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Biological Stimuli-responsive Polymer Systems: Design, Construction and Controlled Self-assembly

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Abstract Biological stimuli-responsive polymers have increasingly attracted attention in recent years because it can satisfy many requirements of applications related with human body while traditional systems do not meet. Due to the importance of this burgeoning field, great efforts have been devoted and, up to now, polymer chemists have made a remarkable success in this prospective research topic. In this review, we systematically generalize the present state of biological stimuli-responsive polymer systems. We highlight several representative examples to specify the current problems and look ahead a clear sense of direction in this area.

Keywords Biological stimuli; Responsive polymer; Controlled self-assembly; Biomedicine

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INTRODUCTION

Stimuli-responsive polymers have attracted significant attention over the past decades because of their promising and powerful applications, such as the fields of nanotechnology[1], advanced materials[2], medical imaging[3], and chemical sensing[4]. This kind of polymers are leading tremendous impact and revolution on the interdisciplinary of nanoscience, material science and even medicine[5, 6]. Stimuli-responsive polymers are defined as a family of 'smart' macromolecules that can undergo changes on chemical structures or phase states in response to an external/environmental signal. These changes usually cause huge transition in the physicochemical properties of the polymers. The signals are derived from chemical factors, including pH variation, redox agents, molecules and chemical components[7–12], and that can be exogenously triggered by physical fluctuations, including temperature, light irradiation, electrical field, magnetic field, and mechanical force[13–17]. Stimuli-responsive polymer can be regarded as a miniature "sense-to-action" device that is able to 'read in' external signal inputs and 'read out' information outputs.

One of the most central applications of stimuli-responsive polymers focuses on drug delivery vehicles[18]. In general, they can self-assemble into nano-/micro-carriers with specific structures and sizes. The common morphologies are spherical micelles and hollow vesicles. Covalently or non-covalently encapsulating drug molecules in their interior, these polymers can serve as intelligent vehicles for achieving controlled/targeted drug transportation and release. One can readily manipulate the external signals to control the parameters (*e.g.* rates, timing, locations and distributions) of disassembly of these polymer vehicles for 'on-demand' delivery.

A representative example was pioneered by Liu and Armes in 2001[19]. They reported a diblock copolymer composed of poly(propylene oxide) (PPO) block and poly(2-(diethylamino)ethyl methacrylate) (PDEA) block. PPO is a typical thermoresponsive polymer which displays water-solubility at low temperature (< 10 °C) while transforms into water-insolubility at above 20 °C; on the other hand, PDEA is a pH-sensitive polymer which can reversibly change its phase state from unimer to aggregate at neutral pH. Thus if one can tune the external temperature or pH value, the PPO-*b*-PDEA block copolymer assemblies can sensitively disassemble. Inspired by this seminal work, up to now, a plethora of stimuli-responsive polymer systems have been established[20–24]. Despite great advances in recent years, there are still several intractable and long-standing issues limiting the development and practicable utility of this field. (i) Chemical contaminations. The majority of chemically responsive polymer systems cause harmful accumulation of chemical residues[25], which can result in potential physiological toxicity and break the normal cell homeostasis. (ii) Cell damage. Except for chemical stimuli, conventional physical stimuli such as UV irradiation and a variety of field forces can inevitably bring about cell damages and genic mutation, which is hard to apply in real clinical therapy. (iii) Low bio-specificity and biodistribution. A desirable nanomedicine needs to meet the conditions of local targeted

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delivery and cell-level precise release[26]. Although the traditional responsive polymer carriers are based on physiological milieu difference between normal cells and pathological cells, it has been shown that, to most of the diseases, the differences are limited and it is difficult for these carriers to specifically distinguish them (Fig. 1, left). Therefore, it is emerging and challenging to explore novel stimulation modes to satisfy the requirements of many applications related to precision biomedicine in human bodies.

Facing to these bottlenecks, recently, a new concept concerning on biological stimuli-responsive polymers has been proposed. This alternative strategy aims to take advantage of endogenous biological signals as stimulus sources, in order to *in situ* trigger disassembly of responsive polymer systems in pathological milieu. In comparison to those conventional stimuli-responsive polymers responding to chemical or physical signals, biological stimuli-responsive polymers boast three particular merits. (i) Biological signal molecules are the natural metabolisms or secretions of cells; because of their endogenesis, there is no accumulation problem of chemical pollutants. Without physiological toxicity, it can effectively avoid cell damage. (ii) Almost all of diseases stem from the metabolic abnormality of biological signals or overexpression of specific proteins at molecular level. Seeking a special polymer system that is capable of selectively responding to disease-related biological signals may hopefully create pathological cell-specific, precise nanomedicine and solve the serious side effects of common medications. (iii) There are a vast scope of biological signal molecules, the studies to which involves biochemistry, molecular biology and cell biology. Developing biological stimuli-responsive polymer models is conducive to bridging these disciplines to polymer science (Fig. 1, right). Given these aspects, the quest to design biological stimuli-responsive polymer systems is an ambitious goal, which can not only address the tricky scientific problems but also take a step in realizing future precision-therapy.

Although the construction of biological stimuli-responsive polymers holds promise for overcoming a series of problems in traditional responsive polymer carriers, two key difficulties require to be solved. The first one is how to selectively sense one certain biological signal by use of polymers because there are numbers of homogenous biomolecules with similar chemical structure and functions co-existing in cells. For example, glutathione (GSH) is a redox-active biosignal, which is responsible to modulate cellular redox atmosphere[27]. However, there are other reductive biomolecules such as cysteine (Cys), homocysteine (Hcy) and thiols in cells[28], all of which have similar reductive ability. To our best knowledge, conventional responsive polymers are hard to selectively recognize GSH in complex intracellular milieu. On the other hand, it is well-known that the critical sensitive limit (CSL) of conventional responsive polymers generally attains to 10^{-6}–10^{-3} mol/L, but in general, the biologically relevant levels of cell signals are ranging from 10^{-9} mol/L to 10^{-6} mol/L, which mismatch each other. In view of these, rationally designing polymer structures with high-selectivity toward single biological signal is crucial. In this review, we summarize the structural design of biological stimuli-responsive polymers and showcase the recent progress on this topic. In the last part, we propose a prospect on the next generation of responsive polymer carriers.

SMALL MOLECULES OF BIOLOGICAL SIGNALS

From the difference of molecular weight of active biomolecules, biosignals can be classified into two main categories, small-molecule-type and macromolecule-type. Small-molecule-type biological signals have great advantages compared with many other chemical or physical stimuli in responsive polymer systems. In general, small-molecule-type biosignals are made up of three subfamilies: cell signaling molecules, bioactivators, and reactive species. All of them have extensive distribution in biological cells, high sensitivity to the regulation of relevant organisms, and favorable cell penetration. Therefore, from the prospect of bio-stimulating sources, the development of small molecule-type biological signals has a great value. However, the similar structure and reactivity among these biological signals pose considerable challenges to utilize such stimulants. Taking H_2S as an example, there are numbers of biological analogues co-existing in our cells such as thiols[23], cysteine (Cys),

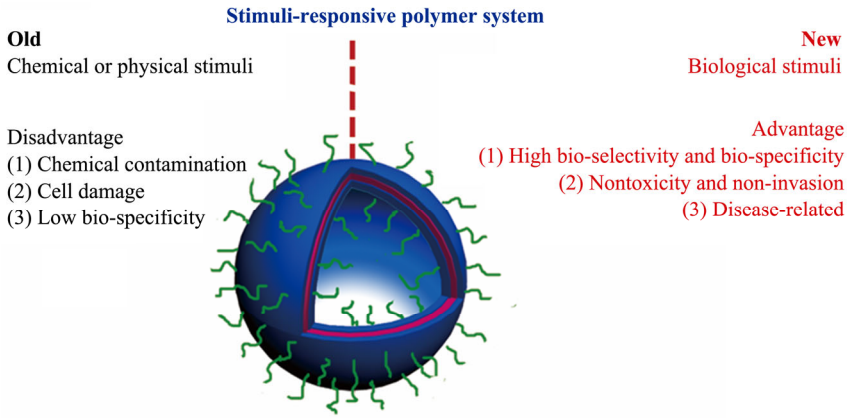

Fig. 1 Systemic comparison of traditional chemical/physical stimuli-responsive polymer and new biological stimuli-responsive polymer systems

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homocysteine (Hcy), and glutathione (GSH), which play similar roles in some cellular processes. Therefore, how to design a polymer structure with specific response to a certain biological signal is the key to solving the key problem.

Cell Signaling

Nitric oxide (NO)

Nitric oxide (NO) is known as the first gaseous neurotransmitter that has been exerted into the stimuli-responsive polymer systems and used to induce responsiveness in synthetic polymer chains in 2014 by Thomas P. Davis and his coworkers. As we all know, NO causes damage to both the environment and human health. However, despite the damage it caused, it is also regarded as an endothelium-derived relaxing factor (EDRF) and a broad-spectrum cell signaling molecule that play a key role in both systemic and specific cellular levels[29] and it is involved in many physiological and pathological processes[30]. Low levels of nitric oxide production are important in protecting organs such as the liver from ischemic damage. However, abnormal concentrations of NO in the human body can lead to cardiovascular disorders, gastrointestinal pain, neurodegeneration and hypertension and other diseases.

Due to the biological role of NO, Thomas P. Davis and his coworkers developed new NO-responsive monomers (*N*-(2-aminophenyl) methacrylamide hydrochloride (NAPMA) and 2-(3-(2-aminophenyl) ureido) ethyl methacrylate hydrochloride (APUEMA)), containing *o*-phenylenediamine functional groups[31] (Fig. 2). Then the two monomers have been polymerized with *N*-isopropyl acrylamide (NIPAM) to form NO responsive block copolymers as truly biomimetic polymers, P(NIPAM-*co*-NAPMA) and P(NIPAM-*co*-APUEMA). In this work, they have proved that the NAPMA functional monomer could react with NO to yield an amide-substituted benzotriazole intermediate which is easy to hydrolyse in an aqueous solution. However, urea-functionalized benzotriazole transformed from APUEMA is quite stable in solutions. Upon exposure to NO, the NAPMA and APUEMA labeled thermo-responsive copolymers exhibited substantial changes in LCST, thus potentially adjust the phase transformation of the polymer chains, further trigger the polymer's self-assembly into nanostructures. This study laid a very important theoretical basis for the NO-responsive drug delivery.

Hydrogen sulfide (H_2S) and hydrogen polysulfide (H_2S_n)

Sulfur-containing biomolecules play an important role in many biological process, among which the most important ones are hydrogen sulfide (H_2S) and hydrogen polysulfide (H_2S_n). H_2S plays an important role in tuning blood vessel dilation, inducing cell apoptosis and resisting inflammation[32], although it has long been regarded as a toxic gas in atmosphere. The latest reports illustrated that H_2S is an important neuromodulator and cell signaling molecule and it can be generated from the decomposition of L-cysteine mediated by cystathionine γ-lyase (CSE). Metabolic disturbance of H_2S is related to various diseases, such as angiocardiopathy and neurodegeneration. Taking into account of the crucial effect of H_2S on cells and great disturbance of other sulfur-containing analogs such as GSH whose concentration is order of magnitude higher than H_2S, exploring a new stimulation mode by H_2S to specifically activate polymer self-assembly behaviors for targeted drug delivery is significant.

