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Cooperative Macromolecular Self-Assembly toward Polymeric Assemblies with Multiple and Bioactive Functions

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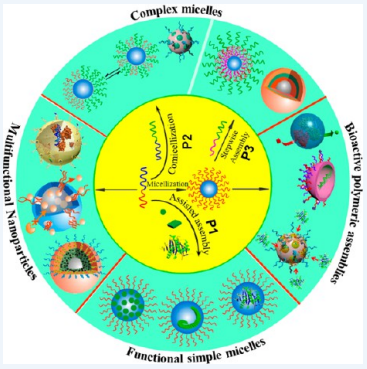
Supporting Information

CONSPECTUS: In the past decades, polymer based nanoscale polymeric assemblies have attracted continuous interest due to their potential applications in many fields, such as nanomedicine. Many efforts have been dedicated to tailoring the three-dimensional architecture and the placement of functional groups at well-defined positions within the polymeric assemblies, aiming to augment their function. To achieve such goals, in one way, novel polymeric building blocks can be designed by controlled living polymerization methodology and advanced chemical modifications. In contrast, by focusing on the end function, others and we have been practicing strategies of cooperative self-assembly of multiple polymeric building blocks chosen from the vast library of conventional block polymers which are easily available. The advantages of such strategies lie in the simplicity of the preparation process and versatile choice of the constituent polymers in terms of their chemical structure and functionality as well as the fact that cooperative self-assembly based on supramolecular interactions offers elegant and energy-efficient bottom-up strategies. Combination of these principles has been exploited to optimize the architecture of polymeric assemblies with improved function, to impart new functionality into micelles and to realize polymeric nanocomplexes exhibiting functional integration, similar to some natural systems like artificial viruses, molecular chaperones, multiple enzyme systems, and so forth.



In this Account, we shall first summarize several straightforward designing principles with which cooperative assembly of multiple polymeric building blocks can be implemented, aiming to construct polymeric nanoassemblies with hierarchal structure and enhanced functionalities. Next, examples will be discussed to demonstrate the possibility to create multifunctional nanoparticles by combination of the designing principles and judiciously choosing of the building blocks. We focus on multifunctional nanoparticles which can partially address challenges widely existing in nanomedicine such as long blood circulation, efficient cellular uptake, and controllable release of payloads. Finally, bioactive polymeric assemblies, which have certain functions closely mimicking those of some natural systems, will be used to conceive the concept of functional integration.

1. INTRODUCTION

Polymers are one of the popular building blocks in self-assembly for versatile functional nanoassemblies, of which morphologies, architectures, and functions are the three key themes that have been intensively pursued during the past decades.[1,2] For instance, amphiphilic block copolymers can associate to form polymeric micelles (PMs) in a block-selective solvent, resulting in several classic morphologies including spherical, wormlike micelles, and vesicles. Significant progress has been made recently to create PMs or, more generally, polymeric assemblies with novel architectures and morphologies that were unimaginable before.[1,3] For practical applications, especially in the biomedical field, a plethora of multifunctional polymeric nanoassemblies has been created which can perform targeting, imaging, and therapeutic function in concert.[4]

Either for controlling the morphology and architecture of polymeric assemblies or for the fabrication of multifunctional nanocarriers, the polymeric building blocks (PBBs) are normally synthesized on a case-by-case basis. Such successes are fueled by advanced living control polymerization and chemical modification techniques, which make it possible to prepare tailored PBBs with predefined block sequence,[5] block ratios, or hierarchical architectures like hyperbranched polymers,[6] molecular bottle brushes, or supramolecular polymers[7] and to incorporate stimuli responsive or crystalline blocks.[8] By these efforts, the number of novel PBBs have been increasing exponentially. However, for practical applications, cost versus benefit is a question that has to be taken into account.[9] Synthesis of tailor-designed block polymers often requires specific chemistries that sometimes work in narrow synthesis parameter windows, or relies on multiple steps that limit their general use. In addition, a gap exists between the seemingly unlimited possibility to design polymeric assemblies with exotic morphologies or architectures and advanced end functions.[3] This is probably due to the fact that the exotic morphologies or architectures of polymeric assemblies are often realized by subtle manipulation of in situ microphase separation of

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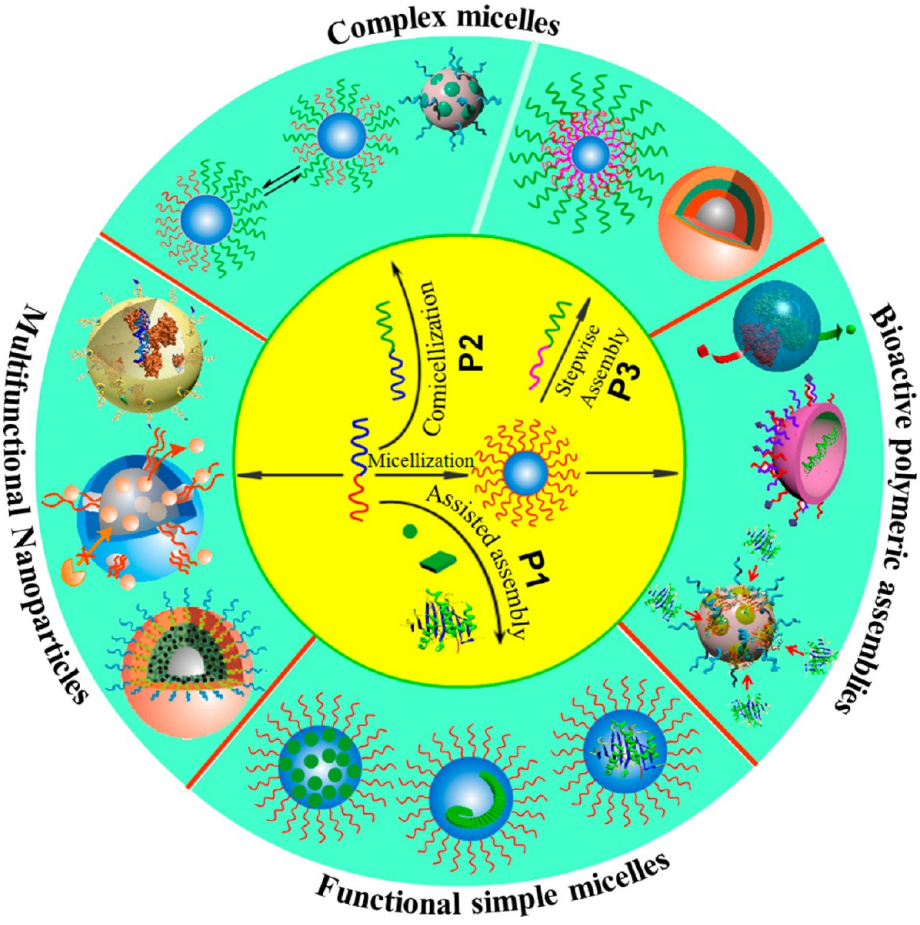

Figure 1. Schematic illustration of the core principles of cooperative self-assembly of multiple PBBs and the resulting functional polymeric assemblies.

