surface of SPIO NPs. Controlled and sustained release of PTX from hybrid micelles was observed in the system. It was remarkable to achieve considerably enhanced T_2 relaxivity (r_2 = 121.1 s^{-1} mM^{-1} Fe) for the clustering of SPIO NPs within micellar coronas as compared with that of surfactant-stabilized single SPIO NPs (r_2 = 28.3 s^{-1} mM^{-1} Fe). Together with preliminary in vivo MR imaging analysis, the hybrid micelles could serve as a T_2-weighted MR imaging contrast enhancer with improved performance. This work indicated that functional polymeric micelles with surface-embedded SPIO NPs at the hydrophilic corona could act as a novel theranostic platform for targeted drug delivery, controlled release, and diagnostic functions.

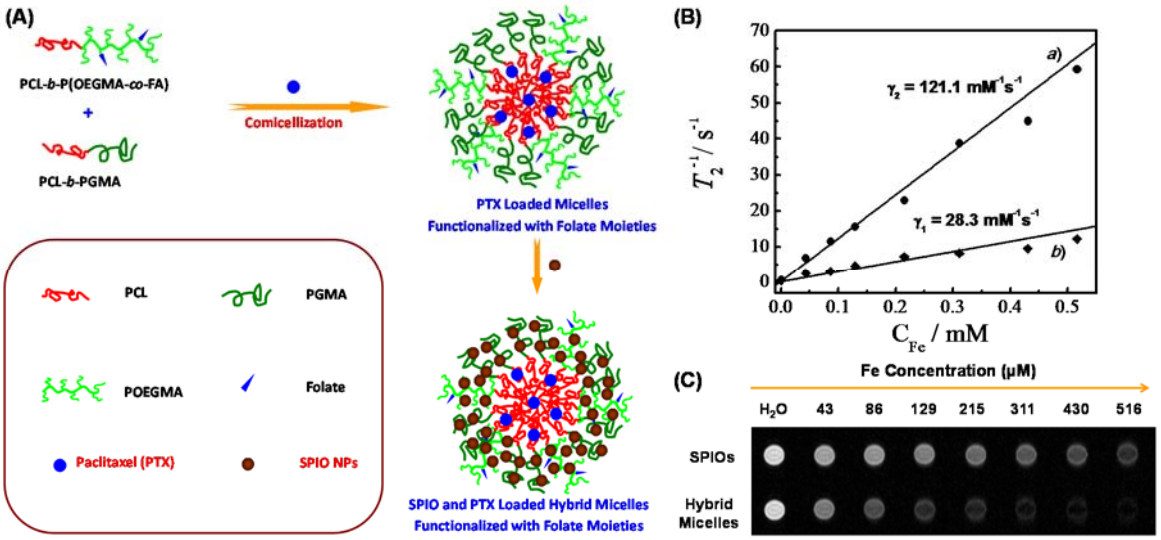

Figure 14. (A) Schematic illustration of the fabrication of hybrid micelles comprising of hydrophobic PCL cores and mixed PGMA/P(OEGMA-*co*-FA) hydrophilic coronas embedded with 4 nm SPIO nanoparticles from PCL-*b*-PGMA and PCL-*b*-P(OEGMA-*co*-FA) diblock copolymers. (B) T_2 relaxation rates ($1/T_2$) as a function of iron concentration recorded for the aqueous dispersion (25 °C) of (a) CTAB-stabilized SPIO nanoparticles and (b) hybrid micelles comprising hydrophobic PCL cores and mixed PGMA/P(OEGMA-*co*-FA) hydrophilic coronas embedded with ~5 w/w% SPIO nanoparticles (4 nm) fabricated from PCL-*b*-PGMA and PCL-*b*-P(OEGMA-*co*-FA) diblock copolymers (5/1, w/w). (C) T_2-weighted MRI images obtained for CTAB-stabilized SPIO nanoparticles and hybrid micelles, respectively. Reproduced from ref. 111. Copyright 2012, American Chemical Society.

The fabrication of intricate magnetic iron oxide NP hybrid superstructures with high transverse relaxation rates are highly desired considering their MR imaging applications. In this context, many efforts have been input in Park's group during the past few years.[112-115] They investigated the self-assembly of magnetic NPs with a typical amphiphilic block copolymer, poly(acrylic acid)-*b*-polystyrene (PAA-*b*-PS) (Figure 15). Depending on solvent compositions, three types of self-assembled nanostructures were obtained: (a) core-shell type polymeric assemblies where NPs were radially arranged at the core-shell interface, (b) spherical micelles where NPs were homogeneously embedded, and (c) polymersomes with the bilayer densely packed with NPs (magneto-polymersomes). Additionally, they found that the introduction of magnetic NPs can significantly affect the morphology of self-assembled