In 2016, Yan's group have developed a new H_2S-sensitive block copolymer bearing *o*-azido-methylbenzoate group (AzMB), (Fig. 3)[33]. Indeed, AzMB is a particular self-cleavable precursor, which can be transformed into benzylamine by H_2S, and the latter is able to cause a series of intramolecular cascade reactions that finally cleave the benzoyl bond. Experimental results indicated that the cleavage of the benzoyl bond of AzMB would yield glycerol methacrylate, altering the polymer amphiphilicity, further leading to a totally disassembly of their self-assembly aggregates. Moreover, Yan and co-workers have proved that the responsiveness can extend further from H_2S to a specific amino acid bioactivator by introducing cystathionine γ-lyase (CSE)—a specific enzyme converting cysteine into H_2S. It is expected that this polymer system could open up a new avenue for constructing H_2S-triggered nanocapsules for drug delivery.

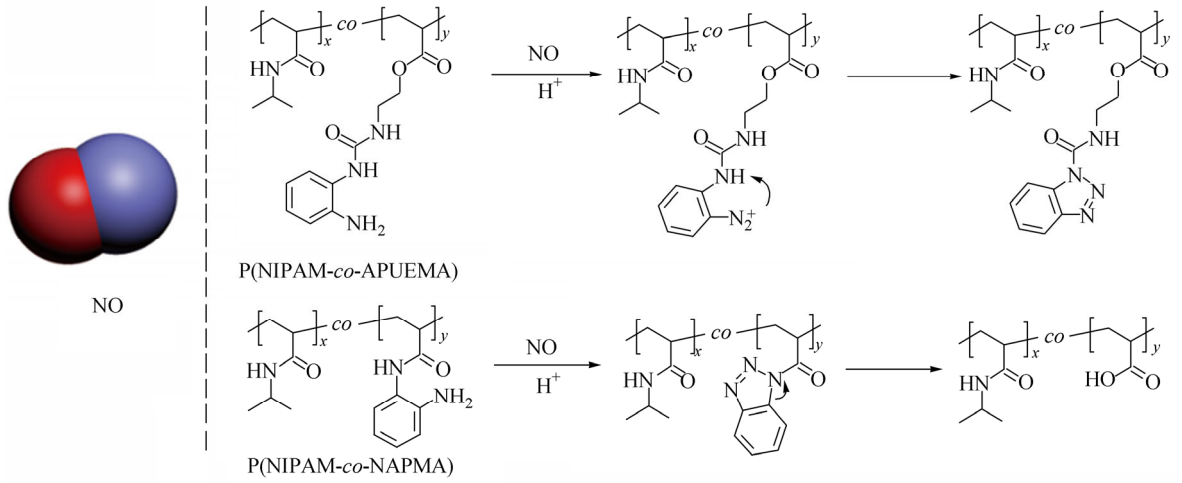

Fig. 2 NO-responsive polymers and their NO-sensitive molecular mechanism

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Similar to the H_2S mentioned above, H_2S_n also plays a decisive role in the biological activities[34–36]. It has been increasingly evidenced that many biological activities are mediated both by H_2S and H_2S_n in recent studies. Compared with H_2S, the concentration of H_2S_n is even lower. Hence, the biggest obstacle for designing a system that can specifically detect and apply intracellular H_2S_n is to construct a polymer structure with high sensitivity and selectivity to intracellular H_2S_n.

Recently, Yan's group designed and synthesized *o*-fluorobenzoate (FBz) linkage by the esterification of 2-fluoro-5-nitroisophthalic acid and benzyl alcohol (Fig. 4). Then FBz-containing polyurethane block was further terminated by the hydrophilic polyethylene monomethyl ether (PEG) to afford the triblock, which could further self-assemble to form vesicular nanostructures[37]. Upon H_2S_n, FBz as a self-cleavable unit can undergo a cascade reduction-cyclization to form cyclic benzodithiolone and sever the benzoyl bond, so that the triblock copolymer was broken down to fragments. However, upon other sulfur-containing analogues which possess only single nucleophilic group, the second-step cyclization reaction is

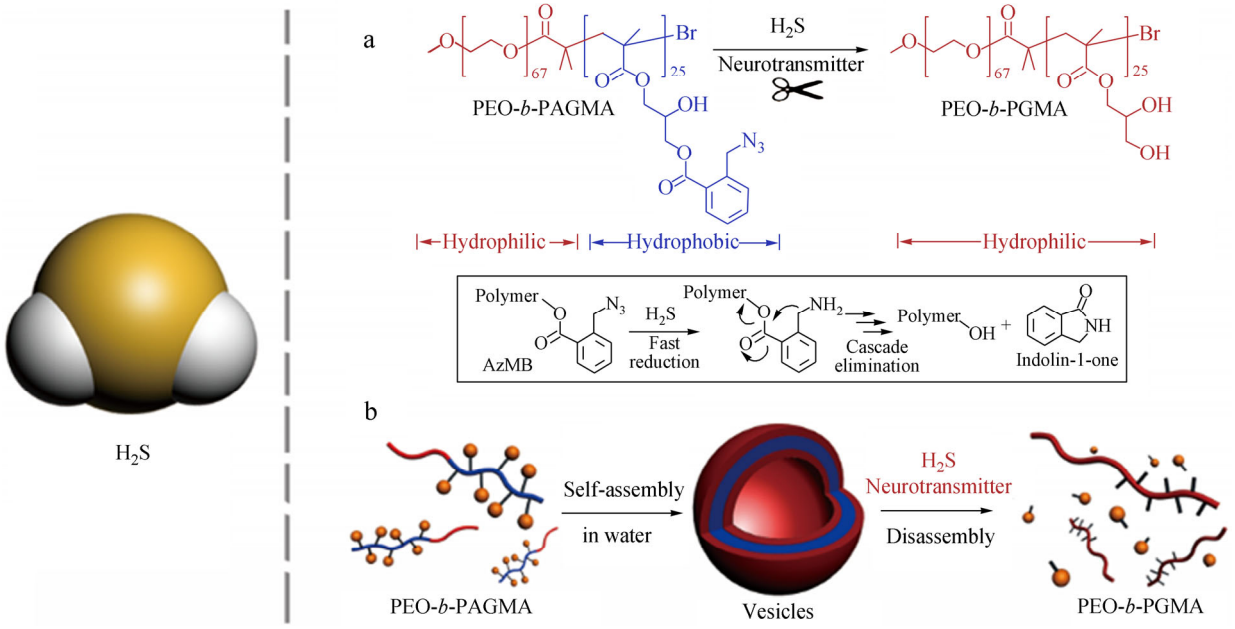

Fig. 3 H_2S-responsive polymersomes and their H_2S-sensitive cascade reduction-elimination cleavage mechanism (Reprinted with permission from Ref. [33]; Copyright (2016) American Chemical Society)

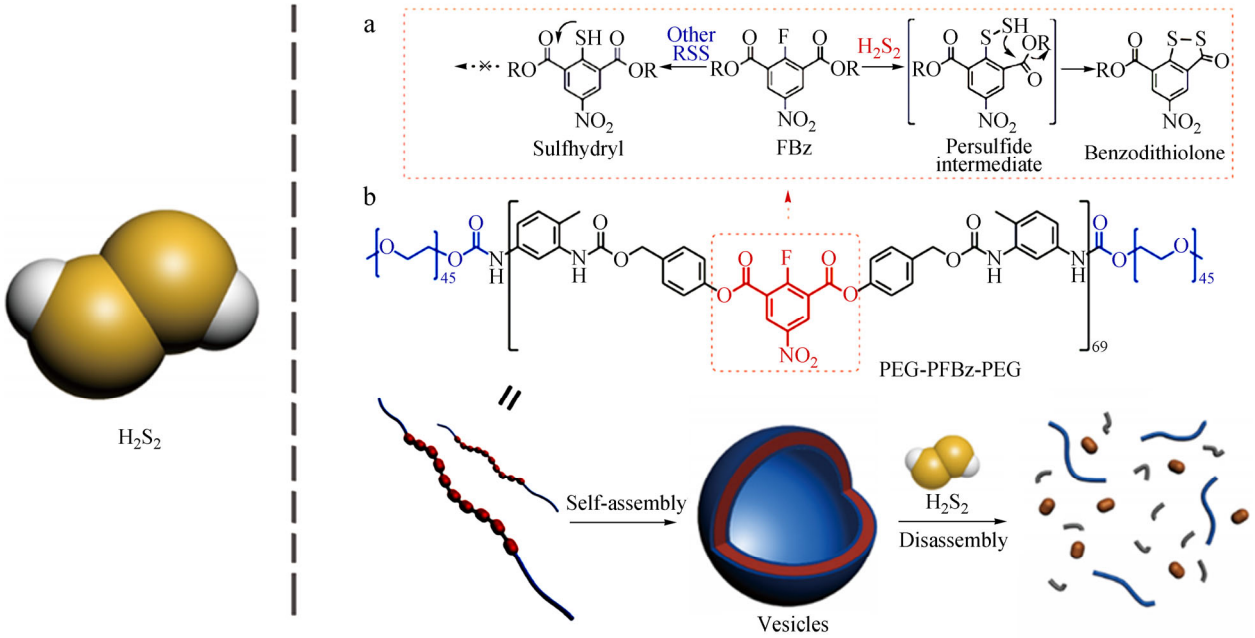

Fig. 4 H_2S_n-responsive polymersomes and their H_2S_n-sensitive cascade reduction-elimination reaction mechanism (Reprinted with permission from Ref. [37]; Copyright (2017) Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim)

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prohibited. Besides, the most exciting thing is that due to the main-chain polymer structure, only minimal amount of H_2S_n is required for the disassembly of the nanostructure, which is different from the side-chain functionality we have designed in the above H_2S-responsive project. The special design of main-chain polymer with FBz linkage endows the system with ultrahigh sensitivity to H_2S_n (even up to 10^{-9} mol/L), while can inhibit the interference of other biosignals in the cell. The triblock could further self-assemble to form vesicular nanostructures, which could be utilized as all-in-one nanoplatforms to explain the H_2S_n-related biology and achieve site-specific therapy in H_2S_n-releated diseases.

Peroxynitrite ($ONOO^-$)

Peroxynitrite ($ONOO^-$), a redox signaling molecule generated from the reaction of nitric oxide and superoxide, inspired much interest due to its central pathogenic factor[38]. The abnormal regulation of $ONOO^-$ is related to various diseases, such as neurodegeneration, ischemia, angiocardiopathy, and cancers[39]. Due to its crucial role in biological process, several groups have developed probes to detect $ONOO^-$ yet[40–44].

In 2016, Yan's group synthesized a phenyl methacrylate monomer bearing a special trifluoromethyl ketone moiety (TFK). Then the block copolymer poly(ethylene oxide)-*b*-poly(trifluoro-3-oxobutyl phenyl methacrylate) was gained to construct a $ONOO^-$ responsive system[45] (Fig. 5). Due to the strong electron-withdrawing TFK unit and an adjacent phenyl ester spacer, $ONOO^-$ was able to trigger cascade oxidation-elimination reactions in which the ketone is first oxidized to the dioxirane intermediate, and then the intermediate undergoes an intramolecular elimination and finally to afford spiroquinone. It should be noted that only the $ONOO^-$ was able to trigger the site-specific chemical cleavage from the polymer chain, which alters the amphiphilicity of the polymer and further leads to controllable disassembly the vesicular structure.

