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Cyclodextrin-based biological stimuli-responsive carriers for smart and precision medicine

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Spurred on by recent progress in nanotechnology and precision medicine, smart drug carriers are entering an entirely new era. Smart drug carriers have been widely studied in recent years as a result of their ability to control drug release under different microenvironments (such as pH, redox, and enzyme) *in vivo*. Host–guest interactions based on cyclodextrins have proven to be an efficient tool for fabricating smart drug carriers. Because of the application of host–guest interactions, many kinds of biological molecules or supramolecular building blocks can combine into an organic whole at the molecular level. In this review, the features, mechanisms of action, and potent applications of biological stimuli-responsive drug carriers based on cyclodextrins are discussed. In addition, some personal perspectives on this field are presented.

Introduction

The Nobel Prize in Chemistry 2016 has been awarded jointly to Jean-Pierre Sauvage, Sir J. Fraser Stoddart, and Bernard L. Feringa, in recognition of the outstanding contributions made by the three scientists in the field of the "design and synthesis of nanometer molecular machines".[1] In the long run, with the development of precision medicine, nanometer molecular machines are set to play a prominent role in the field of smart drug carriers. At the same time, it is hoped that the treatment of cancer and other related diseases will achieve a new dimension. Tumors are common diseases. Generally speaking, they are among the leading causes of death worldwide, and their incidence is increasing as the population ages.[2] Tumors constitute only a small part of the body's tissue mass overall. If we can target anti-cancer drugs to tumor tissues *in vivo*, the local concentration of the drugs will be sharply increased, and the therapeutic effect should be greatly improved. However, the delivery of drugs to specific target tissues or organs *in vivo* and the accurate release of drugs pose problems for doctors and scientists.[3–5] In recent years, spurred by precision medicine, biological stimuli-responsive materials have been studied extensively for smart drug carriers.[6] In drug delivery, the dosage-, spatial- and/or temporal-controlled release of therapeutics significantly enhances the treatment efficacy in a precise manner. One of the foundations of precision medical research is to carry out individualized drug delivery. Accurate delivery can allow the minimum dose of the drug to achieve the highest biological efficacy with the lowest side effects. Smart drug carriers can act as a new weapon for treating tumors.[7,8]

Simply stated, smart drug carriers can give specific feedback when they are stimulated by a series of external signals. The specific feedback is mainly the change in the internal structure of the carriers. The stimulus signals may be diverse. According to their origin, they can be divided into biological stimuli signals and non-biological stimuli signals. Biological stimuli signals include pH, redox and enzymes, and non-biological stimuli signals include temperature, light, magnetic force, ultrasound, and voltage.[9,10]

Recently, nano molecular machine technology has made a series of innovative breakthroughs. Using these technologies, we can build smart drug carriers more quickly, conveniently, and flexibly. Self-assembly supramolecular technology has been one of the hot topics in nano biotechnology for a long time.[11] We can take advantage of the characteristics of non-covalent bond self-assembly technology to construct supramolecular assemblies with novel structures and functions. This is of great significance for the research and design of smart drug carriers.[12,13]

Self-assembly refers to the phenomenon that disordered molecules can spontaneously assemble and organize into ordered structures without external intervention and control.[14] Self-assembly is a critical tool for the preparation of nanostructured materials using small molecular materials.[15] The fabrication of nanostructured materials with a high degree of order and a low incidence of defects by self-assembly is a

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promising technique, which has been widely used in the construction of nanostructured materials with different morphologies.[16–18] Nanostructured materials formed by self-assembly have unique physical and chemical properties, and they can be used as smart drug carrier materials. However, self-assembly is usually a spontaneous thermodynamic process, which is difficult for humans to interfere with and control. Therefore, it is still a challenge to control the size and function of self-assembled structural materials.[19]

The formation of self-assembled nanostructured materials is mainly due to the spontaneous organization or aggregation of small molecular blocks in the presence of noncovalent interactions, which include hydrophobic interactions, hydrogen bonds and van der Waals forces.[20] Molecular recognition is the precondition for self-assembly. If the molecular recognition process cannot be completed, the next step of the self-assembly process cannot continue.[21] Molecular recognition is also the process in which a specific receptor molecule selectively accepts a ligand of a specific size. In this process, the molecules find the position that is most stable and where they are closest to each other, and where the aggregation assembles with a higher-order structure that is beyond the individual behavior of a single molecule.[22] In general, the method of self-assembly is convenient, flexible, and efficient for integrating biological materials with different functions into a single entity. However, it is difficult to meet this requirement by ordinary chemical methods.