deliberately designed block polymers,[3] during which payloads or functional components are difficult to be introduced simultaneously. Therefore, the conventional core−shell structure of polymeric micelles or vesicles is still the main platform of many researchers with practical applications in mind, and postsurface modification is normally used to install their favorite polymeric assemblies with targeting, imaging, and therapeutic capabilities.[10]

Just like general supramolecular chemistry, fabrication of polymeric nanoparticles by self-assembly might learn some tricks from nature, which often builds minimal complex units with determined function from some elementary components, in a bottom-up approach and driven by weak interactions. In this way, new advanced functionalities, that each single constituent component could not produce individually, can appear from the collective and synergistic behavior of multicomponent systems. Many examples in nature, such as the light-harvesting system of the photosynthetic center in plants, viruses, or cells, represent different levels of structural and functional complexity that can be accessed from a limited number of units by combining hierarchical levels of organization. Similarly, in the function oriented polymeric self-assembly, the ideal case might be to screen a library of PBBs with varied function and properties. Cooperative self-assembly of several such PBBs, combining environmental stimuli is then used to fabricate nanoscale polymeric assemblies with defined structure and functionality. Such ideas have been manifested in the pioneering work of several groups who have created several general platforms of multifunctional polymeric assemblies based on a narrow range of PBBs.[4,10] Especially, Zhang et al. have developed special selenium-containing block copolymers which can assemble into polymeric particles and disassemble via responding to multiple stimuli such as dual redox, low dose γ-irradiation, and so forth. Such kinds of micelles have been used as synthetic enzyme mimics and versatile drug-delivery systems for the combination of radiotherapy and chemotherapy.[11]

In this Account, we aim to illustrate the possibilities to fabricate polymeric assemblies with defined structure and enhanced functionality through cooperative self-assembly of conventional PBBs which are relatively easy to obtain. We intend to illustrate several flexible and efficient self-assembling principles exploited by us and many others to implement cooperative self-assembly of PBBs and other functional species (section 2, Figure 1). Derivatives and combination of these principles lead to multifunctional nanoparticles that can address certain aspects of the challenges in nanomedicine (section 3). Integration of environmental stimuli and functional biomolecules can further lead to advanced polymeric assemblies that mimic certain functions of natural systems (section 4).

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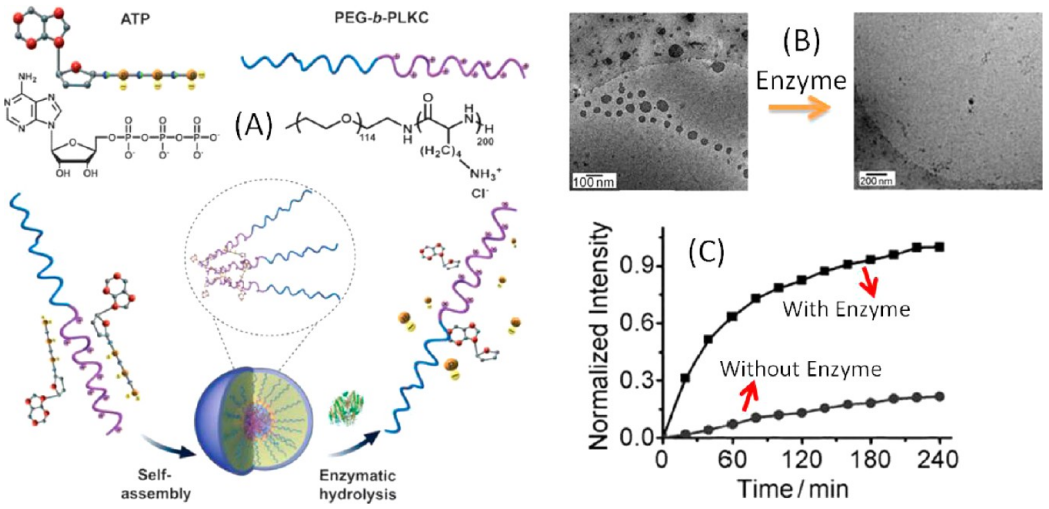

Figure 2. Enzyme-responsive polymeric micelles (A). (B) Cryo-TEM demonstrates the disassembly of the complex micelle due to enzymolysis. (C) Enzyme responsive release behavior. Reproduced with permission from ref 15. Copyright 2010 WILEY-VCH Verlag GmbH & Co. KGaA.

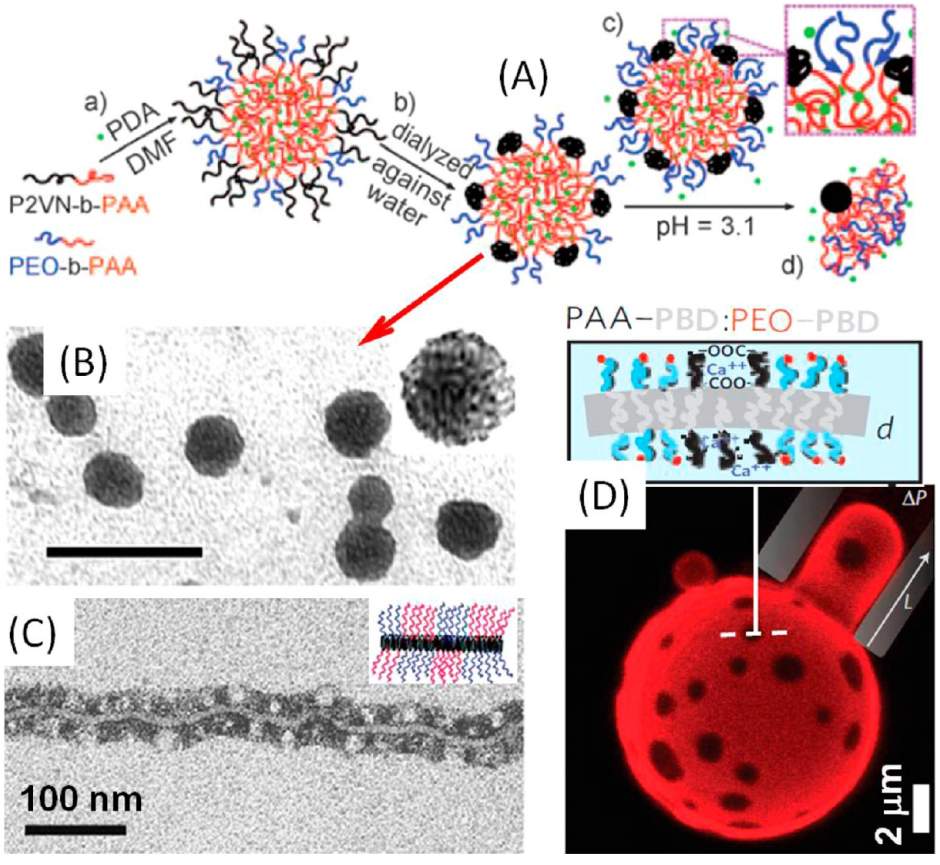

Figure 3. MSPMs and controlled demixing of the mixed shell (A and B). Reprinted with permission from ref 20, Copyright 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (C) Wormlike micelles consisting of a polyethylene crystal core and a mixed PS/PMMA shell. Reproduced with permission from ref 21, Copyright 2012 Elsevier BV. (D) Spotted vesicles formed by ionic bridging lateral segregation in the mixed PAA/PEO shell. Reprinted with permission from Macmillan Publishers Ltd: Nature Materials (ref 22), Copyright 2009.