Carbon monoxide (CO)

Carbon monoxide is also known as a toxic gas, but recent studies have indicated that CO is also continuously produced *via* the breakdown of heme by heme oxygenase enzymes in the body[46, 47]. Similar to the other two major gasotransmitter mentioned above (NO and H_2S), CO is proposed to play significant roles in regulating many biological process and can be used as a medical agent[48]. CO abnormal secretion is related with many human diseases, including angiocardiopathy, neurodegeneration and inflammation[49].

Since CO shows great importance to living organism, Yan's group created a novel palladacycle unit that can response to CO[50] (Fig. 6). Then the palladacycle moiety was reacted with diisocyanate to afford palladacycle-connected polyurethane block (PUPd), which was finally terminated by PEG monomethyl ether. In the polymer chain, palladacycle exists as the halogen-bridged dimer. However, upon CO, the metal-coordination bond of the palladacycle can be inserted by CO to decompose into two unimers. Due to the site-specific scission of the main-chain polymer, the self-assembling micelles show desirable disassembly process and extraordinarily sensitive limit. Besides, the micellar dissociation rate is adjusted by the dose of CO stimulus, thus a controlled release of drugs can be realized by tuning the concentration of CO.

Bioactivator

Adenosine triphosphate (ATP)

ATP, known as the cellular energy, is a central metabolite and critical bioactivator, playing an irreplaceable role in many cell activities. Recent studies have shown that ATP can be regarded as a stimulus to regulate the disassembly of nanocarriers. For example, Aida and his coworkers have constructed a nanotube *via* the coordination of magnesium ion and chaperonin GroEL[51]. In this study, the chaperonin GroEL was site-specifically modified previously, with a

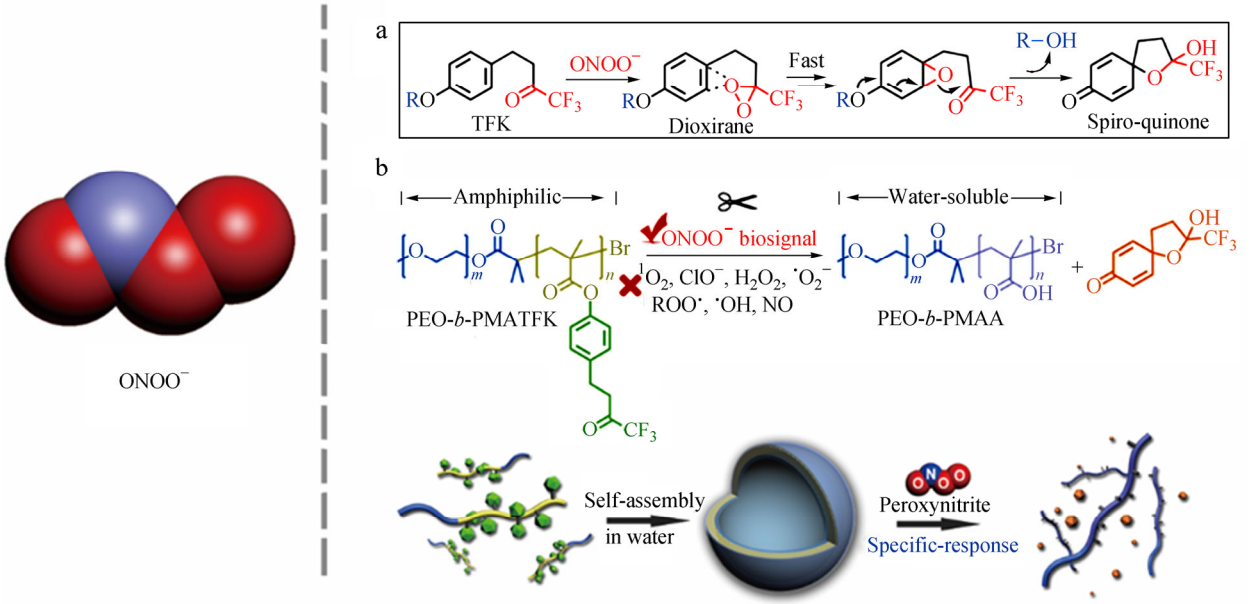

Fig. 5 $ONOO^-$-responsive polymersomes and their $ONOO^-$-sensitive cascade oxidation-elimination cleavage mechanism (Reprinted with permission from Ref. [45]; Copyright (2016) American Chemical Society)

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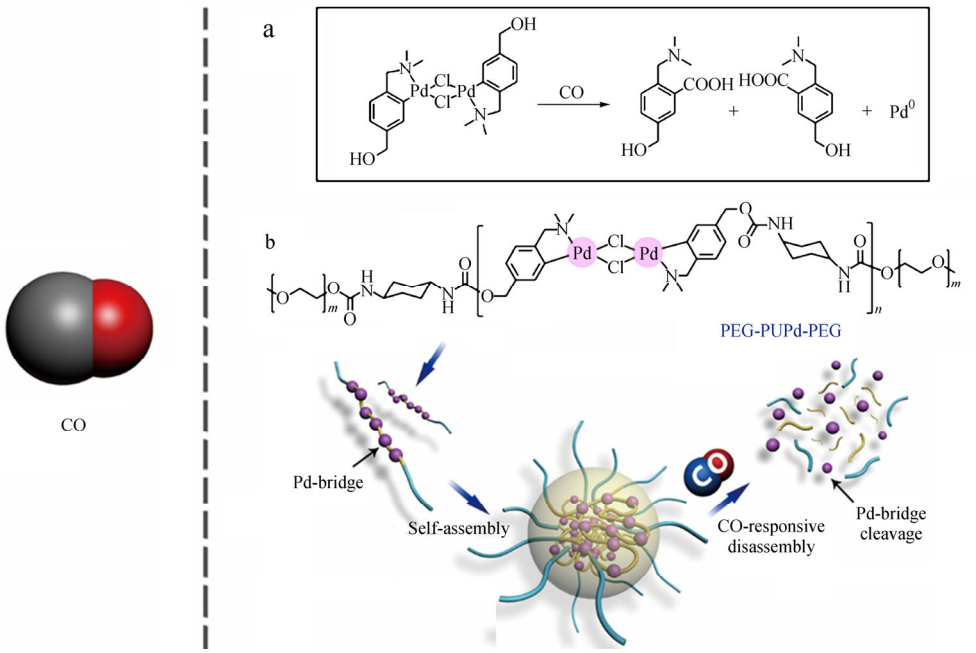

Fig. 6 CO-responsive metal-bridging polymer micelles and its CO-sensitive cascade insertion-elimination (Reprinted with permission from Ref. [50]; Copyright (2017) American Chemical Society)

number of photochromic [spiropyran (SP)/merocyanine (MC)] units in its cavity entrance parts. Influenced by the energy from the hydrolysis of the ATP in the living cell, there was a remarkable change in the structure of the chaperonin GroEL, which in turn generated a mechanical force and triggered the disassembly of the nanotube, leading to the release of guest molecules (Fig. 7).

Hereafter, their group continued to develop a kind of ATP responsive polymer chains, named GumBAn, with multiple guanidinium ion (Gu^+) and boronic acid (BA) appended in the side chains[52]. Their research group took advantage of the difference of bind affinity of polymer chains with ATP compared with trypsin Trp to realize the regulation of trypsin activity through ATP and now it has achieved preliminary research results.

Gu and his coworkers utilized adenosine-5'-triphosphate (ATP) to trigger the controlled release of drugs[53]. They developed polymeric nanocarriers functionalized with an ATP-binding aptamer-incorporated DNA motif, which could realize the conformational change under the stimulation of ATP and then trigger the release of the intercalating doxorubicin (Fig. 8).

Based on the pillar[6] arene, Huang's group recently prepared a stable 1:1 complex WP6⊃ATP due to the differences in the cavity size of the hosts and the charge and length of the guests (Fig. 9). This complex greatly improved the efficacy of anti-cancer drug through the inhibition of ATP hydrolysis[54], which was greatly attributed to the existence of

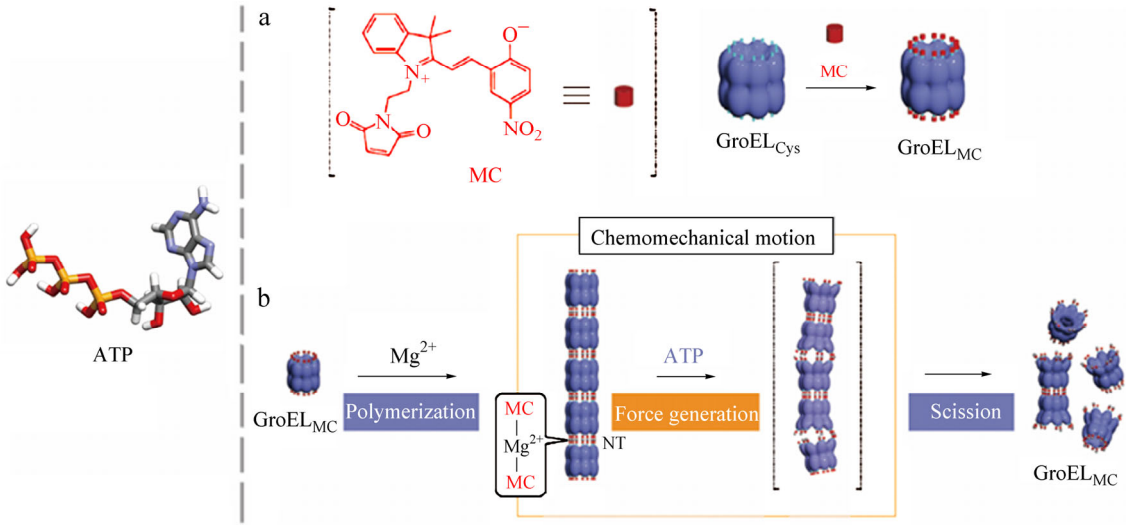

Fig. 7 MC-modified GroEL protein and ATP-responsive GroEL protein nanotubes (Reprinted with permission from Ref. [51]; Copyright (2013) Nature Publishing Group)