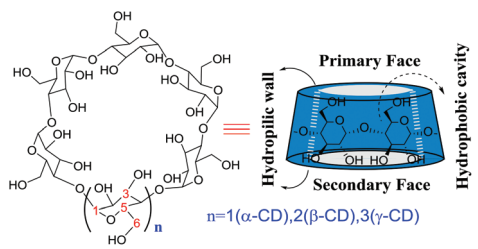

Fig. 1 The spatial structure of cyclodextrin.

Nano functional materials built by the self-assembly method have a variety of biological properties, because many kinds of biological molecules or supramolecular building blocks are integrated into an organic whole by the application of self-assembly. Upon receiving an external stimulus, the nano functional materials can rearrange their structures or morphological characteristics towards their most stable states, because the properties of the biological molecules or supramolecular building blocks, such as their hydrolysis, protonation, conformation, hydrophilicity, or solubility, have changed.[23]

Cyclodextrins (CDs) are commonly composed of five or more α-D-glucopyranoside units in a ring linked by α-1,4-glycosidic bonds. The most frequently used cyclodextrins contain six, seven, or eight glucopyranoside monomers, namely, α-, β-, and γ-cyclodextrin, respectively. Generally, cyclodextrins have a truncated cone structure whose exterior surface is hydrophilic and whose interior cavity is hydrophobic (Fig. 1).[24,25] In pharmaceutical science and technologies, cyclodextrins have been successfully employed to form inclusion complexes with drugs through host–guest interactions.[26,27] Recently, cyclodextrins and their derivatives have been extensively utilized to construct supramolecular nanomedicines. For example, Mark E. Davis *et al.* first reported a diverse class of CD oligomers coupled through cationic linkers, and successfully used them as vectors for siRNA delivery in Phase 1 clinical trials for treatment of melanoma in humans.[28] In addition, they constructed a polymeric nanoparticle comprising cyclodextrin-poly(ethylene glycol) copolymer conjugated to camptothecin (CRLX101) nanomedicine, and Phase 2 clinical trials are ongoing.[29] Cyclodextrin also has the effect of lowering cholesterol and can be used in the treatment of cardiovascular disease[30] (Table 1).

The hydrophobic cavity inside the cyclodextrin can spontaneously combine with various compounds, such as adamantane,[31,32] ferrocene[33] and benzimidazole compounds.[34] These spontaneous combination processes occur mainly through noncovalent interactions and self-assembly, which provide easy and facile approaches for building smart drug carriers.[35,36] Cyclodextrin self-assembly can avoid multiple syn-

Table 1 Clinical trials of cyclodextrins and their nanomedicines (data from clinicaltrials.gov)

Drug	Disease	Phase	Status	Information provided by	Clinical ID	Start date–last updated date
CRLX101[a]	Solid tumor	Phase I/II	Completed	NewLink Genetics Corporation	NCT00333502	May 2006–Apr 2017
		Not provided	Completed	City of Hope Medical Center	NCT01612546	Nov 2012–Jun 2015
		Phase II	Terminated	University of Chicago	NCT01803269	Jan 2013–Jun 2016
		Phase I/II	Recruiting	National Cancer Institute	NCT02769962	May 2016–Jun 2017
CALAA-01[b]	Solid tumor	Phase I	Terminated	Calando Pharmaceuticals	NCT00689065	May 2008–Oct 2013
Cyclodextrins	Obesity	Not provided	Completed	University of California	NCT00682916	May 2008–May 2017
	Sphingolipidosis	Phase I	Completed	Vtesse Inc.	NCT01747135	Jan 2013–Apr 2017
		Phase II/III	Recruiting	Vtesse Inc.	NCT02534844	Sep 2015–Jan 2017
	Cardiovascular disease	Phase II	Completed	National Institutes of Health Clinical Center	NCT01131299	Mar 2010–Oct 2016

[a] CRLX101 consists of a sugar molecule cyclodextrin linked to a chemotherapy drug, camptothecin. [b] CALAA-01 is a targeted nanocomplex that contains anti-R2 siRNA.