2. COOPERATIVE SELF-ASSEMBLY OF MULTIPLE POLYMERIC BUILDING BLOCKS: DESIGNING PRINCIPLES

2.1. Self-Assembly of Block Copolymers Assisted by Functional Molecular Species (P1)

In parallel with the efforts to further expand the structure and functionality of core−shell polymeric micelles through modern polymerization techniques and advanced chemistry, many researchers have constructed micellar assemblies through the assembly of block copolymers, especially double hydrophilic block polymers, assisted by other functional species (hereafter referred as coassembling agents (CAAs)) (P1 in Figure 1). CAAs include multivalent ions, drug molecules, low molecular weight surfactants, polyelectrolytes, or synthetic homopolymers of various architectures.[12−14] Biopolymers such as proteins and

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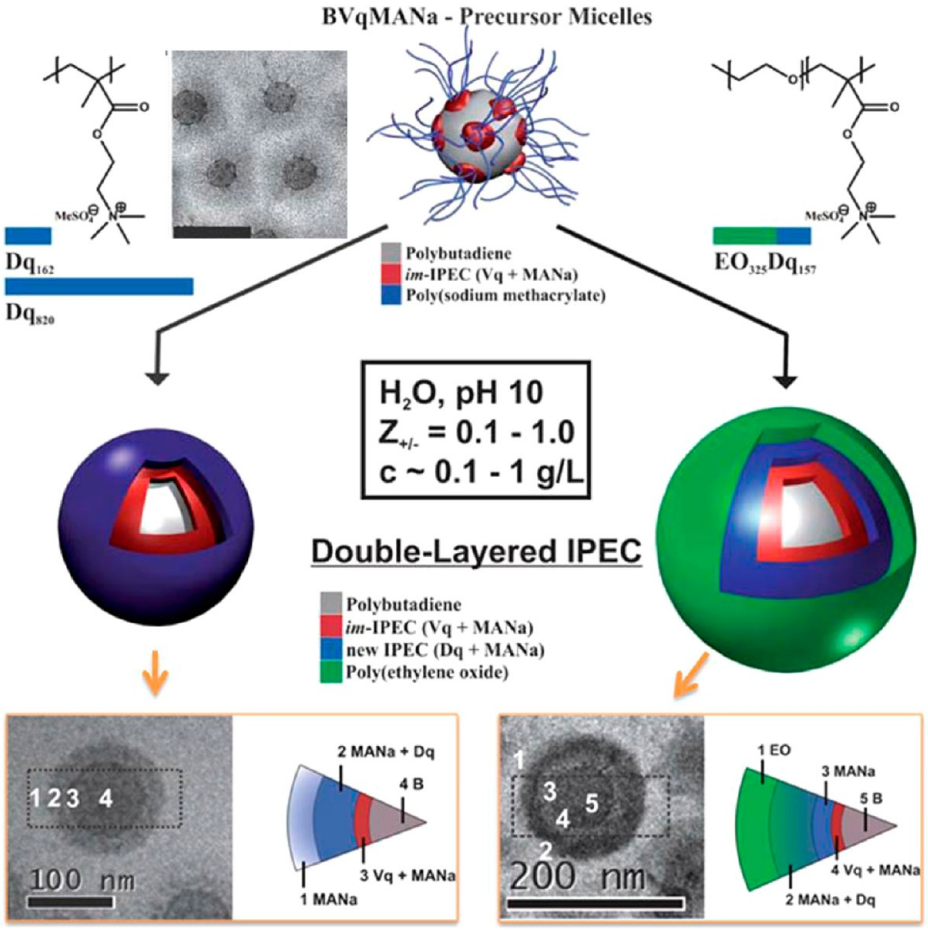

Figure 4. Multilayered core–shell–shell–corona assembles from PB-*b*-P2VPq-*b*-PMANa block terpolymer micelles and either PDMAEMAq homopolymers (left) or a PEO-*b*-PDMAEMAq diblock copolymer (right). Reproduced with permission from ref 28, Copyright 2010 Royal Society of Chemistry.

DNA/RNA have also been widely recruited as CAAs.[4] Weak interactions, such as electrostatic, host–guest recognition, and hydrogen bonding, are the main driving forces of the self-assembly while CAAs can also play the role of chemical cross-linkers.[14] The key advantage of this principle is that polymeric micellization can be expanded to a wide range of polymers that otherwise cannot form micelles. Furthermore, although the resulting architecture of the final complex normally has the core–shell structure, the micellar core possesses advanced functions brought by the CAAs that have their own excellent photo-responsive, catalytic properties or therapeutic functionalities. The core can be further endowed with pH, redox, sugar, enzyme, or temperature responsiveness through which payloads can be released.[4,14,15] Zhang's group used adenosine 5′-triphosphate (ATP) to induce the micellization of PEG-*block*-poly(L-lysine hydrochloride) (PEG-*b*-PLKC) via the complexation of ATP with the PLKC block, and the resulting micelles have enzyme-responsiveness due to the hydrolyzation of ATP by phosphatase (Figure 2).[15] We have created several kinds of micelles with tunable chiral tetrakis-(4-sulfonatophenyl)-porphyrin (TPPS) aggregates in the micellar core through TPPS induced micellization of PEG-*block*-poly(4-vinylpyridine) (PEG-*b*-P4VP) in acidic aqueous media. If ionic metallic TPPS (e.g., ZnTPPS) was used, the resulted polymeric micelles can effectively protect metallic TPPSs against demetallization and act as photosensitizers in aqueous media.[16]

2.2. Co-Micellation of Multiple Block Polymers (P2)

Polymer blends consisting of several dissimilar polymers represent an efficient way to create new materials which take the best out of the constituent polymers. Similarly, it is templating to coassemble several block polymers into a complex polymeric micelles through which the structural and chemical diversity of the constitute polymers are conveniently incorporated into the final micelles (P2 in Figure 1). Herein, we mainly focus on mixed shell polymeric micelles (MSPMs) with at least two polymer blocks coexisting in their shell, obtained by co-micellation of multiple block polymers (Figure 3A).[17] For instance, co-micellation of poly(*tert*-butyl acrylate)-*block*-poly(*N*-isopropylacrylamide) (PtBA-*b*-PNIPAM) and PtBA-*block*-poly(4-vinylpyridine) (PtBA-*b*-P4VP) resulted in MSPMs with a mixed shell consisting of PNIPAM and P4VP.[17] This strategy offers a very convenient way to tune the outermost surface of polymeric assemblies, which is responsive for the stabilization against aggregation and interactions with the surrounding environment. While payloads can still be incorporated into the micellar core, predefined amounts and type of functional groups with targeting or recognition functions can be easily installed onto the micellar surface via co-micellation of prefunctionalized polymeric components that have desired functional species.[18,19] Another key advantage of the MSPMs lies in the fact that the compatibility of the two shell-forming polymeric blocks can be manipulated to induce demixing or