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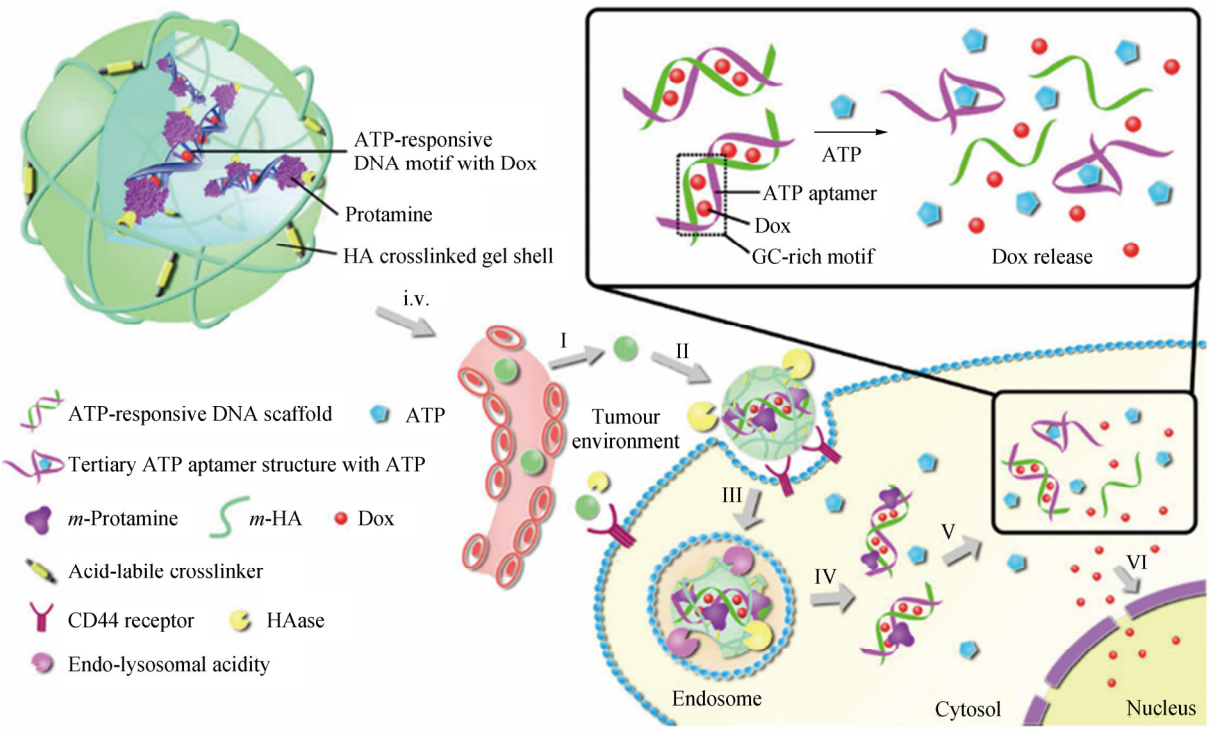

Fig. 8 ATP-responsive nanocarriers constructed by specific DNA aptamers and their controlled drug delivery in cells (Reprinted with permission from Ref. [53]; Copyright (2014) Nature Publishing Group)

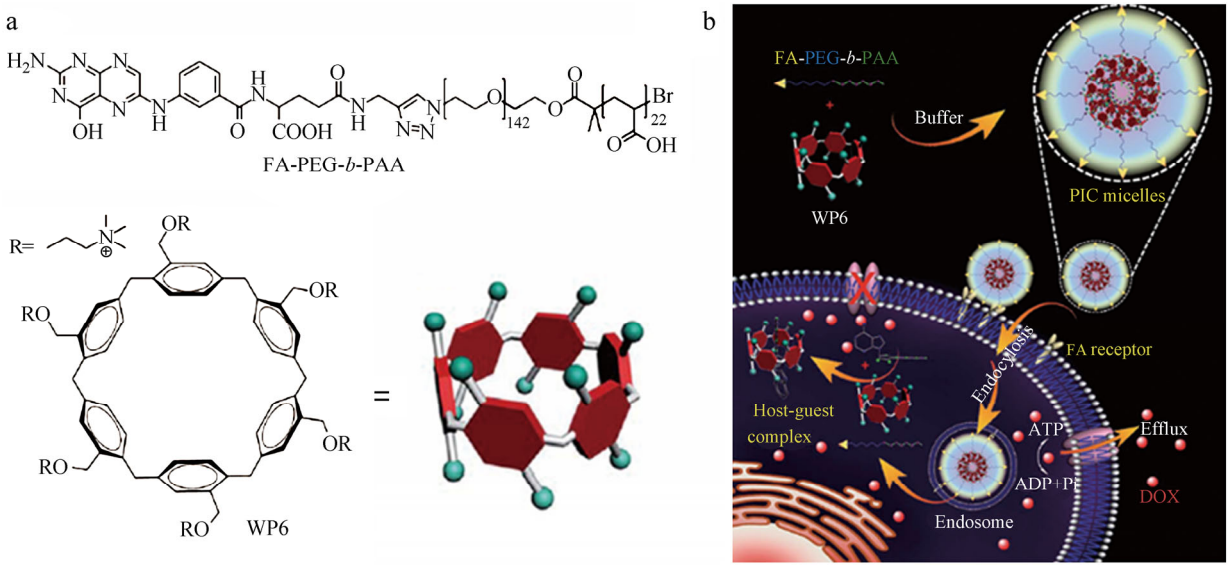

Fig. 9 FA-PEG-*b*-PAA/WP6 supramolecular amphiphiles and their self-assembly micelles for ATP-sensitive disassembly for drug delivery in cells (Reprinted with permission from Ref. [54]; Copyright (2016) The Royal Society of Chemistry)

the hydrophobic cavity of WP6. The WP6 can be further decorated by the diblock copolymer (FA-PEG-*b*-PAA) functionalized with a folic acid, increasing the targetability of the polyion complex (PIC) micelles to overexpressing KB cell.

Yan's group has also made some achievements in ATP stimuli-responsive polymers. They used ATP as a trigger to successfully regulate the assembly behavior of the synthetic polymers. Inspired by the native ATP binding protein, their group designed a hydrophilic triblock appended with a biomimetic ATP recognition unit[55]. The ATP recognition unit was composed of a biguanidine (GA) spacer and a *β*-cyclodextrin (CD) host moiety. With the synergistic effect of the host-guest interaction between the adenine and cyclodextrin and the hydrogen bond of the triphosphate-guanidine, the synthesized unit could realize the specific response to ATP molecules, and inhibit other intercellular ATP analogues (such as adenosine diphosphate ADP, adenosine monophosphate AMP, cytosine triphosphate CTP, triphosphate guanosine GTP, *etc.*). With the continuous

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addition of ATP, the polymer chain became increasingly hydrophobic attributed to the complex of ATP and bio-inspired ATP receptor. Thus, by regulating the concentration of ATP, the proportion of the hydrophobic segments in the hybrid complex can be tuned, so as to accurately control the self-assembled nanostructures in diverse dimensionalities and geometries (Fig. 10).

Carbon dioxide (CO_2)

CO_2 has received widespread attention as it is known as greenhouse gases. However, more and more studies have shown that it is a major product of metabolism, associated with some certain physiological diseases[56]. Moreover, the accurate monitor of CO_2 level in biological arteries and venous will provide an important basis for the diagnosis[57, 58]. In addition, CO_2 has good biocompatibility and biomembrane permeability[59], so it can be used as an appropriate stimulus in the physiological environment.

CO_2 can selectively react with certain functional groups (such as tertiary amino groups, amidine groups, and guanidine groups) to convert these groups into hydrophilic species. It should be noted that the reversible process can be easily achieved in the atmosphere of inert gas (argon or nitrogen), which means this reaction can achieve multiple "on-off" cycles without any accumulation of chemicals[60]. Therefore, for the polymer assembly system, CO_2 is truly a mild and green stimulating source. Due to the great advantage of CO_2 as stimulus in the polymer science, many research groups take efforts in this area, as well as our groups. Since the beginning of 2011, Yan's group has been devoted to the study of polymer assembly and disassembly with CO_2 as the stimulus and have been successfully developed a series of functional applications, including biomimetic nanocontainers, biomimetic cells and protein separation.

In 2011, Yuan and Yan *et al.* successfully realized the CO_2 stimuli-response in aqueous solution by utilizing the amphipathic block copolymers with highly reactive amidine pendent groups[61]. The block copolymers could spontaneously self-assemble into vesicles with a diameter of ~110 nm. However, as CO_2 was passed through the solution, the size of these vesicles expanded to 205 nm due to the increasing degree of protonation of the polymer chains, triggered by CO_2. It is worth noting that when the solution is under the atmosphere of argon, the size of vesicles recovered to the original size reversibly (Fig. 11). This process is similar to the breathing feature of the protocell.

Inspired by the previous work studied, Zhao and Yan *et al.* designed and synthesized a new class of triblock copolymer. In this study, they changed the place of CO_2-sensitive block and hydrophobic polystyrene block, and successfully realized the organelle deformation mimicry (Fig. 12). This polymer chain consisted of outer hydrophilic poly(ethylene oxide) (O) and poly((2-diethylamino)ethyl methacrylate) CO_2-sensitive block (A), hydrophobic polystyrene (S) as bridging block[62]. Fixing the length of O and A blocks, the OSA copolymers could self-assemble to three initial nanostructures, such as spherical micelles, worm-like micelles and huge vesicles, by varying the length of the S block. For the polymer $O_{113}S_{30}A_{52}$, which has the shortest S block, the size of the sphere micelles could continuously increase from 24 nm to 45 nm, similar to the respiration of the lipid bubble. For the polymer $O_{113}S_{72}A_{61}$ containing longer S block, the curly nanofiber stretched as the continuous addition of CO_2, which was similar to the elastic telescopic movement of microfilaments. For the polymer $O_{113}S_{211}A_{59}$ containing the longest block, the vesicles could realize compartmentalization and form dozens of irregular vacuoles. To some extent, this transformation was similar to the endocytosis of the lysosomal. The key to various deformations was that CO_2-sensitive block was hidden in the hydrophobic core and was restricted by the S block. When the A block was ionized by CO_2, it could absorb more water although it could not dissolve. From this work, our group drew a conclusion that the synergy of core-forming chain restricted hydration and corona-forming chain repulsion was an effective way to regulate the shape of assemblies.

In 2011, Zhang's group synthesized a homopolymer *via* RAFT polymerization with suitable hydrophobic backbone

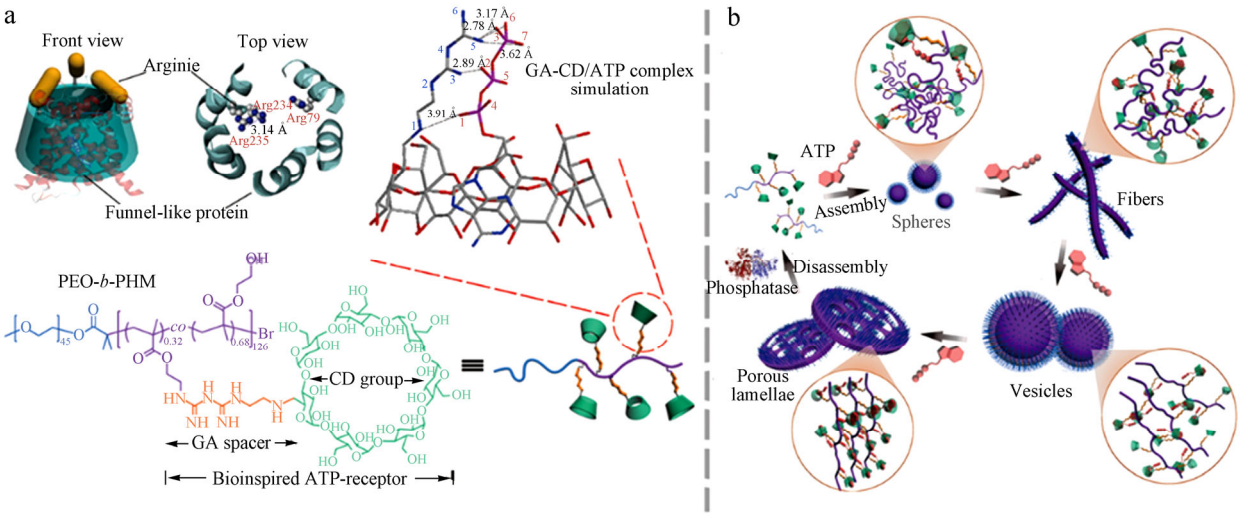

Fig. 10 Chemical structure of PEO-*b*-PHM bearing biomimetic ATP-receptor side groups and their reversible self-assembly evolution triggered by ATP (Reprinted with permission from Ref. [55]; Copyright (2015) The Royal Society of Chemistry)