thesis steps and a complicated purification process during the fabrication of smart drug carriers. By mixing the building blocks in solution under non-specific conditions, smart drug carriers can be formed through intrinsic self-assembly. Because of the cyclodextrin self-assembly by dynamic interactions, smart drug carriers then have reversibility, allowing convenient dissociation and reconstitution.[37] Thus, smart drug carriers based on cyclodextrins have adaptive capability in response to external stimuli that can trigger a structural change. In recent years, a number of bio-responsive drug carriers with different properties and morphologies have been formed by the self-assembly of cyclodextrins. They can accurately control the time and space of drug release under the stimuli of pH, redox, or biological enzymes. There are two main reasons to explain the control of drug release based on cyclodextrins in response to stimulus signals. On the one hand, the changes in the properties of the guest molecules allow the guest molecules to escape from the cavity of the cyclodextrins. On the other hand, the changes in the physical and chemical properties of other materials in the carriers lead to the release of the drugs from the carriers. In general, the self-controlled drug carriers can accurately release the drug to specific sites, which dramatically improves the drug bioavailability and significantly reduces the side effects of the drug.

The construction and application of cyclodextrin-based nanomedicines have been extensively reviewed elsewhere. This review will focus only on smart and precision medicine utilizing various types of cyclodextrin-based biological stimuli-responsive nanomedicines, including pH-, redox-, enzyme-, and multiply responsive systems. In addition, some personal perspectives on this field are presented.

pH-Responsive system

Using the change in the pH of the surrounding environment to control drug release is currently one of the main strategies for the design of passive-targeting drug carriers. Tumor tissues are in a pathological state for a long period, and the pH around tumor tissues is usually lower than around normal tissues. Through the skilful construction of drug delivery carriers, such carriers release drugs only at the site of tumor tissues. This not only reduces the damage to normal tissues, but also can improve the bioavailability of the drugs.[38]

It is known that cyclodextrin exhibits a pH-sensitive host–guest interaction with benzimidazole (BM).[39] At physiological pH (~7.4), BM has a hydrophobic nature and can form a stable inclusion complex with CD *via* host–guest interactions. However, when BM is protonated, under acidic conditions (pH < 6), the binding constant of the BM/CD complex decreases dramatically and thus the BM is disassociated from the cavity of CD.[40] The main reason is that, under neutral conditions, benzimidazole exists in an unionized state, and it is not easily dissolved in water. However, under acidic conditions, benzimidazole becomes protonated, and it is then easily dissolved in water. According to the "like dissolves like" rule (any substances that have the same polarity dissolve into each other), benzimidazole can automatically enter the hydrophilic environment from the hydrophobic cavity of cyclodextrin.[41]

Chaoliang He *et al.* successfully constructed pH-responsive supramolecular amphiphilic micelles by using the host–guest interaction between benzimidazole-terminated poly(ethylene glycol) (PEG-BM) and cyclodextrin-modified poly(L-lactide) (CD-PLLA) (Fig. 2).[42] The disassembly process of the supramolecular micelles was initiated in acidic environments. An anticancer drug, doxorubicin (DOX), was loaded into the supramolecular micelles as a model drug. The release rate of the model drug from the supramolecular micelles was clearly accelerated as the acidic environment was reduced from 7.4 to 5.5. The reason was that the benzimidazole is protonated in acidic conditions, and it detaches from the hydrophobic cavity of cyclodextrin.[34,40] The DOX-loaded PEG-BM/CD-PLLA supramolecular micelles showed an enhanced intracellular drug release efficiency in HepG2 cells, and a significantly reduced systemic toxicity, and they exhibited higher tumor inhibition efficacy compared to free DOX after intravenous injection into nude mice. Moreover, the DOX-loaded PEG-BM/CD-PLLA supramolecular micelles showed a blood clearance efficiency significantly lower than that of free DOX. Consequently, the pH-responsive PEG-BM/CD-PLLA supramolecular micelles hold potential as smart drug carriers, especially for anticancer drug delivery.