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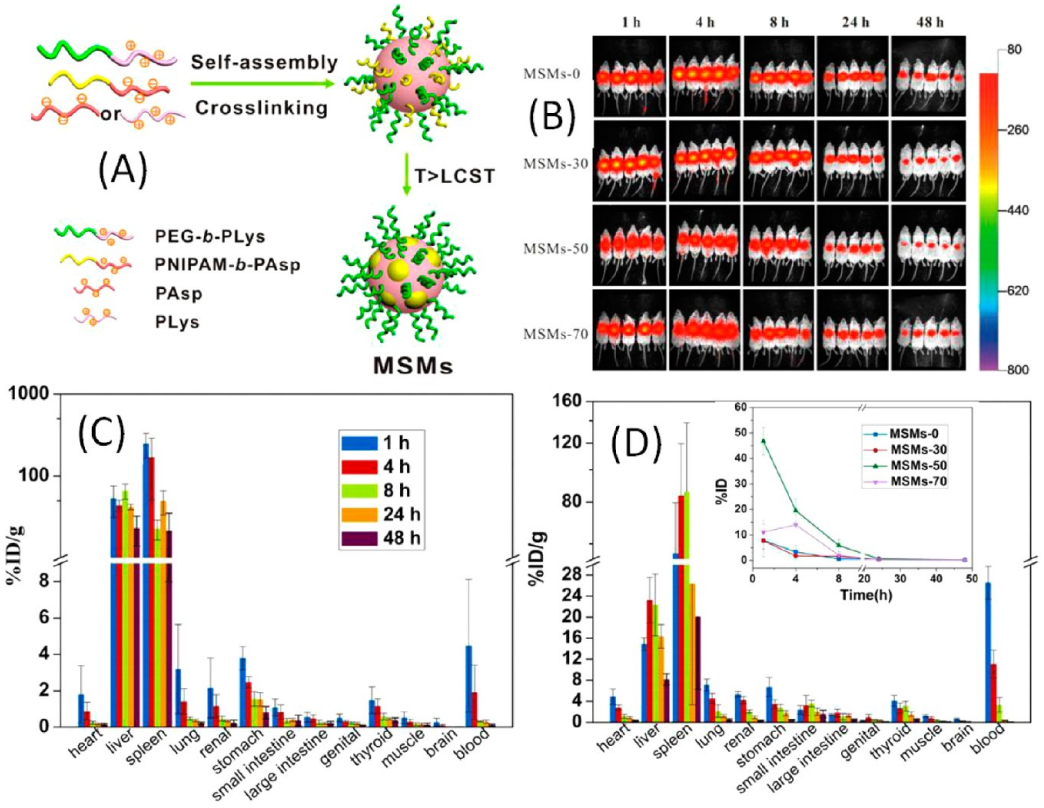

Figure 5. Blood circulation of MSPMs with a microphase separated surface. (A) Schematic illustration of the formation of the MSPMs. (B) Gamma-camera images of MSPMs in mice after i.v. (C and D) In vivo biodistribution of simple micelles with only PEG in the shell (C) and MSPMs with a 50:50 (w/w) PEG/PIPAM mixed shell (D) labeled with ^{125}I. Inset of (D): Blood clearance curves of MSPMs in mice after i.v. Reproduced with permission from ref 31. Copyright 2013 American Chemical Society.

phase segregation, resulting in domains of a nanometer size in the shell (Figure 3).[20−22] Controlled segregation of the shell blocks has been intensively practiced recently, aiming to prepare micelles with Janus or patchy nature and to induce their subsequent hierarchical self-assembly.[23,24]

2.3. Stepwise Assembly of Polymeric Precursor Micelles with Other Polymers (P3)

Principles P1 and P2 can be used to introduce heterogeneity and functionalities into the core or surface of conventional core−shell polymeric assembles, respectively. To create nano-assemblies with enhanced function or sophisticated architecture, the core−shell polymeric micelles themselves can be used as building blocks. Herein, we summarize the idea to construct complex nanoassemblies through in situ complexation of the shell of a precursor micelle with a secondary free (block) polymer, driven by hydrogen boning, electrostatic interactions, or other noncovalent interactions, resulting in core−shell−corona (CSC) or onion type micelles (P3 in Figure 1).[25] We have demonstrated that the PAA shell of the micelles formed by PS-*b*-PAA can further complex with the P4VP block of PEG-*b*-P4VP, induced by hydrogen bonding between the two blocks, resulted in spherical CSC micelles with a PS core, a PAA/P4VP complex shell, and a PEG corona.[26] CSC micelles belong to multicompartment polymeric micelles (MPMs), an intriguing class of self-assembled aggregates with segregated domains, where two (or more) types of distinct regions coexist.[27] Thanks to the vast possibilities of combining different precursor polymeric particles with secondary functional polymers, this strategy is a very versatile way to prepare CSC micelles with orthogonal properties within a single nanoparticle.

It is noted here that the above three designing principles is not the complete list of the possibilities to accomplish cooperative assembly of multiple PBBs. Whereas, by combining these three designing principles, we have reported a series of polymeric assemblies with enhanced architectures and morphologies (see the Supporting Information for more examples). Many groups have used, either explicitly or implicitly, similar designing philosophy to create many other advanced polymeric assemblies.[2] Muller and co-workers created a core−shell−shell−corona assembly by first fabricating core−shell−corona micelles from polybutadiene-*block*-poly(1-methyl-2-vinyl pyridinium)-*block*-poly(sodium methacrylate) (PB-*b*-P2VPq-*b*-PMANa) block terpolymers (Figure 4).[28] Subsequent complexion of the PMANa corona with the poly(*N*,*N*-dimethylaminoethyl methacrylate) (PDMAEMAq) block of PEO-*b*-PDMAEMAq results in well-defined core−shell−shell−corona aggregates with five distinguishable layers. By playing with many other parameters, the same group has reported many kinds of micellar assembly with elegant structure.[24]

3. MULTIFUNCTIONAL POLYMERIC NANOCARRIERS WITH ENHANCED FUNCTIONALITY

Structure services functionality. To fulfill the elevated requirements in many fields such as nanomedicine, the final goal of any polymeric assembly design in terms of architectures and morphologies is to impart new functions into a single micellar platform, resulting in multifunctional polymeric nanocarriers (MFPNs) that can simultaneously target specific disease parts, facilitate in vivo imaging for diagnosis, and deliver therapeutic payloads in a controlled way.[4] There are many ways to realize