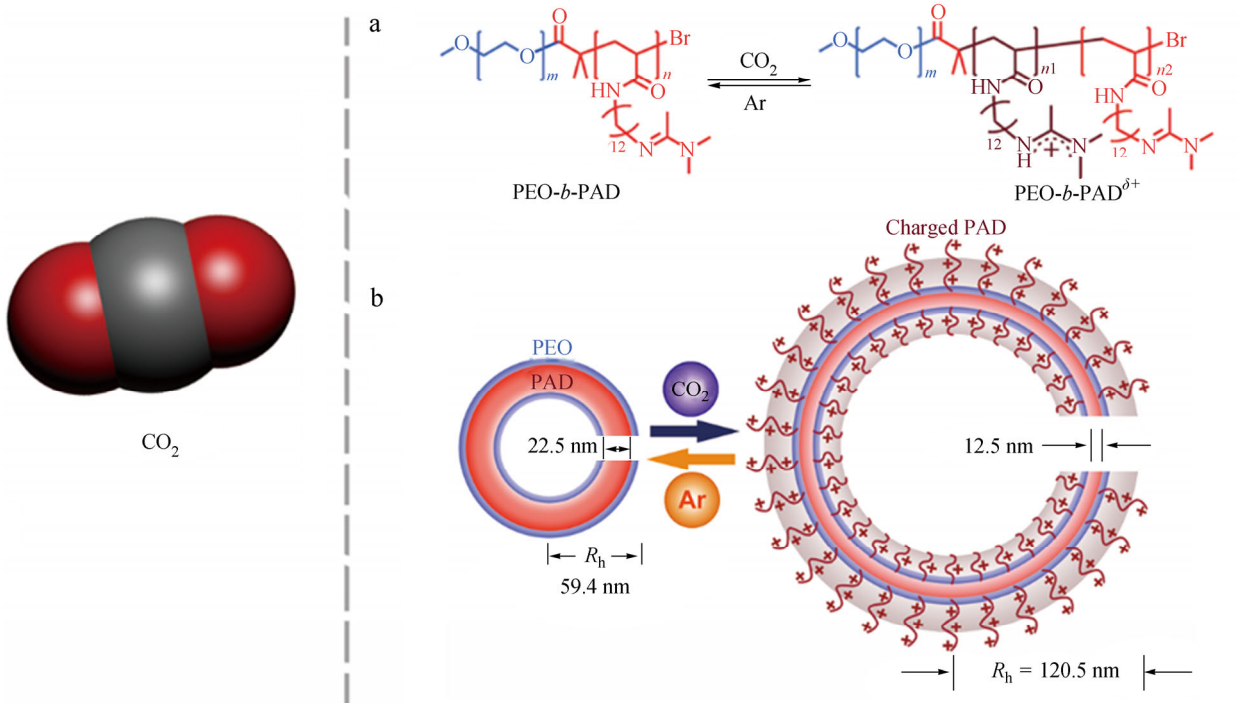

Fig. 11 Chemical structure of PEO-*b*-PAD diblock copolymer with CO_2-sensitive amidine group and their CO_2-tunable self-assembly into "breathing" vesicles (Reprinted with permission from Ref. [61]; Copyright (2011) Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim)

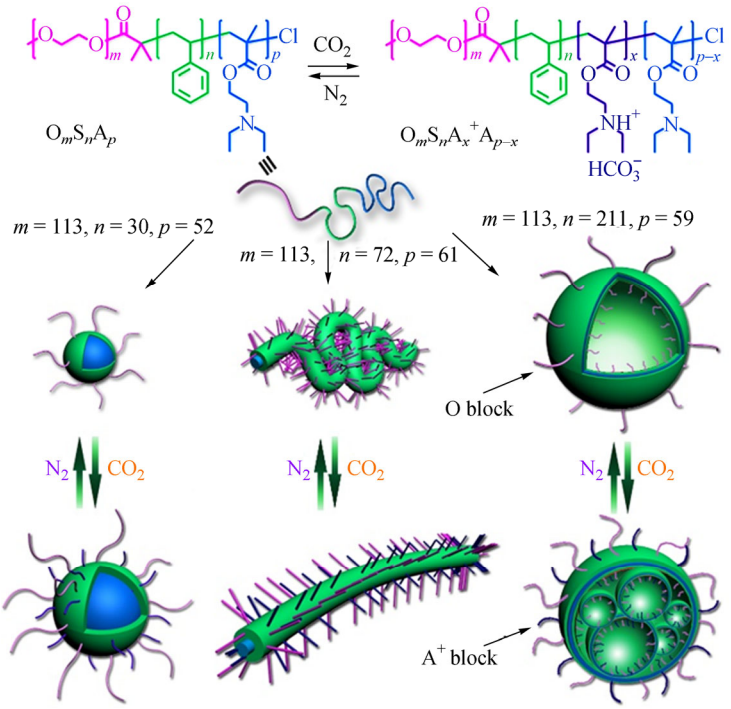

Fig. 12 Chemical structure of OSA triblock copolymers with CO_2-sensitive tertiary amine group and their CO_2-driven multiform shape transformation (Reprinted with permission from Ref. [62]; Copyright (2013) American Chemical Society)

and amidine pendants derived from polystyrene[63]. As described above, amidine group was able to be protonated by CO_2, thus the homopolymer exhibited a hydrophobic-hydrophilic transition in solution upon the exposure of CO_2 and N_2. The system with a green trigger may find potential applications in sensors, "smart'' surfaces, drug delivery. Yuan and coworkers developed a class of a supramolecular triblock stimuli-responsive copolymer PNIPAM-*b*-PCL-*b*-PDMAEMA with good biocompatibility[64]. Compared with other CO_2-responsive polymers with breakage and formation of chemical bonds, the construction of PCL and PDMAEMA segments adopted a supramolecular method utilizing host-guest inclusion complexation between β-cyclodextrin and adamantane, which could avoid the wide polydispersity

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obtained through the general copolymerization strategies (*e.g.* ATRP and ROP), and allow a greater degree of freedom due to the near-spherical structure of adamantane. PNIPAM-*b*-PCL-*b*-PDMAEMA had both CO_2 and temperature responsive segments incorporated into the design of dual stimuli-responsive polymers. After introducing CO_2 into the water, the tertiary amine groups could be protonated and form a charged ammonium bicarbonate, leading to the vesicular assemblies swelled obviously. This process could be reversed and repeated more than 4 times under N_2/CO_2 passing through alternatively. While raising the temperature, the assemblies showed a conversion between vesicles and spherical micelles due to the PNIPAM segment.

Oxygen (O_2)

As we all know, O_2 is the most important cell metabolite and it is irreplaceable for cell breathing. However, up to now, O_2-responsive polymer has relatively little been attended. In 2014, the first O_2-sensitive functionality was reported by Jeong[65]. He demonstrated that the pentafluorophenyl end-capped poly(ethylene glycol) was able to sense O_2. Inspired by the Jeong' work, Zhu and his coworkers introduced the O_2-sensitive functionality to polymer side chains[66]. Their group synthesized a CO_2- and O_2-responsive polymer *via* ATRP protocol, which consisted of one hydrophilic poly(ethylene glycol) (PEG) block, one hydrophobic CO_2-responsive block poly(*N*,*N*-diethyl-aminoethyl methacrylate) (DEA) and one O_2-responsive poly(2,2,2-trifluoroethyl methacrylate) (FMA) block. The triblock copolymer could self-assemble into nanoaggregates, which was able to expand its size under the exposure of O_2. The increment of aggregated size is mainly attributed to membrane swelling, as O_2 could only slightly increase water solubility of the highly hydrophobic FMA without charge accumulation (Fig. 13).

Glucose

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic disorders in which there are high blood sugar levels over a prolonged period. Now, diabetes, regarded as a global disease, affects the development of public health enterprise all over the world. Hence, researchers have exploited a series of glucose-responsive polymer systems to deliver insulin. Among all these systems, the most attracting one is the polymer based on the boronic acid and its derivatives. The reasons are listed below. (1) It is convenient to introduce boronic acid and its derivatives into different systems and these systems are much more stable compared with protein-based macromolecular systems, such as glucose oxidase with Concanavalin A[67]. (2) The corresponding polymer assemblies are easy to be modified and functionalized *via* the addition of different molecules[68–70]. Besides, it can help to protect the preloading protein drugs from the deterioration by the exterior environment[71]. For example, Li and his coworkers developed a series of amphiphilic block and random phenyl boronic acid-based glycol-polymers *via* RAFT polymerization[72]. The study demonstrated that these two kinds of polymer chains are able to self-assemble to form stable nanoparticles and could be used as nanocarriers for drug delivery. As shown by statistics, random copolymer nanoparticles, compared with block copolymers, exhibited faster release rate and bigger storage capacity. The introduction of glycol-block was capable of enhancing the biocompatibility of the polymer system based on the boric acid and its derivatives, to ensure that this polymer system can be applied in the biomedical area.

Kim and his coworkers made a great breakthrough in constructing a polymer system that can response to monosaccharides (including glucose) at neutral pH[73]. The polymer system is composed of hydrophilic poly(ethylene oxide) block and poly(styreneboroxole) (PBO), polymerized *via* RAFT polymerization method. In PBS buffer, the polymer chains were capable of self-assembling into nanoparticles. Due to the specific binding effect between boronic acid and vicinal diol in monosaccharides, the assembly disrupted, further leading to the release of the preloaded insulin (Fig. 14). It is expected that this system could be used as a sensing probe and a nanocarrier in the glucose-related diseases, like diabetes.

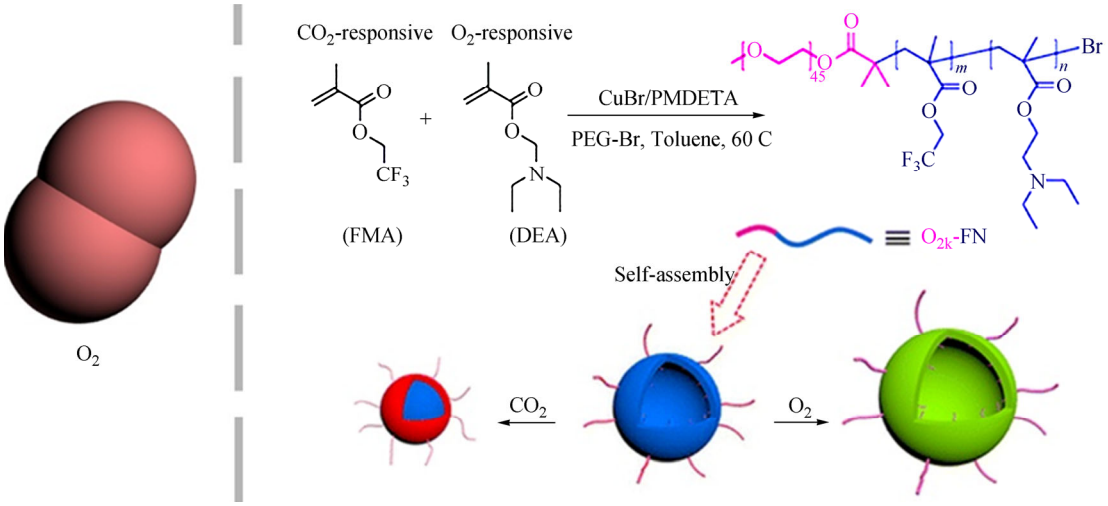

Fig. 13 Chemical structure of O_{2k}-FN triblock copolymers with CO_2-sensitive and O_2-sensitive group and their size transformation (Reprinted with permission from Ref. [66]; Copyright (2014) American Chemical Society)