Another strategy is the introduction of acid-sensitive chemical bonds in the internal structure of drug carriers, such as ester bonds and amide bonds. When the carrier is placed in an acidic environment, the chemical bonds in the carrier are broken, which causes the carrier to split. To efficiently deliver antineoplastic drugs to tumor tissues and increase their treatment efficacy at the tumor location, Kaiyong Cai *et al.* constructed a polyethylene glycol (PEG) shielding and tumor microenvironment, triggering a cascade of pH-responsive hollow mesoporous silica nanoparticles (HMSNs) as a drug carrier (Fig. 3).[43] 3-(3,4-Dihydroxyphenyl) propionic acid (DHPA)-functionalized CD was conjugated onto the surfaces of

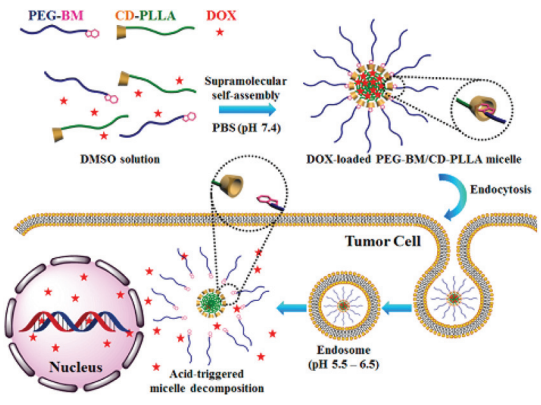

Fig. 2 Schematic illustration of the formation and triggered drug release process for supramolecular micelles in response to the intracellular microenvironment.[42] Reproduced with permission, Copyright 2015 American Chemical Society.

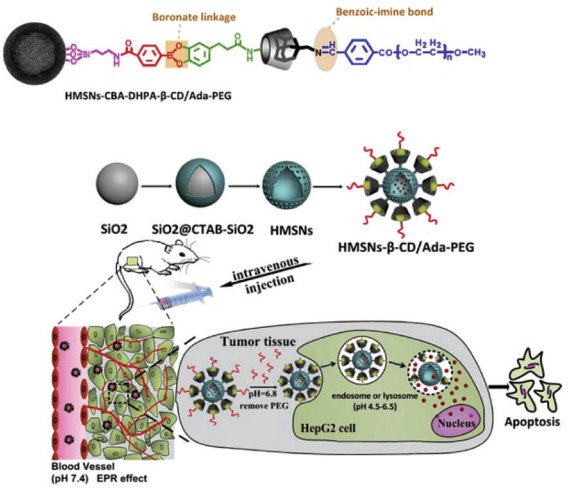

Fig. 3 Schematic illustration of the drug delivery process of the HMSNs-β-CD/Ada-PEG system in a tumor microenvironment *in vivo*.[43] Reproduced with permission, Copyright 2016 Elsevier.

HMSNs by acid-sensitive boronic acid–catechol ester bonds. Subsequently, PEG-grafted adamantane (Ada) was anchored on an HMSNs-β-CD nanocarrier by the host–guest interaction of Ada and CD. The association constants for adamantane derivatives and CD are of the order of 10^4–10^5 (M^{-1}), so they can be assembled together through the host–guest interaction.[44] The *in vitro* tests showed that the drug carrier was biocompatible. After the drug carrier had permeated into the tumor location by the enhanced permeability and retention (EPR) effect, the acid-sensitive benzoicimine bonds between the PEG and Ada were cleaved under weakly acidic conditions in the tumor microenvironment (pH 6.8), while the dissociated PEG protective layer facilitated cellular uptake of the drug carrier. Previous studies demonstrated that PEGylation of nanocarriers can prolong the circulation of nanocarriers and then passively target tumor sites *via* the EPR effect.[45] Then, for intracellular drug delivery, the acid-sensitive boronic acid–catechol ester bonds became cleaved under the low endosomal pH (4.5–6.5) conditions, leading to efficient tumor cell death. The *in vivo* results confirmed that drug-loaded HMSNs obviously inhibited tumor volume and weight growth, with only minimal toxic side effects. This strategy presents a good example of the rational design of a new generation of smart drug carriers that are triggered *via* the tumor microenvironment.