nanostructures compared to pure block copolymers. For example, the self-assembly of micelle-forming block copolymers typically afforded magneto-polymersomes instead of magneto-micelles. Remarkable effects of as-prepared hybrid nanostructures on the overall magnetic relaxation properties were also demonstrated, exhibiting considerably different T_2 relaxivity with 154 ± 1, 64 ± 1, and 167 ± 1 s^{-1} mM^{-1} for core-shell magneto-assemblies, magneto-micelles, and mixture of magneto-micelles and magneto-polymersomes, respectively. These results highlighted the importance of self-assembling morphologies, NP arrangement, and overall nanostructure sizes on MR signal enhancement.

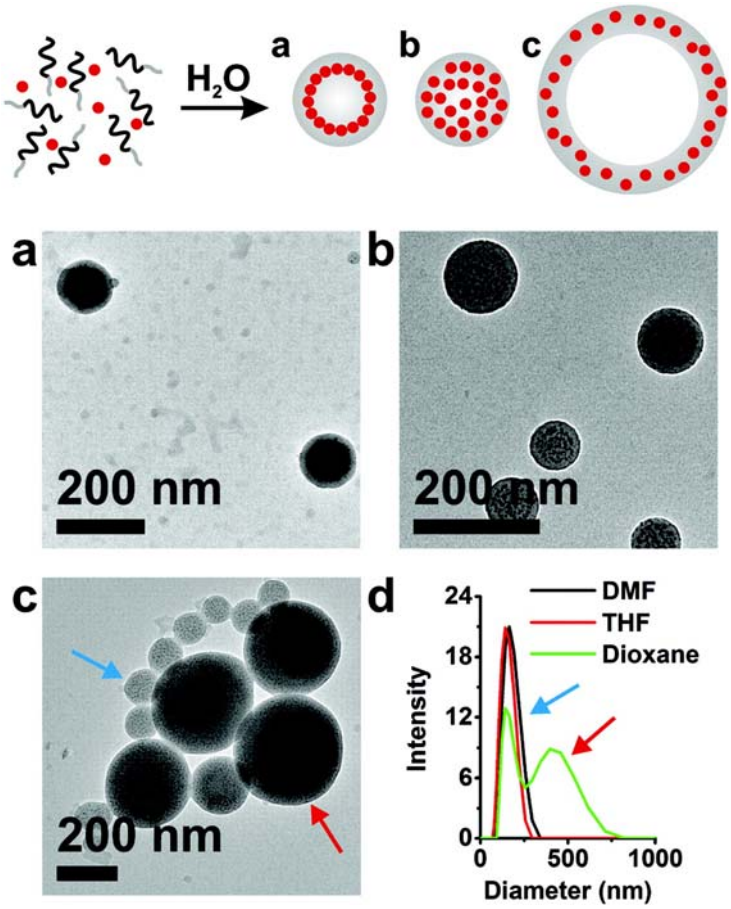

Figure 15. Schematic illustration for the self-assembly of magnetic NPs with PAA-*b*-PS block copolymers in various solvents and the corresponding self-assembled morphologies. (a) Magneto-core shell assemblies formed when DMF/THF mixture (96.8% DMF) was used as the initial cosolvent for polymers and nanoparticles. (b) Magneto-micelles self-assembled in THF. (c) Magneto-polymersomes self-assembled in dioxane/THF (96.8% dioxane). (d) DLS data for the assemblies formed with three different solvents. Reproduced from ref. 112. Copyright 2011, American Chemical Society.

They further investigated the size-dependent fabrication of hybrid polymersomes from magnetic NPs and PAA-*b*-PS amphiphilic diblock copolymers (Figure 16).[116] Polymersomes densely packed with magnetic NPs in the polymersome membrane (magneto-polymersomes) were fabricated on the basis a series of iron oxide NPs with different sizes. They found that NP localization within polymersome membranes was also dependent on the size of magnetic NPs. Small NPs were dispersed in a bilayer of polymersome, larger ones formed well-ordered superstructures at the interface between inner and outer layer of the bilayer membrane. Size increment of magnetic NPs can promote the fabrication of magneto-polymersomes. Meanwhile, the size of self-assembled polymersomes was also effectively modulated by varying the size of incorporated NPs. The size-controlled self-assembly was attributed to the polymer chain entropy effect and the size-dependent distribution of NPs within polymersome bilayers. Moreover, the T_2 relaxivity of magneto-polymersomes considerably increased with increasing NP sizes and decreasing polymersome dimensions. The highest value can reach up to 555 ± 24 s^{-1} mM^{-1} for 241 ± 16 nm polymersomes.