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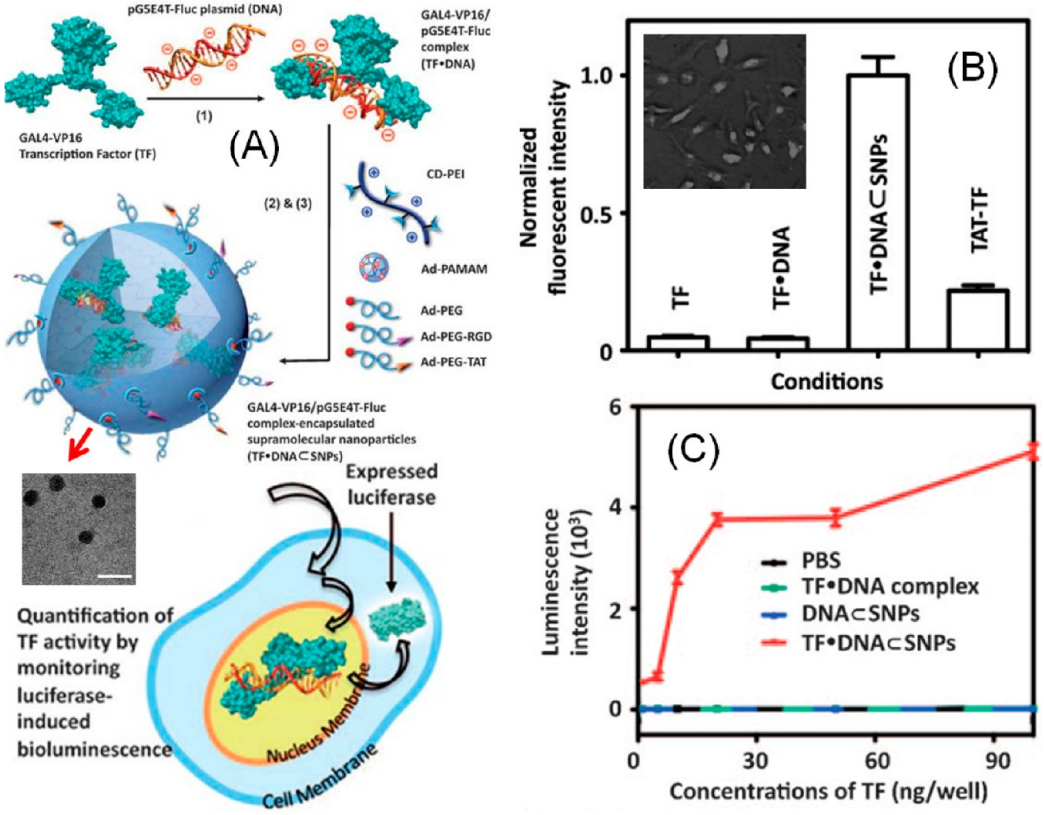

Figure 6. Supramolecular nanoparticles (SNPs) for protein delivery. (A) Self-assembly approach for the preparation of transcription factor (TF)-incorporated SNPs and the delivery of TF into the nuclei of the cell. (B) Delivery efficiency of Cy5-labeled TF·DNA⊂SNPs compared with other nanoparticles. Inset: Cy5-labeled TF was localized in the HeLa cell nuclei after incubating with TF·DNA⊂SNPs. (C) Bioluminescence intensity as a result of luciferase expression. Reproduced with permission from ref 34, Copyright 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

MFPNs, during which self-assembly of novel polymers designed on a case-by-case basis or advanced conjugation chemistry are involved. With the following examples, we shall demonstrate the possibility to fabricate MFPNs through fully cooperative self-assembly of multiple building blocks, guided by the several designing principles summarized in the last section. These examples partially address challenges widely existing in nanomedicine such as long blood circulation, efficient cellular uptake, and controllable release of payloads.

3.1. Nanoparticles with Tunable Surface Properties for Prolonged Blood Circulation

Multifunctional nanoparticles (NPs) based tumor targeting and therapeutic strategies rely largely on passive targeting achieved by the enhanced permeability and retention (EPR) effect during blood circulation. However, due to nonspecific serum protein (opsonins) adsorption on the surface of NPs, specific recognition by macrophages and subsequent blood clearance of the NPs will be initiated after intravenous injection (i.v.), leading to low particle concentration in blood and short contact time with the target tumor site. The main trick to prolong blood circulation of NPs is to make the surface of NPs antifouling, for example, by PEGylation.[29] Such stealth strategies sometimes conflict with the requirement of other subsequent steps of the delivery procedures, including enhanced accumulation in the tumor site, penetration through the whole tumor, cellular internalization, and rapid or sufficient intracellular drug release. To balance such dilemma, in situ surface charge transformation or detachable shells has been used to tune the surface properties of polymeric assemblies.[18,30]

Recently, we fabricated a series of MSPMs with approximately the same size, charge, and core composition but with varied PEG/PNIPAM ratios in the shell through cooperative self-assembly of several kinds of polyionic block copolymers in aqueous medium (P1+P2) (Figure 5).[31] The effect of the surface heterogeneity on the in vivo biodistribution was systematically investigated through in vivo tracking of the ^{125}I-labeled MSPMs determined by Gamma counter. Compared with PEGylated simple micelles, MSPMs with proper microphase separated surface exhibits significant improvements in prolonging blood retention and reducing accumulation in liver and spleen (Figure 5D). In addition, the in vivo biodistribution can be manipulated by simply varying the ratio of hydrophobic and hydrophilic segments on the surface of nanoparticles. Furthermore, to address the conflict of the requirements of long blood circulation with other procedures in the targeted drug delivery, the PNIPAM chains can be replaced with other stimuli responsive chains that are end-functionalized with cancer-targeted functional groups. Such groups hide under the PEG sheath during blood circulation, due to the collapse of the responsive chains, through which undesired interactions with serum proteins or healthy cells can be avoided. As soon as they approach the tumor site, the targeting functional groups can be exposed to interact with the tumor cell induced by the pH change, as elegantly demonstrated by Bae et al.[32]

3.2. Functional Nanoassemblies for Efficient Delivery and Internalization of Biomolecules

Proteins and DNAs are natural medicines, which work through regulating cellular behavior without the safety concerns of other

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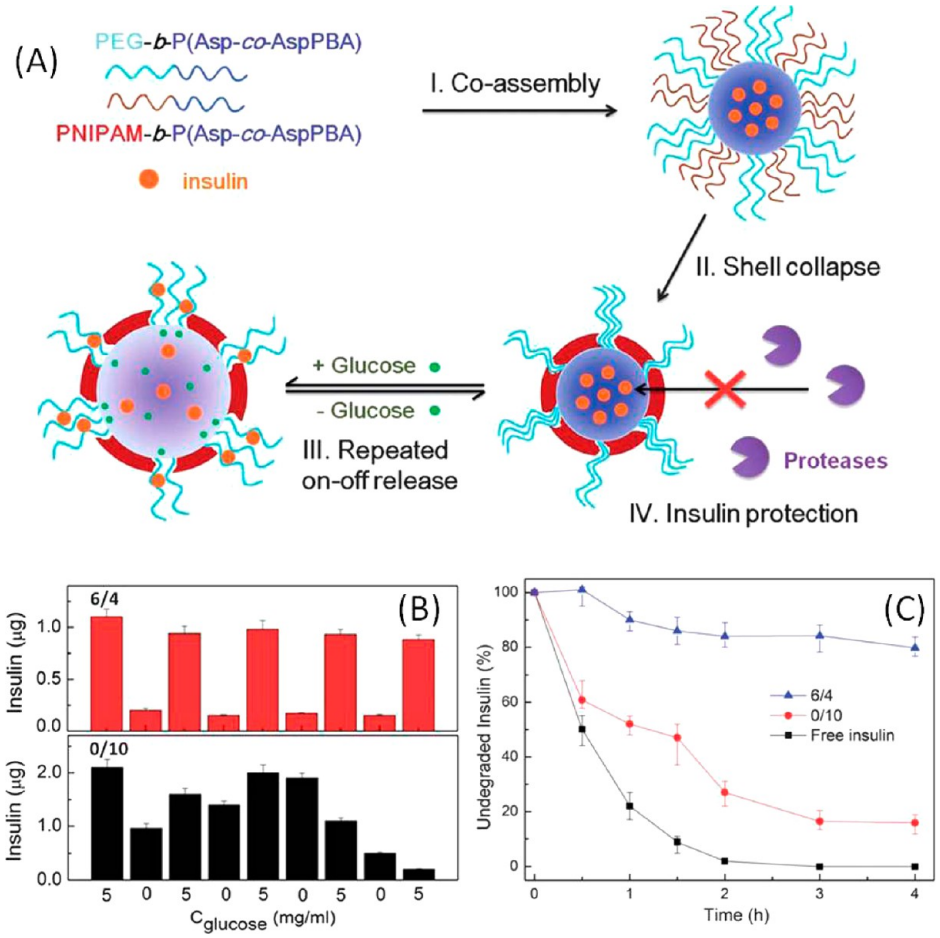