Jun Feng *et al.* reported a type of hyperbranched-linear supramolecular amphiphile and its assembled vesicles for the combined achievement of drug encapsulation and gene delivery.[46] Amine compounds attached to cyclodextrin-centered hyperbranched polyglycerol (CD-HPG-TAEA) and linear adamantane-terminated octadecane (C18-AD) were arranged to spontaneously interlink together. Subsequently, the hyperbranched-linear supramolecular amphiphile self-assembled into nanoscale vesicles. An antineoplastic drug, doxorubicin hydrochloride (DOX·HCl), was loaded into the vesicles as a model drug. The release of DOX·HCl from the nanoscale vesicles could be controlled by adjusting the environmental pH, favoring fast drug liberation intracellularly in response to the acidic tumor microenvironment. The results indicated that the vesicle had obviously cleaved under acidic conditions (pH = 5), and the drug release rate was nearly 80%. However, the drug release rate was only about 30% at pH 7.2. The smart nanovesicles showed superior serum-tolerant transgene delivery ability and significantly lower cytotoxicity compared to polyethyleneimine. The drug-loaded smart nanovesicles could co-deliver gene payloads into cells, offering the advantage of the accumulation of two payloads within the nuclei. The strategy provides new insight into the development of a new generation of cationic supramolecular vesicles for stimulus-responsive drug and gene transport.

In our research group, a targeted gene carrier for cancer-specific delivery was successfully developed through a "multilayer bricks–mortar" strategy.[47] This strategy has many advantages, such as the lack of the need for special reaction equipment, mild reaction conditions, simplicity of operation, environmental friendliness, and easy batch preparation. The gene carrier was composed of adamantane-functionalized folic acid (FA-AD), an adamantane-functionalized poly-(ethylene glycol) derivative (PEG-AD), and cyclodextrin-grafted low-molecular-weight branched polyethylenimine (PEI-CD). The gene carrier, based on multivalent host–guest interactions, could be an effective, targeted, and low-toxicity carrier for delivering nucleic acid to target cells (Fig. 4). Similarity, an efficient tumor-targeting drug carrier was designed by the bioconjugation of folic acid to cyclodextrin through a polyamine cationic spacer.[48] The folic acid–polyamine-β-cyclodextrin that is presented may be a promising active tumor-targeting carrier candidate *via* folate mediation. In addition to having efficient gene or drug loading and targeted delivery, this smart biocompatible carrier system showed obvious degradation and consequent release of the gene or drug in tumor cells, and could selectively induce tumor cell death. The controlled gene or drug delivery system demonstrated its use as a potential theranostic material.

The pH-responsive system based on cyclodextrins can precisely control the release of intracellular anticancer drugs and significantly improve the bioavailability of anticancer drugs; it can deliver an efficient inhibition of tumor growth and a reduction in systemic toxicity, enhancing selectivity to tumor cells while showing extremely low cytotoxicity toward normal

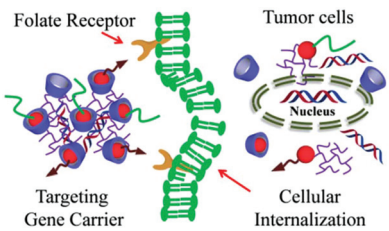

Fig. 4 Schematic illustration of polycation/pDNA complexation.[47] Reproduced with permission, Copyright 2015 John Wiley and Sons.

cells. The variation in pH is the key design rationale for the development of stimuli-responsive carriers. However, it is difficult to achieve pH-response-based controllability, owing to the complexity of the biological environment.

Redox-responsive system

There exists a large difference in redox potential between tumors and normal tissues, which is the basic principle of the design of redox-responsive systems.[49,50] Typical stimuli explored by a redox-responsive system mainly include reactive oxygen species (ROS) and glutathione (GSH). Driven by the need for on-demand drug delivery and fuelled by recent developments in smart and precision medicine, a redox-responsive system based on cyclodextrin has gained increasing research interest.

Reactive oxygen species (ROS) play an important role in signal transduction and metabolism. However, the excessive production of ROS in cells or tissues often leads to oxidation stress that has implications in a series of diseases, including cancer, aging, atherosclerosis, and inflammation. ROS are oxidizing substances, including hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide (O_2^-), and hydroxyl radicals ($OH^\bullet$).[51–53] ROS are produced by endogenous mitochondrial metabolism or through the action of NADPH-utilizing enzymes.[54] A moderate level of ROS associated with the pathophysiology is normal, but an excessive ROS concentration will defeat the antioxidant defense system, leading to oxidative stress. Prolonged exposure to high levels of ROS will cause irreversible functional alterations or irreparable damage to nucleic acid, proteins, lipids, and hydrocarbons.[55,56] ROS-responsive drug carriers for the treatment of cancer, atherosclerosis, inflammatory response, and other diseases have aroused intense interest.[57] The purpose of the ROS-responsive drug carrier design is to release the drug into the region of ROS abundance in a targeted way, which may lead to both enhanced therapeutic efficiency and reduced side effects.