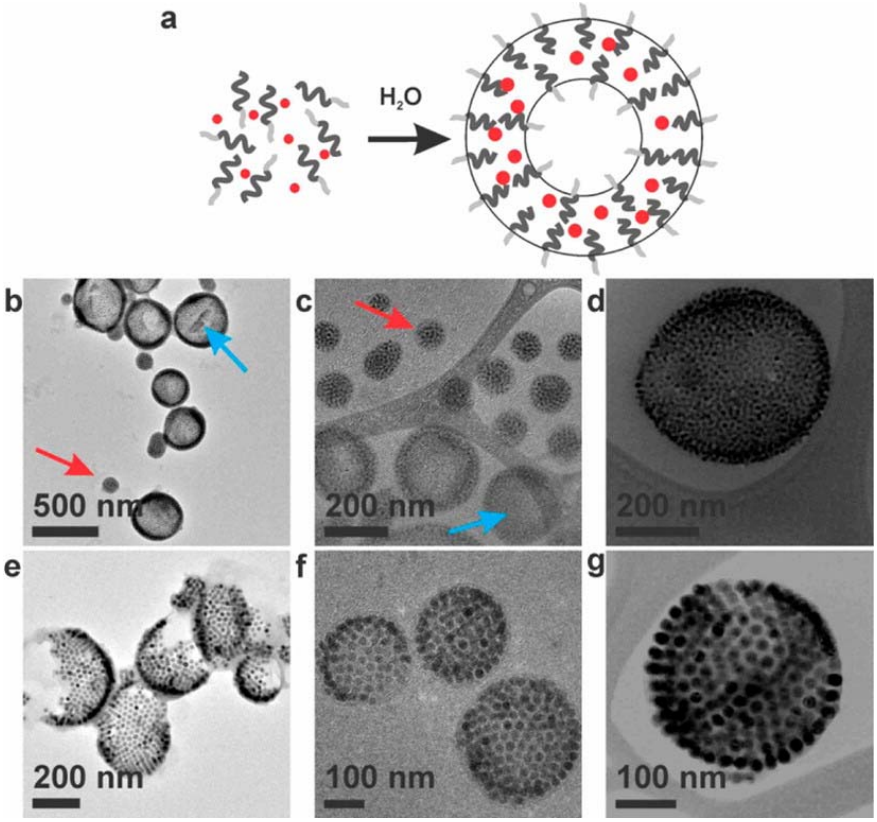

Figure 16. (a) Schematic representation of the self-assembly of magneto-polymersomes from iron oxide NPs and amphiphilic block copolymers, PAA-*b*-PS. Light gray lines, dark gray

lines, and red dots represent PAA, PS, and iron oxide nanoparticles, respectively. (b-d) Conventional TEM (b), cryo-TEM (c), and cryo-STEM (d) images of magneto-polymersomes self-assembled with 10.8 nm iron oxide nanoparticles. Blue and red arrows indicate polymersomes and micelles, respectively. (e-g) Conventional TEM (e), cryo-TEM (f), and cryo-STEM (g) images of magneto-polymersomes assembled with 19.9 nm particles. Cryo-TEM images show intact magneto-polymersomes, indicating that the broken polymersomes observed in normal TEM (e) are due to the drying process. Reproduced from ref. 116. Copyright 2014, American Chemical Society.

On the other hand, Boyer, Davis and coworkers[117] recently reported *in situ* formation of magnetic NPs within preformed polymeric nanostructures (Figure 17). They employed polymerization-induced self-assembly (PISA) to fabricate POEGMA-*b*-PMAA-*b*-PS nanostructures, where PMAA is poly(methacrylic acid). Subsequently, carboxylic acid moieties in middle block were used to anchor Fe(II) and Fe(III) mixed irons. Iron oxide NPs were then in-situ formed at the hydrophobic/hydrophilic interface within polymeric nanostructures. The resultant hybrid superstructures were evaluated as negative MRI contrast agents, displaying significantly high and different transverse relaxivities for distinct nanostructures, which were up to 582 mM^{-1} s^{-1} for hybrid spherical micelles, 412 mM^{-1} s^{-1} for hybrid rods, and 277 mM^{-1} s^{-1} for hybrid vesicles respectively.[118] This work extended PISA-based polymeric nanostructures to the fabrication and theranostic applications for polymeric magnetic superstructures.