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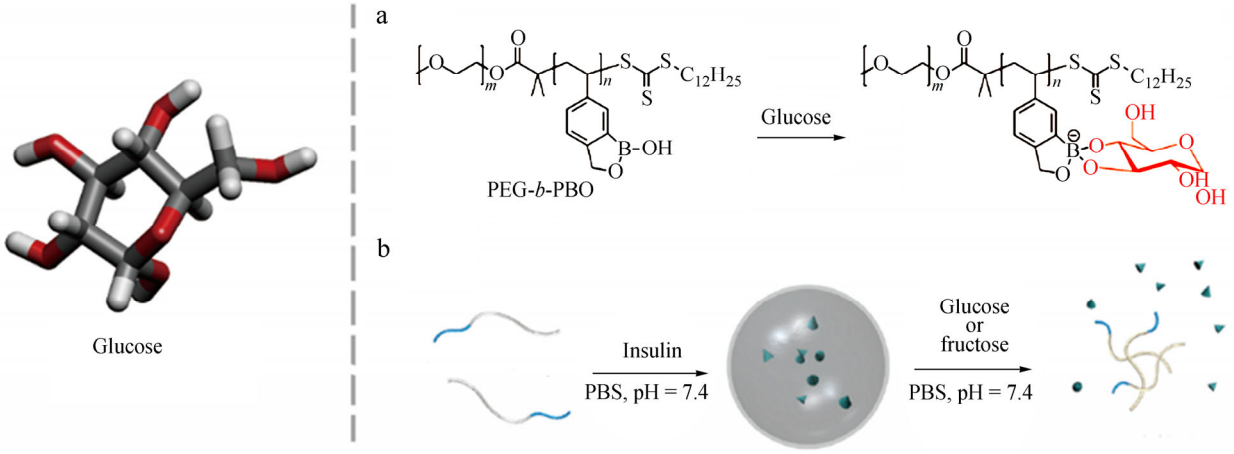

Fig. 14 Chemical structure of PEG-*b*-PBO diblock copolymers with glucose-sensitive boroxole group and their glucose-responsive disassembly of polymer vesicles (Reprinted with permission from Ref. [73]; Copyright (2012) American Chemical Society)

Reactive Species

Compared with the reductant-responsive polymer systems, little research has been done on the field of the oxidant-responsive polymer systems. However, this class of assembly is really suitable for the delivery of antigen-presenting cells (APCs) due to the high concentration of the oxidant (H_2O_2) in the antigen-presenting cells[74]. Frechet and his coworkers developed a biocompatible H_2O_2-responsive polymer, in which dextran, a water-soluble polysaccharide, was modified with arylboronic esters at its hydroxyls[75]. The nanoparticles assembled from the modified dextran were sensitive to H_2O_2. The study showed that under the stimulation of 1 mmol/L H_2O_2, the half-life of the nanoparticles was 36 min, while the nanostructures were stable over one week in the absence of H_2O_2. They demonstrated that this system could provide a significant improvement to MHC I presentation from DCs compared with non-oxidation degradable nanovectors.

Studies have shown that glutathione (GSH) and oxidized glutathione (GSSG) are the most abundant redox pairs in animal cells. The concentration of GSH in the cytoplasm and nucleic acid reaches 10 mmol/L under the catalysis of glutathione reductase, but the extracellular concentration of GSH is between 2 and 20 mmol/L[76]. *In vivo* experiments in mice suggested that the concentration of GSH in tumor tissue is at least four times compared with that of normal tissue[77]. Herein, it would be of great importance if we can take advantage of the GSH concentration difference to construct a novel GSH responsive drug delivery system. Zhong's group developed a biodegradable diblock copolymer based on poly(ethylene glycol) block and poly(2,4,6-trimethoxybenzylidene-pentaerythritol carbonate) block[78]. The polymer chain could realize dual-response to both redox and pH. The polymer chain self-assembled into micelles averaged at 140 nm, where Dox could be encapsulated. It has been proved that 24.5% Dox was released within 21 h from the polymer micelles in physiological conditions. However, the release of Dox at pH = 5.0 or 10 mmol/L GSH (pH = 7.4) could even reach up to 62.8% and 74.3%. Under pH 5.0 and 10 mmol/L GSH, 94.2% Dox could be released within 10 h from the micelles. MTT assay showed that the IC_{50} of the micelles to HeLa/RAW cells was 0.75 and 0.60 μg/mL, respectively.

Xu's group established a dual redox responsive system from diselenide block copolymers. They used a novel diselenide-containing polyurethane blocks (PUSeSe) as hydrophobic bock and hydrophilic poly(ethylene oxide) grafted at the two flanks[79] (Fig. 15). The PEG-PUSeSe-PEG block copolymers were capable of self-assembling to form stable micelles in aqueous solution and were highly sensitive to redox stimulus, attributed to the cleavage of diselenide. The nanostructure disrupted even under the trigger of low level of H_2O_2 or GSH, leading to the release of the drugs. It is highly expected that this system can open up a new avenue to establish controlled drug delivery system.

On the basis of the success in the last work, Xu's group continued to exploit a new responsive system based on the stimulation of singlet oxygen ($^1O_2$)[80]. The porphyrin derivative was added as a chromophore to absorb red light and further produce singlet oxygen. The production of singlet oxygen of the system cleaved the diselenide bond, leading to the cleavage of the polymer chains, further resulting in the disassembly of the polymer aggregates. With the disruption of the nanostructures, the encapsulated drugs in polymeric micelles released.

BIOLOGICAL MACROMOLECULES

Protein and Enzyme

In recent five years, researchers have taken a lot of efforts to explore certain proteins as stimulus to trigger the assembly systems. However, there are few reports about the non-enzymatic protein as a trigger of the self-assembly system. Most of the proteins used in this area are enzymes.

Enzyme-responsive polymer assemblies are widely favored in recent years due to their special characteristics, such as good biocompatibility, high selectivity[81] and many others. In addition, there is always an overexpression of certain enzymes in pathological cells, therefore the concentration of certain enzymes in a various types of tumor cells is higher than that in normal cells. It is possible for us to construct an enzyme-responsive polymeric system and drug delivery system[82].

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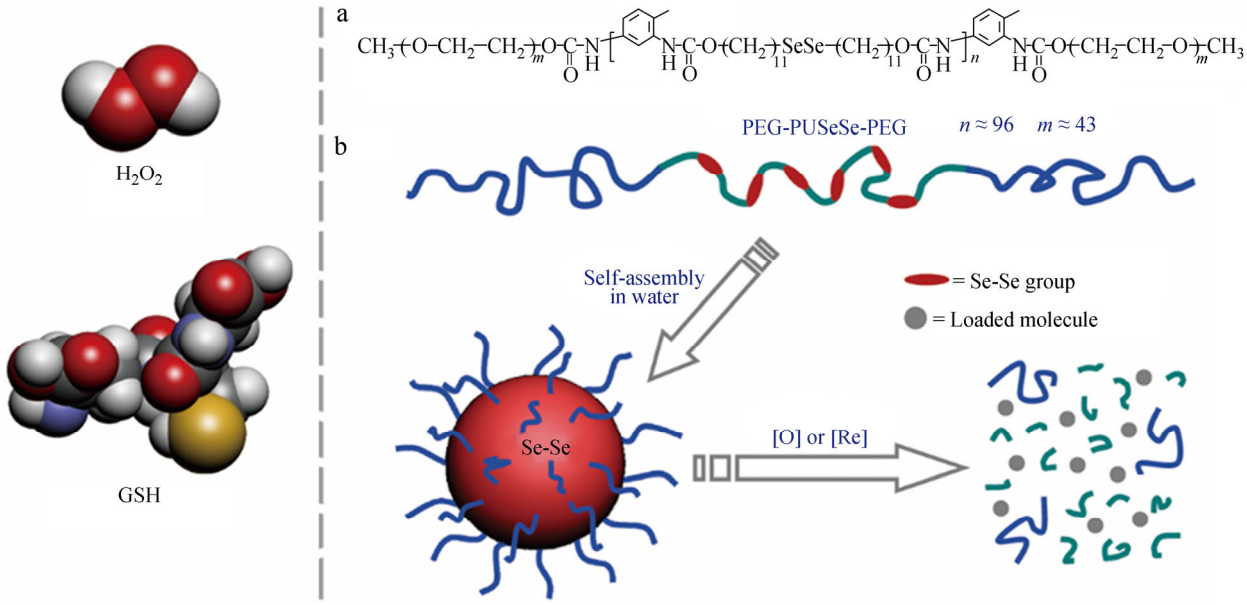

Fig. 15 PEG-*b*-PUSeSe-*b*-PEG triblock copolymers with H_2O_2 and GSH dual-sensitive diselenide connection and their dual redox-responsive disassembly of polymer micelles (Reprinted with permission from Ref. [79]; Copyright (2010) American Chemical Society)

In 2013, Khan and his coworkers designed an amphiphilic diblock copolymer connected by an azobenzene linkage, which was highly sensitive to enzyme azoreductase[83]. This polymer assembled into a micellar structure in water without azoreductase. However, with the treatment of azoreductase and coenzyme NADPH, the azobenzene junction could be cleaved and the nanostructure disrupted. The results suggested that azobenezene is a useful non-natural structural motif for the preparation of enzyme responsive polymer nanoparticles (Fig. 16).

Roy J. Amir has done a lot of work in the study of the enzyme-responsive degradation rate. In 2014, he exploited that the length of the hydrophilic block was of great importance to the stability of the enzyme-responsive nanoparticles[84]. The longer the hydrophilic PEG block was, the more stable the self-assembled aggregate was, thus the slower rate the enzyme-trigger degradation rate was.