Borate esters are easily cleaved under oxidizing conditions and are often used in smart drug carriers. Boron-modified cyclodextrin is an excellent drug carrier design material. Jianxiang Zhang *et al.* reported that cyclodextrin conjugated with boronic ester (Ox-bCD) was an efficient drug carrier in both *in vitro* and *in vivo* experiments.[58] As shown in Fig. 5, 4-phenylboronic acid pinacol ester (PBAP) was chemically conjugated onto hydroxyl groups of CD to synthesize oxidatively responsive CD (Ox-bCD). Then, core shell nanoparticles were formed between Ox-bCD and poly(ethylene glycol)-distearoylphosphatidylethanolamine (DSPE-PEG) or PEG-adamantyl (Ada) through a self-assembly/nanoprecipitation method, taking advantage of the hydrophobic interaction between the boronic segments of Ox-bCD and DSPE, and guest–host interaction between adamantane and cyclodextrin, respectively. *In vitro* and *in vivo* tests then showed that the core shell nanoparticle was highly biocompatible, and it was further loaded with a hydrophobic antineoplastic drug docetaxel (DTX).

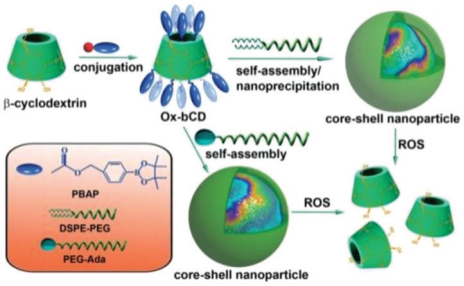

Fig. 5 Schematic illustration of the construction and ROS-responsive course of cyclodextrin-based core shell nanoparticles.[58] Reproduced with permission, Copyright 2015 John Wiley and Sons.

Follow-up experiments indicated that the DTX could be completely released within 4 hours under 1.0 mM H_2O_2 conditions, whereas only 21% of the drug was released in the absence of H_2O_2. Through a protonation mechanism, the borate linker in the nanoparticles was broken, so the DTX could be successfully released from the core shell nanoparticles in the ROS environment. The DTX-loaded core shell nanoparticles showed higher tumor inhibition efficacy, with little effect on normal tissues, indicating their therapeutic advantages and safety as smart drug carriers.

The intracellular glutathione (GSH) of a tumor is another stimulus signal for smart drug carriers. The concentration of GSH in tumor tissues is at least four times higher than that in normal tissues, so the difference in concentration of GSH can be used to trigger the drug release.[59] Some compounds contain disulfide bonds which are easily broken by GSH. The design of a redox-active drug carrier relies on the chemistry of disulfide bonds, which are cleaved in a reducing environment due to reduction of the disulfide to thiol groups, but which are stable in an oxidizing extracellular environment. Because of its easy modification and molecular recognition property, cyclodextrin has been widely used in the construction of a disulfide-containing redox-response drug carrier.

A multi-functional cationic smart gene carrier was fabricated by exploiting the cyclodextrin-based supramolecular modular approach (Fig. 6).[60] The smart gene carrier was composed of two pre-functionalized modules: (1) a host module: a polymer (PCD-SS-PDMAEMA) consisting of a poly (β-cyclodextrin) backbone and disulfide-linked poly(2-(dimethylamino) ethyl methacrylate) arms, anticipated to function by packaging genes and then releasing them upon the cleavage of disulfide bonds in a glutathione microenvironment; and (2) a guest module: adamantane and folic-acid-terminated polyethylene glycol (Ad-PEG-FA), anticipated to act to improve biocompatibility and folate-mediated cellular targeting specificity for tumor tissues. Through the host–guest interaction between the cyclodextrin units of the host module and the adamantane groups of the guest module, the PCD-SS-PDMAEMA and Ad-PEG-FA module self-assembled, forming a supramolecular pseudo-copolymer (PCD-SS-PDMAEMA/PEG-FA). The smart gene carrier can effec-