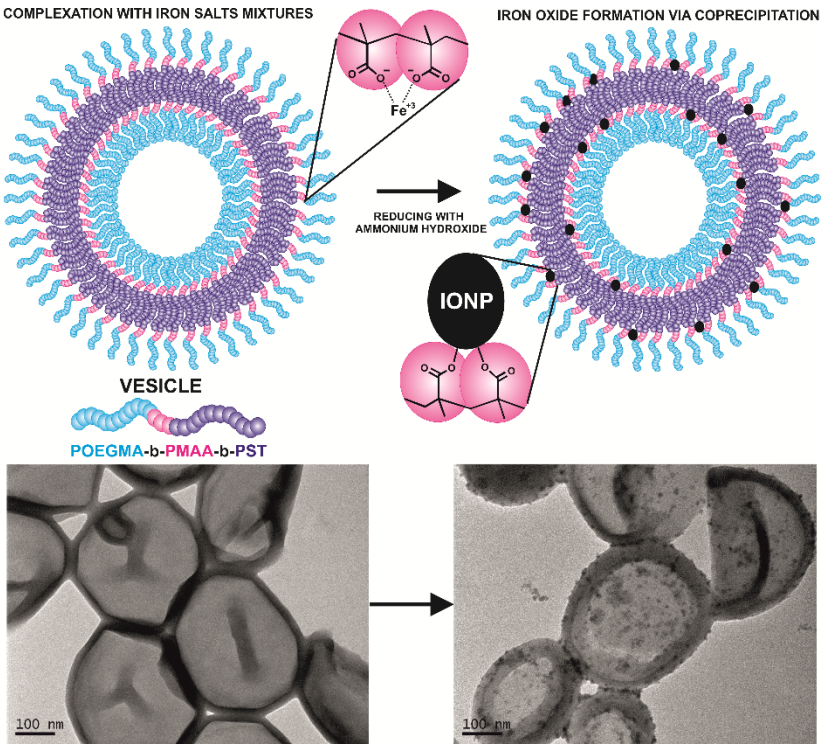

Figure 17. Schematic illustration and TEM images for in-situ formation of magnetic iron oxide NPs within polymeric nanostructures preformed via polymerization-induced self-assembly. Reproduced from ref. 117. Copyright 2014, American Chemical Society.

In this section, we discussed recent advances in block copolymer-assisted magnetic iron oxide NP assembly and their potential biomedical functions. In terms of their applications in bio-imaging and drug delivery, it is highly desirable to further explore novel stimuli-responsive moieties, targeting ligands, and chain topological effects of block copolymers. Furthermore, the effect of shape and size of inorganic NPs on the self-assembling process and bio-functional performance need to be further elucidated. Finally, it is imperative to assay the biocompatibility and toxicity of inorganic nanoparticle based hybrid assemblies.

4. Conclusions

In the past few years, a number of novel strategies have emerged towards the fabrication and biomedical exploration of stimuli-responsive polymeric assemblies as well as their hybrid superstructures. Many other intricate polymeric self-assembly systems such as the fabrication of two-dimensional nanostructures[119-123] and polymer-DNA complex assemblies [124] have been also developed, but not discussed in detail here. This perspective focuses on five burgeoning research hotspots, i.e., polyprodrug-based self-assembly and therapeutic

applications, stimuli-responsive microstructural modulation for polymersomes, host-guest recognition assisted fabrication of synthetic polymeric assemblies, hybrid superstructures fabricated from block copolymers and inorganic NPs. Challenges and future developments in these five research directions have already been described at the end of each section. In summary, the reproducible and controlled mass fabrication and biomedical explorations of polymeric assemblies as well as hybrid superstructures with well-defined physicochemical properties and tailor-made functions are longstanding challenges. Future endeavors in this field are further proposed as described below.

Firstly, from the point of view of chemical compositions and chain architectures of polymers, current investigations concerning stimuli-responsive polymeric assemblies and hybrid superstructures have focused on linear polymers. Many other chain topologies such as star copolymers, multiblock copolymers, dendrimers, and hyperbranched polymers can be further explored. In addition, the integration of cell or tissue-targeting ligands, new stimuli-responsive motifs, and novel molecular design as well as new self-assembling mechanisms can surely enrich current systems.

On the other hand, considering the practical applications of responsive polymeric assemblies and hybrid superstructures, the possibility of controlled large-scale fabrication and facile operation as well as examination of the stability of NP assemblies deserves much more attention in future works. Otherwise, all current endeavors are just under the scope of laboratory experimental exploration.

Finally, much more efforts can be input to interrogate the *in vivo* fate of polymeric assemblies and hybrid nanostructures as well as their degraded residues. Overall, the research field described in this perspective is worthy of systematic and in-depth future explorations, aiming to design and construct novel stimuli-responsive polymeric assemblies and corresponding hybrid nanostructures with optimum biomedical performance, which calls for more revolutionary innovations and novel emerging concepts. We are keen to see more remarkable progresses in this promising field.

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