Inspired by the previous results, their group wished to investigate that whether the number of hydrophobic end-groups can affect the property of the self-assembled aggregates. Recently, their group synthesized a series of amphiphilic PEG-dendron hybrids with different numbers of enzyme- cleavable end groups[85] (Fig. 17). The results showed that the number of dendrons greatly affected the sensitivity of polymers to enzymes. The stability of self-assembled aggregates increased significantly as the number of dendrons increased, and the enzymatic sensitivity ranged from highly responsive micelles to practically

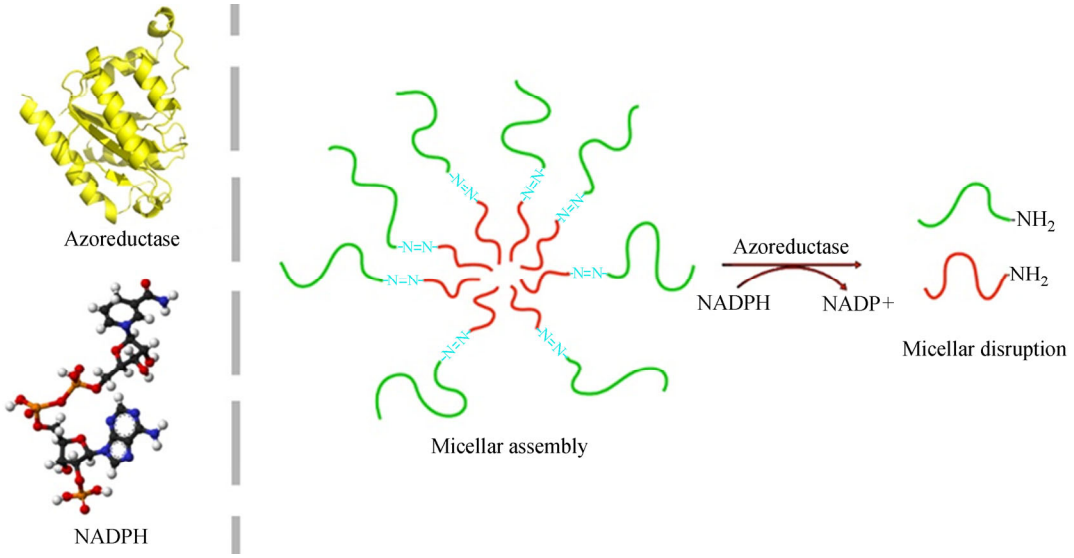

Fig. 16 Amphiphilic diblock copolymer with azoreductase sensitive azobenzene linkage (Reprinted with permission from Ref. [83]; Copyright (2013) American Chemical Society)

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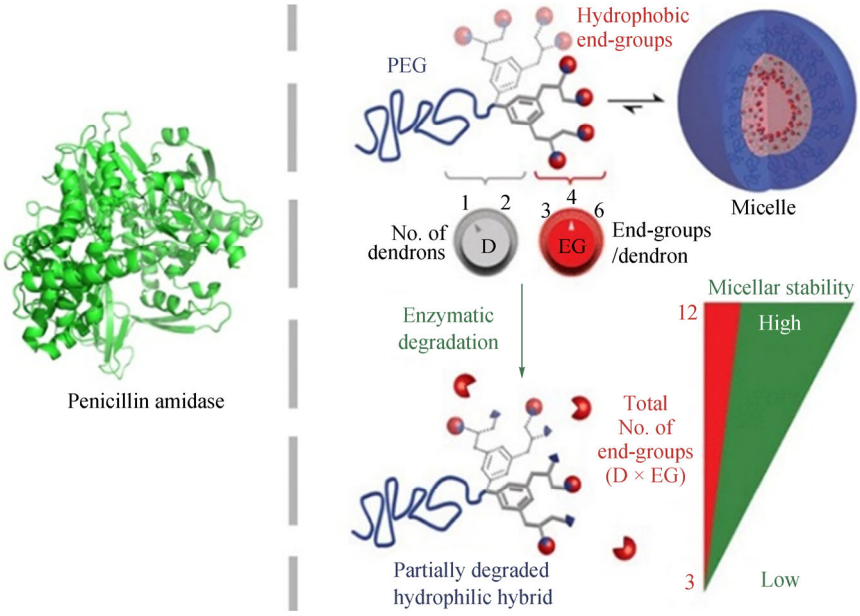

Fig. 17 Amphiphilic PEG-dendron hybrids with different numbers of amidase enzyme-sensitive end groups (Reprinted with permission from Ref. [85]; Copyright (2017) American Chemical Society)

nondegradable ones. This important discovery can guide us to design proper polymer structures of enzymatic degradation systems to realize the precise control of encapsulated drugs.

Jayakannan and his coworkers developed a new polymer with aliphatic polyester as backbones polymerized from a multifunctional monomer converted by natural L-aspartic acid and BOC urethanes as pendants[86] (Fig. 18). The pendant was sensitive to pH, and the backbone was sensitive to the lysosomal enzyme, thus this system could realize dual-response to both pH and enzyme. The polymer chain was capable of self-assembling into stable nanoparticles, which have good capacity to several anticancer drugs, such as doxorubicin (DOX), topotecan (TPT), and curcumin (CUR). At low pH, the pendants protonated to cationic species and the nanoparticles disassembled in the protonation process. In addition, the aliphatic polyester backbone could be cleaved when exposed to the lysosomal enzymes, leading to a release of the encapsulated drugs.

Zhang's group developed phosphatase-responsive polymer micelle by ATP/ionomer complex. As we all know, ATP contains hydrophobic adenine units in physiological condition, and their phosphonate bonds can be hydrolyzed by phosphatases and the negatively charged molecules converted to neutral adenine. In this study, the authors used the electrostatic interaction between cationic block copolymers and ATP, constructing supramolecular micelles. With the addition of phosphatase, ATP began to hydrolyze and the supramolecular complexes could be dissociated, leading to the collapse of the assembling aggregates[87] (Fig. 19).

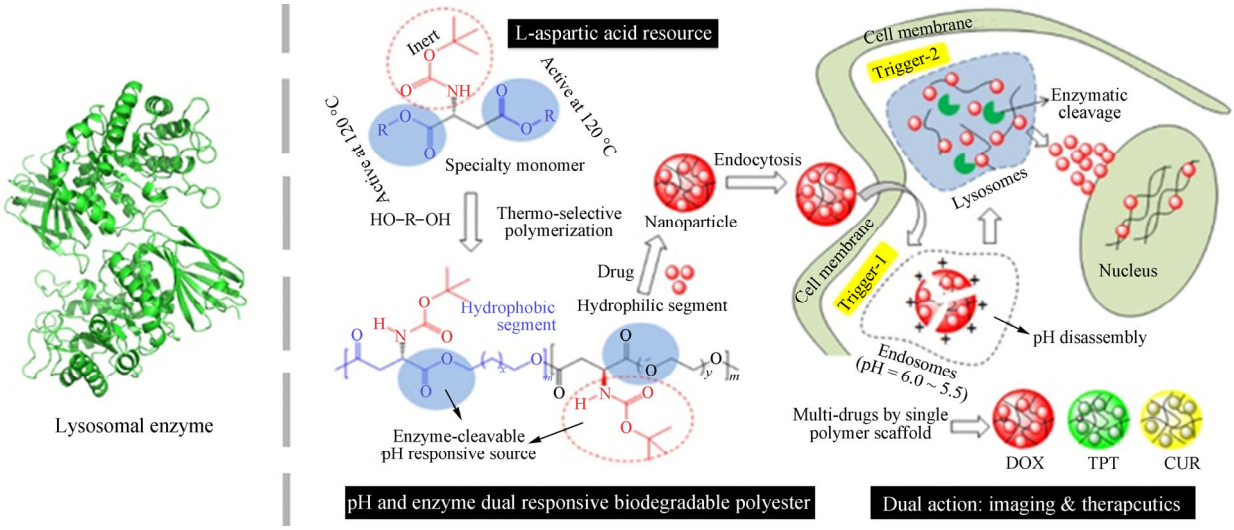

Fig. 18 Lysosomal enzyme-responsive polymer and its enzyme-sensitive molecular mechanism (Reprinted with permission from Ref. [86]; Copyright (2016) Wiley Periodicals, Inc)

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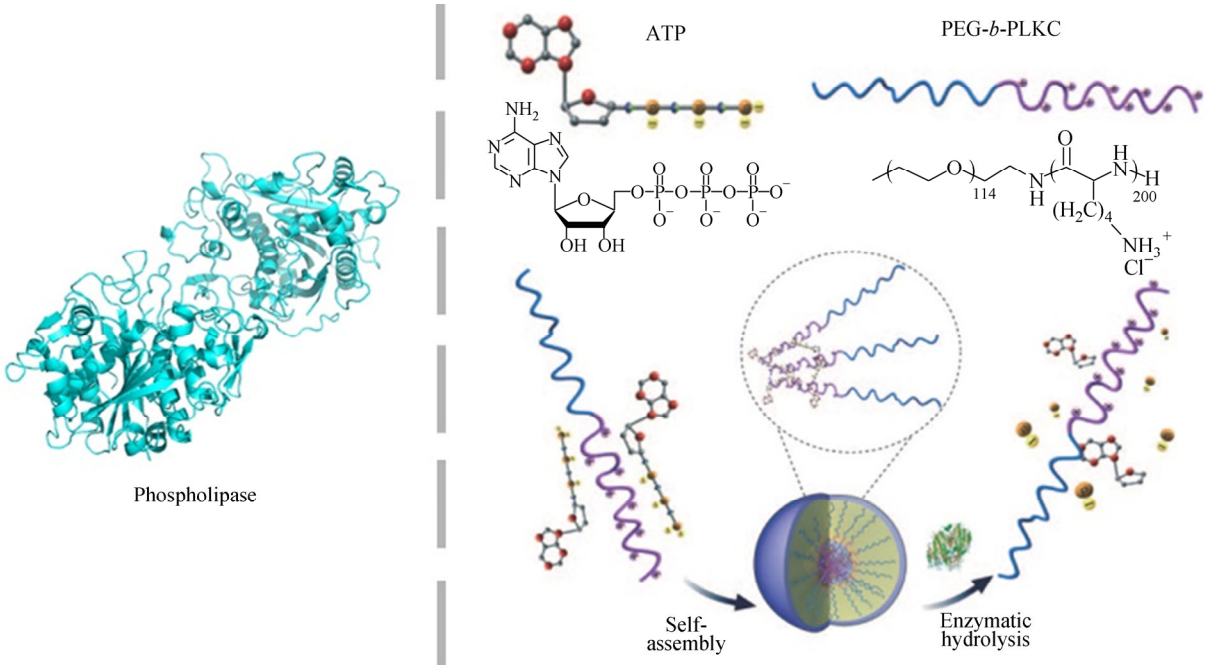

Fig. 19 Phosphatase-responsive polymer micelle by ATP/ionomer complex (Reprinted with permission from Ref. [87]; Copyright (2010) WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim)

An antibiotic misuse has produced more virulent and resistant strains, which pose huge threats to global health. Thus, exploring the enzyme-responsive polymeric systems are of great importance to bacteria detection, in which enzymes are relevant to the acquisition of bacterial resistance. In 2016, Liu and his coworkers developed a general strategy to construct bacterial strain-selective delivery nanocarriers[88]. The nanocarriers were based on the self-assembly of the block copolymers, which were composed of hydrophilic poly(ethylene glycol) (PEG) block and hydrophobic block containing enzyme-cleavable self-immolative side linkages. With specific enzymes (penicillin G amidase (PGA) and b-lactamase (Bla)), the side linkage was cleaved, triggering the transformation of morphology and release of loaded cargo. As all of the enzyme-responsive vesicles can share the same backbone scaffold attached with different types of enzymatic linkage in the side chain, it is believed to construct a new system against virulent resistant pathogens (Fig. 20).