Figure 7. Glucose-responsive MSPMs for controlled release and protection of insulin under physiological conditions (A). (B) Repeated on−off release of insulin from MSPMs in response to external stepwise glucose treatment. (C) Enzyme attack is avoided by the size selection of the channel-like structure. Reproduced with permission from ref 38, Copyright 2012 Royal Society of Chemistry.

medicines. Cellular delivery of such kinds of biomolecules faces two major challenges: (1) retaining their stabilities and functions over the delivery process and (2) efficient cellular uptake. To address such challenges, Tseng and co-workers have built a flexible and versatile modular self-assembly approach for the preparation of supramolecular nanoparticles (SNPs) from a small collection of conventional polymeric building blocks through a multivalent molecular recognition.[33] Simply by varying the mixing ratios of the perspective polymeric building blocks, such a self-assembly strategy enables control upon the sizes, surfaces chemistry, zeta potentials, and payloads of the resulting SNPs. In addition, automatic procedures based on miniaturized high throughput screening platform such as microfluidic techniques can be used to optimize the functionality of the end SNPs for each specific application.

Based on this principle, transcription factor (TF)-incorporated supramolecular nanoparticles have been build which can efficiently delivery TFs into the nuclei of cells to regulate gene transcription in cell circuitry (Figure 6).[34] For this, TF was first coupled with a DNA plasmid with matching recognition sequence specific to a TF, resulting in TF-DNA complex with anionic characteristics. In a one-pot mixing process, such TF-DNA complex was further mixed with other building blocks, including adamantane grafted first-generation polyamidoamine dendrimer (Ad-PAMAM), β-cyclodextrin-grafted branched polyethylenimine (CD-PEI), and Ad-functionalized polyethylene glycol (Ad-PEG), leading to suprananoparticles (TF·DNA⊂SNPs) through a multivalent molecular recognition based on Ad and CD. TF·DNA⊂SNPs consists of a TF-DNA-encapsulated PEI/PAMAM hydrogel core and a PEG shell which is end-functionalized with either membrane penetrate peptides such as TAT or cell surface recongonization motifs such as RGD, endowing the particles with delivery specificity and cell transfusion capability, respectively. By varying the mixing ratios of the three polymeric building blocks, either the size of particles or the surface properties, that is, the charge properties and ratio of TAT/RGD, can be optimized. Compared with the conventional protein delivery strategy, TF·DNA⊂SNPs exhibit unprecedented performance for delivery intact TF. Most importantly, the intracellular TF delivered by TF·DNA⊂SNPs retained its high activity inside the cell (Figure 6C).

3.3. Polymeric Micelles with Controllable Permeability Behaviors for the Protection and Repeated On−Off Release of Payloads

Uncontrollable burst release and protease degradation of the payloads are the common issues facing nanostructured delivery systems. By manipulating the stimuli responsive phase separation of the mixed PNIPAM/PEG shell of the previously discussed MSPMs,[17] we have demonstrated the possibility to influence the release behavior of the payloads loaded inside the core of such nanocarriers.[35] Above the LCST, PNIPAM chains

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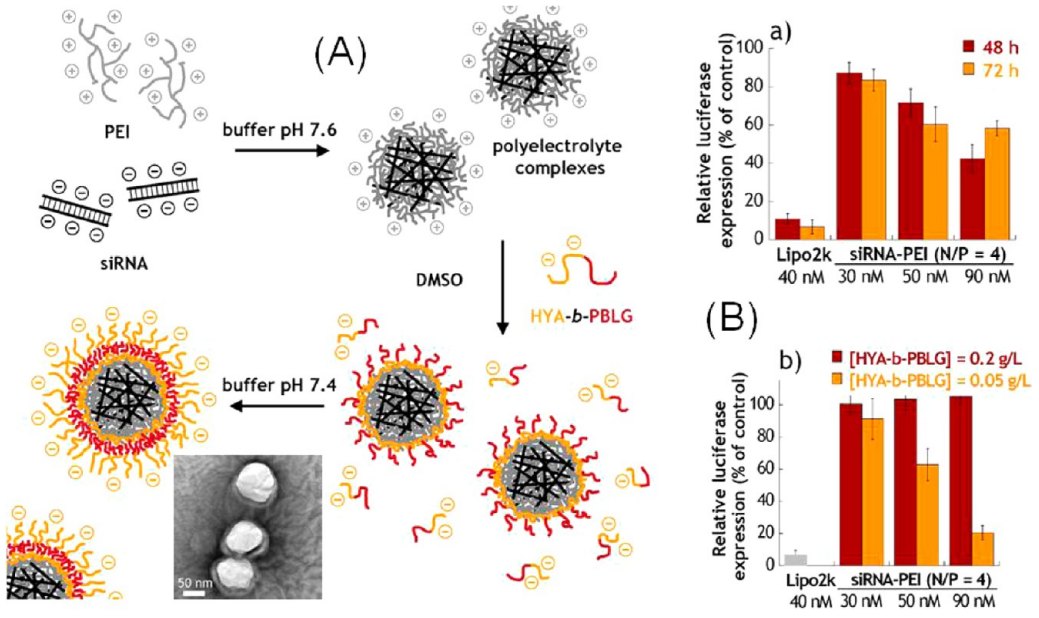

Figure 8. Viruslike particles through combing RNA−polyelectrolyte complexation and block copolymer self-assembly in a single process (A). (B) Relative gene expression of firefly luciferase after incubation of HeLa cells with siRNA complexes prepared in different conditions. (a) siRNA−PEI complexes alone (N/P = 4). (b) siRNA−PEI complexes modified with HYA-*b*-PBLG copolymer (at 72 h). Reproduced with permission from ref 40. Copyright 2012 American Chemical Society.

in the shell will segregate and collapse onto the core, resulting in micelles with a collapsed shell and a stretched PEG corona.[36] If the amount is enough, collapsed PNIPAM forms a continuous membrane around the micellar core, which can stabilize the whole micelle to such a degree that the core can swell without disassembling the micelles.[36] Furthermore, besides offering steric stability and biocompatibility, the stretched hydrophilic PEG chains in the collapsed PNIPAM layer create areas with high permeability which connect the inner core to the outer milieu, similar to protein channels embedded in a cell membrane.[37] The size of the area with high permeability can be fine-tuned such that size-selection effect can be realized, during which only molecules with proper sizes can pass through while large molecules like proteases can be hindered. Therefore, the micellar core can be protected from enzyme attack to prevent the premature release of the loaded payloads while other species can still access the micellar core (Figure 7). Combining these advantages together, we have designed a glucose-responsive complex micelle that enables repeated on−off release and efficient protection of insulin (Figure 7).[38]

4. BIOMIMETIC POLYMERIC ASSEMBLIES BY COOPERATIVE SELF-ASSEMBLY OF MULTIPLE BUILDING BLOCKS

To eventually address challenges faced by modern applications, especially in biomedical fields, sophisticated nanoscale carriers should evolve into fully integrated vehicles, which can sense targets and accordingly adapt their functions. Such devices will also have certain processing capabilities, modulating their properties and functions in response to internal or external stimuli. In other words, the future nanocarriers should mimic certain functionality of some minimum units in nature. Such goals have been approached from different perspectives while macromolecular self-assembly is quiet fruitful.[2] In this section, several examples will be discussed to demonstrate the possibility to create bioactive polymeric assemblies, based on the several designing principles as illustrated in section 2.

4.1. Viruslike Particles Obtained from Stepwise Self-Assembly of Multiple Building Blocks

Viruses normally consist of a protein capsid made of multiple copies of coat proteins, into which the genomic RNA or RNA is installed. Many efforts have been dedicated to designing various kinds of "viruslike particles" (VLPs) or "artificial" viruses to mimic the excellent gene delivery capability of the viruses while avoiding their inherent immunogenic problem.[39] Based on the designing principles of P1 and P3, Schatz et al. recently built polymeric assemblies which closely mimic the virus morphology and functions (Figure 8).[40] Excess surface positive charges of the RNA−PEI nanocomplexes were used to complex with the negatively charged hyaluronan block of hyaluronan-*block*-poly(γ-benzyl L-glutamate) (HYA-*b*-PBLG) in DMSO, a good solvent for both hyaluronan and PBLG blocks, resulting in assemblies with a RNA−PEI core, a PEI/hyaluronan polyelectrolyte shell, and a PBLG corona. A key step is the change of the amphiphilicity of HYA-*b*-PBLG by switching to aqueous buffer at pH 7.4, during which PBLG become hydrophobics and the PBLG corona and the PBLG blocks of excess HYA-*b*-PBLG co-collapse, resulting in a polymeric complex with multilayer CSC-like structure. The authors think the layer formed by glycoprotein-mimic HYA-*b*-PBLG is virus-capsid-like. siRNA-PEI complexes encapsulated in such CSC-like micelles can facilitate the release of siRNA molecules in the cytoplasm and have a higher gene silencing activity than naked complexes.

4.2. Artificial Chaperone Based on Polymeric Assemblies

Misfolding and irreversible aggregation of proteins is the main challenge facing by modern biotechnology and is the reason for protein misfolding diseases, such as Alzheimer's disease.[41] In nature, a few families of protein complexes, called molecular chaperones, can help with in vivo proper folding of nascent polypeptides with high efficiency and generality.[42] We have

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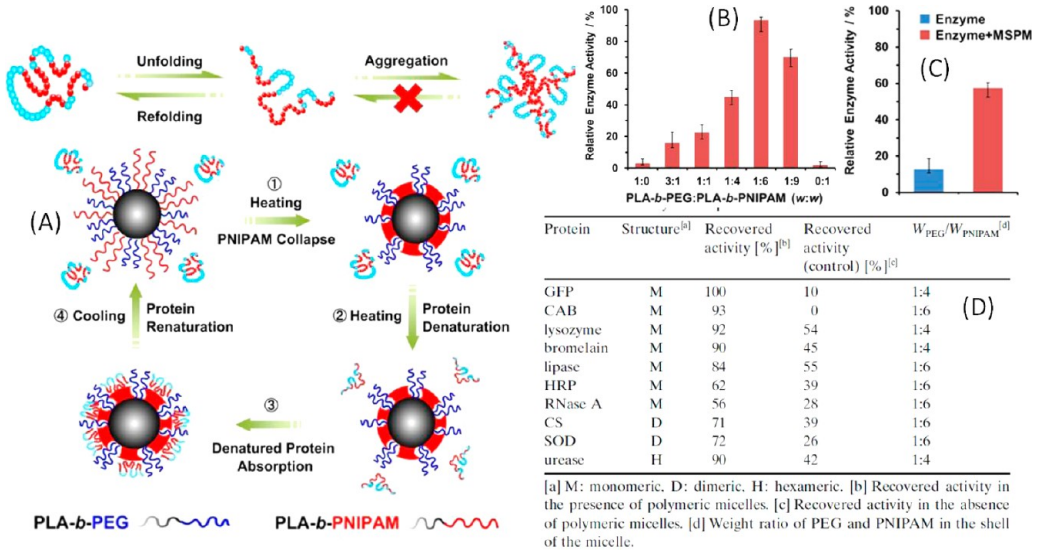

Protein	Structure[a]	Recovered activity [%][b]	Recovered activity (control) [%][c]	W_{PEG}/W_{PNIPAM}[d]
GFP	M	100	10	1:4
CAB	M	93	0	1:6
lysozyme	M	92	54	1:4
bromelain	M	90	45	1:4
lipase	M	84	55	1:6
HRP	M	62	39	1:6
RNase A	M	56	28	1:6
CS	D	71	39	1:6
SOD	D	72	26	1:6
urease	H	90	42	1:4

(D)

[a] M: monomeric, D: dimeric, H: hexameric. [b] Recovered activity in the presence of polymeric micelles. [c] Recovered activity in the absence of polymeric micelles. [d] Weight ratio of PEG and PNIPAM in the shell of the micelle.

Figure 9. MSPM-based artificial chaperones. (A) Mechanism of stabilization and refolding of heat denatured proteins assisted by MSPM-based artificial chaperones. (B) Recovered enzyme activity (REA) of thermally denatured CAB in the presence of MSPMs with different PEG/PNIPAM ratio. (C) Long term stability of CAB that are stored at 40 °C for 2 weeks. (D) REA of thermally denatured enzymes with various structure complexities in the presence of MSPMs. Reproduced with permission from ref 43, Copyright 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