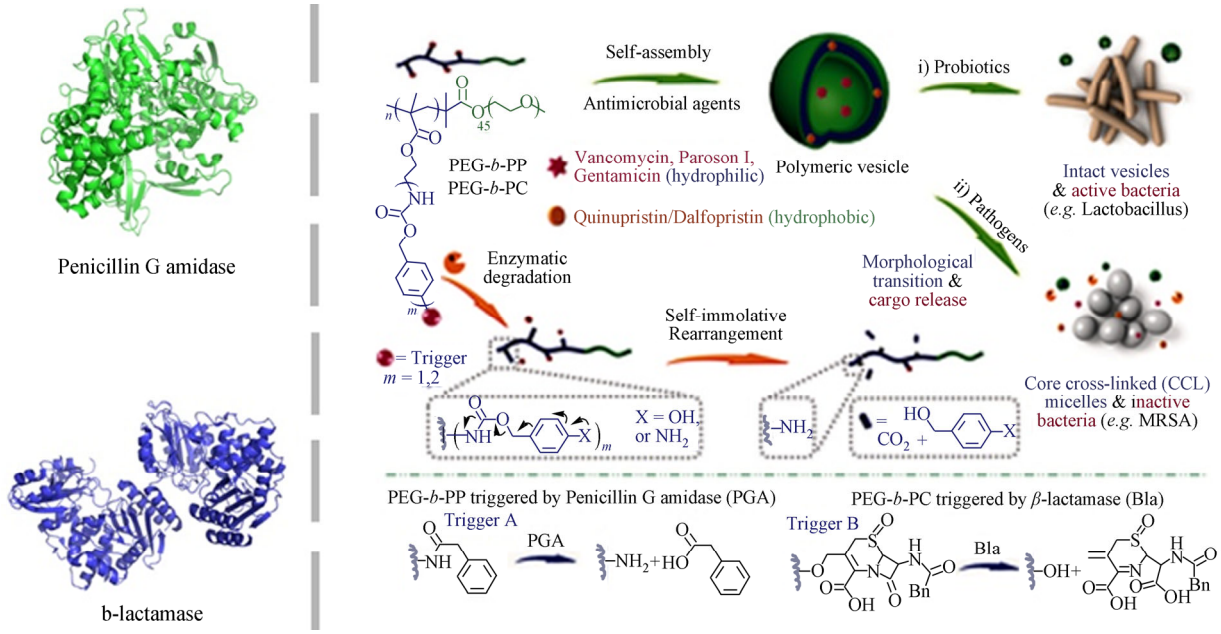

Fig. 20 Penicillin G amidase (PGA) and b-lactamase (Bla) enzyme-responsive polymeric systems (Reprinted with permission from Ref. [88]; Copyright (2016) WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim)

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With the foundation of the previous work, Liu's group continued to study to further prove the robustness and versatility of the strategy in constructing enzyme-responsive polymer vesicles they proposed[89]. In this work, Lipase and Nitroreductase, widely distributed in bacteria, were chosen as the trigger to design and prepare an enzyme-responsive polymeric vesicle for the transport of antimicrobial agents. The two systems could also achieve the remarkable transformation of morphology and the release of loaded cargo.

DNA

DNA gradually becomes an ideal structure material in the DNA nanotechnology in the past several years due to its special characteristics, such as good biocompatibility, controlled programmability, and convenient preparation[90]. The DNA modified with organic and inorganic materials has been used to construct supramolecular DNA assays, building rich functions of DNA nanostructures[91]. For example, with these three-dimensional DNA structures, researchers have successfully achieved the DNA assembly of cylindrical structure, precise regulation of relevant parameters of DNA nanotubes and the creation of hydrophobic environment in its cavity[92].

Sleiman and his coworkers reported a new mode of assembly in DNA technology[93] (Fig. 21). They developed a DNA cube with dentritic alkyl-DNA conjugates. The hydrophobic residues of two neighboring cubes could engage in to form intermolecular 'handshake' due to the intermolecular interaction, forming monodisperse micelles. These micelles were able to encapsulate drugs and deliver them in the presence of specific DNA sequence. As there is often an overexpression of gene in diseased cells, including cancer cells, DNA cube is expected to be used to transport the drugs to the diseased cells in the future.

After that, they continued to design a dynamic DNA cube[94] (Fig. 22). This cube could be selectively unzipped to a flat two dimensional structure from a 3D structure when exposed to a specific nucleic acid sequence. They have proved that the DNA cube shows great stability towards nucleases and robust cellular uptake even in drug-resistant cell lines. After the modification of hydrophobic and hydrophilic dendritic DNA chains, the DNA cube changed a lot in the behavior of cellular uptake while maintaining its ability to open up with the specific sequence and stability in biological environments. This study can help us to design a drug delivery system in diseased environment.

SUMMARY AND OUTLOOK

As a new family of smart polymers with excellent bio-selectivity and bio-specificity, biological stimuli-responsive polymers and their self-assembly are undergoing blooming development. With active efforts from research worldwide, many new methods on structural design and strategies on assembly process have been introduced in this field to build 'perfect' polymer nanocarriers, enabling this nanomedicine with high targetability, precise cell-specificity, toxicity-free, and ultrasensitivity. The kind of nanocarriers not only circumvent the tricky problems that traditional responsive carriers do not solve, but also offer us an all-in-one nanoplatform to execute multiform theranostic

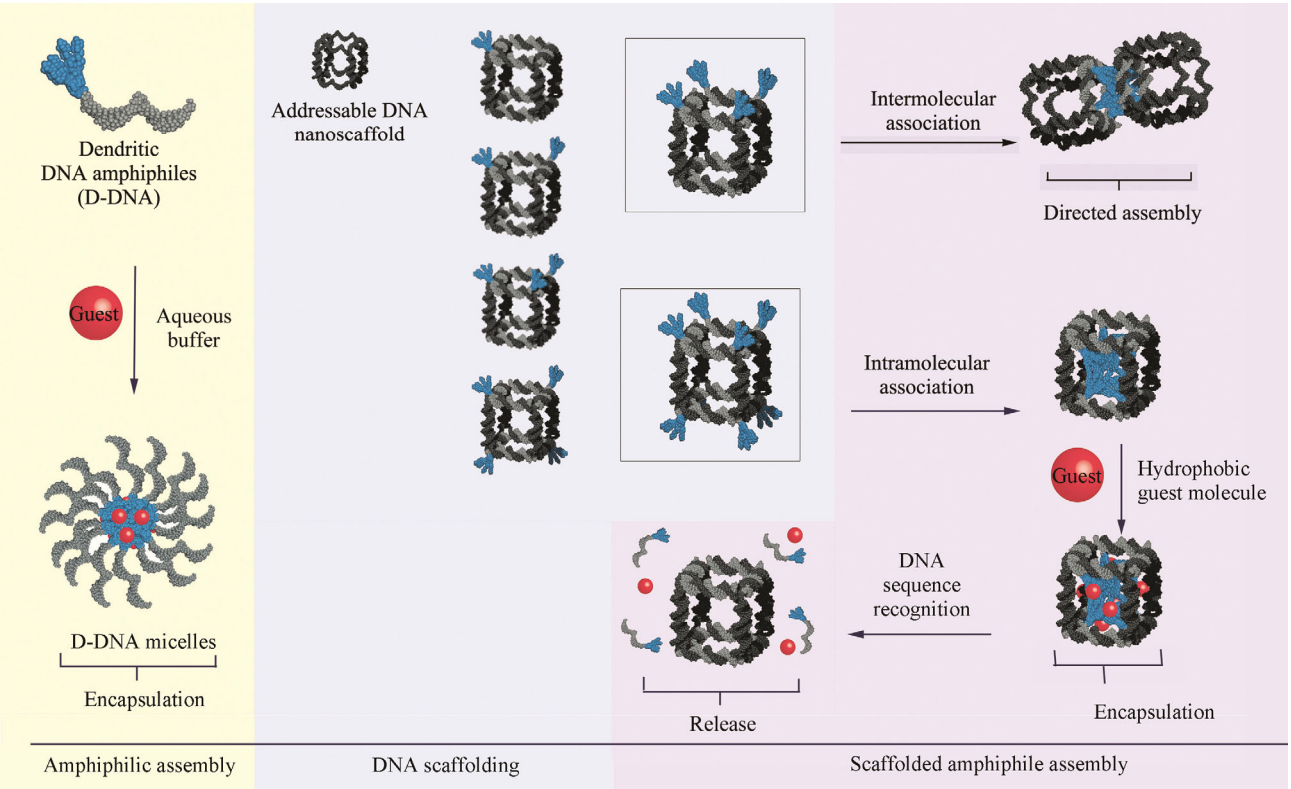

Fig. 21 DNA-responsive "nanocube" carriers (Reprinted with permission from Ref. [93]; Copyright (2013) Nature Publishing Group)

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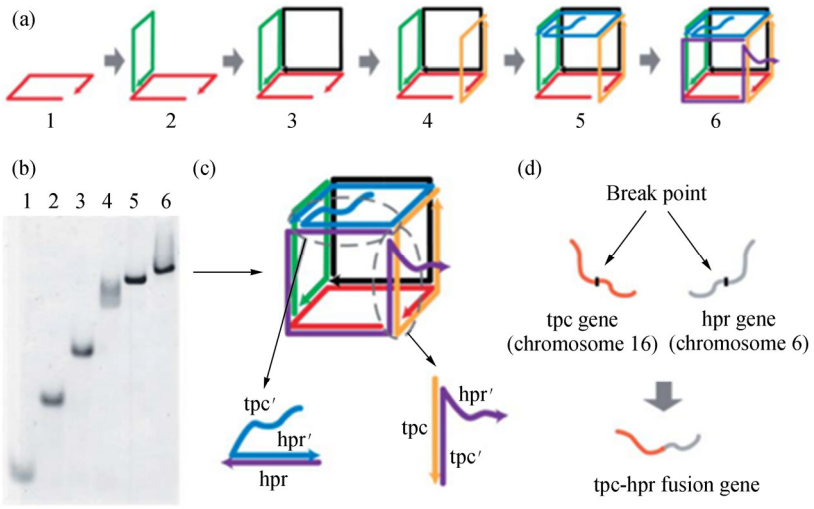

Fig. 22 Specific-responsive dynamic DNA "nanocube" (Reprinted with permission from Ref. [94]; Copyright (2015) The Royal Society of Chemistry)

functions. As more and more researchers are getting involved in this topic, we believe that the biological stimuli-responsive polymers will lead the future advances of responsive polymer materials. Despite burgeoning progress, we must be clearly aware of the current challenges and the following aspects deserve our continuous attentions:

(1) How to simplify the polymer structural design? In comparison to traditional responsive polymers, the chain structures and functional groups of biological stimuli-responsive polymers are relatively complex. For instance, to small-molecule biosignals, if one desires to recognize the simplest diatomic molecule (CO), we have to introduce metal-bridging structure which involves multistep synthesis. Therefore, the pursuit of much simpler polymer structures while keeping high bioresponsive ability is a synthetic hurdle to overcome. To address this issue, a possible direction is to integrate protein into polymer to construct protein-polymer hybrids. The main reason is that many proteins possess specific molecular ligands to recognize. Protein-polymer hybrids are possible to respond more complicated biosignals whereas only implement into simple bio-conjugated structure.

(2) How to expand to the spectrum of biological signals? Besides the above biosignals, there are a lot of biomolecules with intricate structures including vitamins, hormones and metabolites. However, it is difficult to find suitable structure to selectively distinguish them. Thus extending the repertoire of biological stimuli-responsive polymers is crucial.

(3) How to exponentially amplify the biosignal sensitivity by polymer topology? Because of the low CSL of biological signals (~10^{-9} mol/L), the polymer is necessary to be supersensitive. A new strategy is to use self-immolative main-chain polymer[95] in which probably one signal molecule can result in a complete destruction of polymer assemblies. This method may efficiently amplify the low levels of biosignals.

With these challenges, polymer chemists in this field need to aggressively explore new structural design strategies and self-assembly methods. At present, biological stimuli-responsive polymers have showed potent abilities and particular advantages in various applications. We anticipate that it will continue its rapid pace of moving forward and be utilized in a broad scope of disciplines, especially in smart materials and biomimetic materials. With more and more efforts put in, it is expectable that the biological stimuli-responsive polymers will offer new visions on future responsive macromolecules.

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