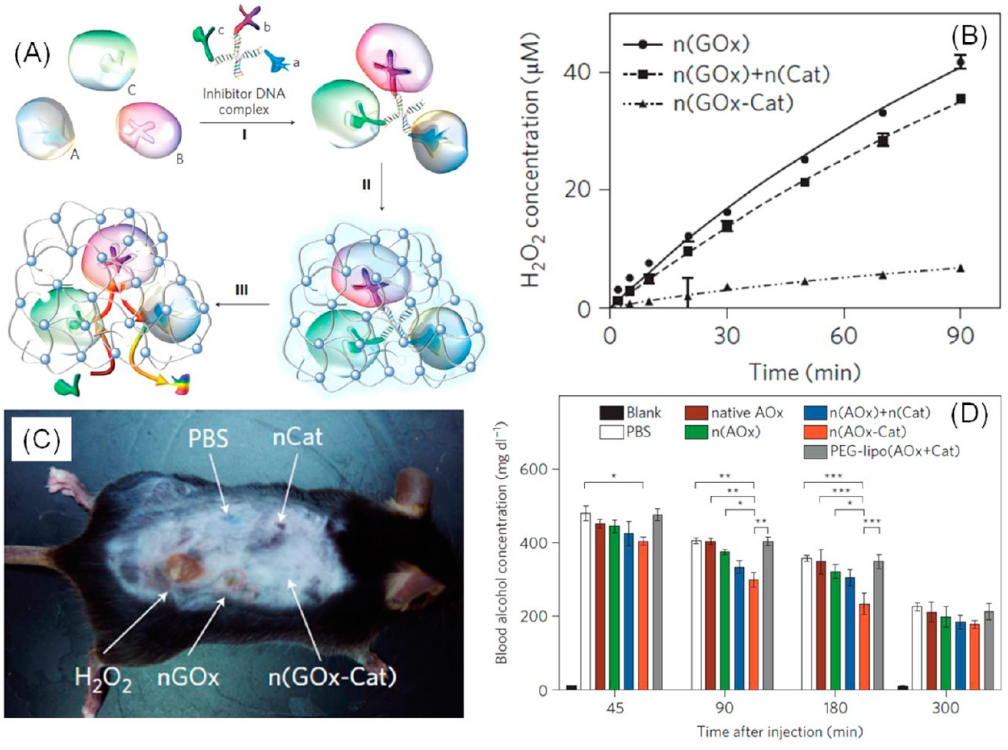

Figure 10. (A) Schematic illustration of the synthesis of a model triple-enzyme nanocomplex by DNA-directed assembly and nanoencapsulation. (B) H_2O_2 concentration in the glucose oxidation reaction catalyzed by n(GOx), n(GOx)+n(Cat), or n(GOx-Cat). (C) Photograph of a mouse cutaneously injected with PBS, n(Cat), H_2O_2, n(GOx), and n(GOx−Cat) at different sites. (D) Blood alcohol concentration of an alcohol intoxicated mouse after injection with PBS, native AOx, lipo(AOx+Cat), or n(AOx-Cat). Reproduced with permission from Macmillan Publishers Ltd: Nature Nanotechnology (ref 47), copyright 2013.

demonstrated that the previously discussed mixed shell polymeric micelles (MSPMs) can be used to avoid heat denaturation of proteins in a way similar to the chaperone-GroEL-GroES (Figure 9).[43]

The MSPM-based artificial chaperone was fabricated through self-assembly of a binary mixture of amphiphilic diblock copolymers, resulting in nanoparticles with a shell consisting of mixed PEG and PNIPAM chains. PNIPAM chains can be induced by heating to form hydrophobic domains surrounding the core while the micelles are stabilized against aggregation by the PEG chains. Several aspects of such MSPMs are comparable with the natural chaperone-GroEL-GroES complex

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which works through trapping unfolded proteins onto the hydrophobic areas inside the cavity of its barrel like structure.[42] First, the hydrophobic PNIPAM domains are similar to the active sites of the GroEL-GroES and can be reversibly turned "on" or "off" via switching temperature, just like the ATP-driving functional state change of the GroEL-GroES. Second, the MSPM performs their chaperone-like function in the "on-demand" fashion of the GroEL-GroES. MSPMs are in the "dormant" state and will not interfere with the function of the proteins under physiological conditions (Figure 9). Upon heating to the protein denaturation conditions, the MSPMs self-transform into their functional states and capture the partially unfolded intermediates of proteins onto the collapsed PNIPAM domains to prevent irreversible interprotein aggregation. When the refolding conditions are satisfied, the MSPMs release the captured proteins by the hydrophobicity and hydrophilicity transformation of PNIPAM during which refolding can occur. In addition, the nanoscale size the MSPMs is advantageous by offering large specific surface area to interact with proteins. Such system works for a wide range of enzymes consisting of either single or multiple subunits (Figure 9D). Similar polymeric assemblies possessing chaperone-like bioactivities have been exploited to assist the protein delivery and to delay the fibrillation of Alzheimer's disease associated amyloid β protein.[44,45]

4.3. Biomimetic Multiple Enzyme System

In the many multiple enzyme systems existing in organisms, enzymes with synergic and complementary functions are confined in the sophisticated subcellular compartments and work in a consecutive and synergetic way.[46] In consecutive reactions catalyzed by multiple enzymes, such confinement ensures effective chemical transformation and transport of molecules among enzymes, eliminates toxic metabolic wastes, and minimizes the diffusion of intermediates among the enzymes. There are vast efforts to mimic such multiple enzyme systems.[46] Recently, functional enzyme nanocomplexes with well-controlled enzyme composition and spatial arrangement in a core−shell configuration have been created by us and collaborators (Figure 10).[47] A tailor-designed DNA-inhibitor scaffold was used as the template to assemble several enzymes with complementary functions into a nanocomplex, through noncovalent specific binding (I in Figure 10A). In this way, spatial arrangement and close-proximity architecture of multiple enzymes are realized. The nanocomplex was then encapsulated within a cross-linked polymeric nanocapsule, through in situ free radical polymerization at room temperature, using conventional water-soluble monomers, cross-linkers, and initiators (II in in Figure 10A). The DNA-inhibitor scaffolds can be subsequently removed, resulting in core−shell nanocapsules containing a multiple-enzyme core and a permeable polymeric shell (III in Figure 10A). Such functional artificial enzyme nanocomplexes exhibit improved catalytic efficiency and enhanced stability when compared with free enzymes. Furthermore, the colocalized enzymes display complementary functions, whereby toxic intermediates generated by one enzyme can be promptly eliminated by another enzyme. For instance, in the glucose oxidase (GOx)-catalase nanocomplex (n(GOx-Cat)), the oxidase can be used for various therapeutics while catalase plays the role to eliminate the toxic H_2O_2. Such a system can effectively protect the skin from damage during oxdase relative therapeutics (Figure 10C). Another nanocomplex containing alcohol oxidase and catalase (n(AOx-Cat)) could reduce blood alcohol levels in intoxicated mice, offering an alternative antidote and prophylactic for alcohol intoxication (Figure 10D). We think the multiple enzyme nanocomplex is an elegant example of the possibility to fabricate polymeric complexes with advanced and biomimetic functionality through combination of conventional synthetic polymeric and biological building blocks with inherent functions.

5. CONCLUSIONS AND OUTLOOK

In this Account, we summarize several straightforward principles that can be exploited to optimize the architecture of polymeric assemblies, to impart new functionality into the end particles, and to realize complex nanoassemblies with bioactive functions that are similar to some natural systems. The advantages of these works are the simplicity of the preparation process and versatile choice of the constituent polymers in terms of their chemical structure and functionality as well as cooperative self-assembly offers elegant and energy-efficient bottom-up strategies. No significant synthetic endeavors are required to prepare specific types of polymeric building blocks to achieve the same level of control over the functionality. Combination of the principles behind each example discussed herein may help produce truly tailor-designed nanoparticles which can satisfy the increasingly sophisticated requirement of modern applications. In the future, focus might be directed to "adaptable" or intelligent bioactive polymeric nanodevices, which can vary their structural and associated functional properties on demand as a response to environmental parameters. Given the vast library of polymers and biopolymers that are currently or potentially available, novel classes of polymeric nanocomplexes could be built for a broad range of applications.

■ ASSOCIATED CONTENT

Supporting Information

Other examples of advanced polymeric assemblies from our group and references. This material is available free of charge via the Internet at http://pubs.acs.org.

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