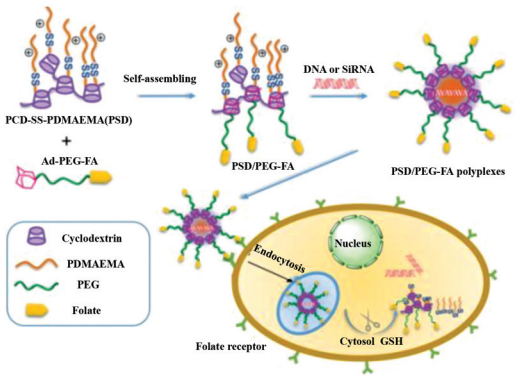

Fig. 6 Schematic illustration of the self-assembly process, receptor-mediated specific cellular uptake, and GSH-triggered intracellular gene release from the smart carrier.[60] Reproduced with permission, Copyright 2016 American Chemical Society.

tively compact genes into stable nano-sized polyplexes resistant to enzymatic digestion, and trigger gene release in a glutathione environment, obviously improving hemocompatibility, and specifically targeting folic acid receptor-positive tumor tissues. Most importantly, the cyclodextrin-based smart gene carrier, endowed with these pre-designed functions, showed excellent transfection efficacy. The reason for the excellent transfection efficacy of gene delivery is that the disulfide is broken in the tumor microenvironment. This research presents a good example of simplicity, convenience and efficiency, a modular approach for conferring multiple functions on a smart carrier to deliver therapeutic genes into target cells safely and efficiently.

Chen Jiang *et al.* constructed a redox-responsive superamphiphile constructed from an inclusion complexation of β-CD and a disulfide-linked azobenzene dimer.[61] As the guest molecule, the azobenzene dimer could be included into the cyclodextrin's cavity from both ends to form a novel bola-type superamphiphile, which could be further assembled into a vesicular structure in aqueous solution. The hidden disulfide bonds in the structure were easily broken in the GSH environment, leading to the release of the drug from the vesicular structure. With this phenomenon it can be seen that the vesicular structure has high potential in the precise control of drug release.

Although exciting specificity and accuracy have been shown by the redox-responsive systems that have been developed based on cyclodextrins, it is difficult to achieve specific redox molecular mechanism-based controllability, because the concentration of redox substances in tumor cells is extremely low and changes very rapidly. A fundamental understanding of the spatial and temporal patterns of redox substances offers an essential foundation for designing more effective and precise carriers.[62] In addition, the disulfide bond is particularly unstable at elevated temperature or under intense light irradiation,[63] and this problem should be noted when designing redox-responsive carriers.

Enzyme-responsive system

The regulated expression of enzymes in tumors has important implications for targeting the tumor microenvironment.[64,65] Having enzymes act as triggers has sparked great interest in developing an enzyme-responsive system based on cyclodextrin for tumor-specific drug delivery, due to the advantage of selecting for specific substrates.[62]

The increased expression of certain local enzymes in tumor tissues, such as esterase and matrix metalloproteinase (MMP), not only can be deemed as a biomarker for the early diagnosis and prognosis monitoring of disease, but it also represents a method for enzyme-triggered drug release in cancer.[7] Because of the abnormal increase in the concentration of these enzymes, it can be exploited for specific cleavage to achieve enzyme-triggered drug release at the desired tumor sites or intracellular compartments.

Won Jong Kim *et al.* reported a novel cyclodextrin-based smart drug delivery system, formed by host–guest interactions between a copolymers–cyclodextrin conjugate (pCD) and a copolymers–paclitaxel conjugate (pPTX) (Fig. 7).[66] The PTX or CD was conjugated to the copolymers of maleic anhydride *via* ester bonds. Because the molecular size of paclitaxel coincides with the cavity of cyclodextrin, the pCD and pPTX block units could be self-assembled to form a nanostructured drug carrier. The multivalent inclusion complexes conferred high stability on the nano-assembly, which efficiently delivered paclitaxel into the targeted cancer cells *via* both passive and active targeting mechanisms. Because esterase is highly expressed within the cancer cell, the ester linkages between paclitaxel and the copolymers of maleic anhydride could be degraded. This novel cyclodextrin-based smart drug delivery system exhibited significantly inhibited tumor growth and greatly extended the

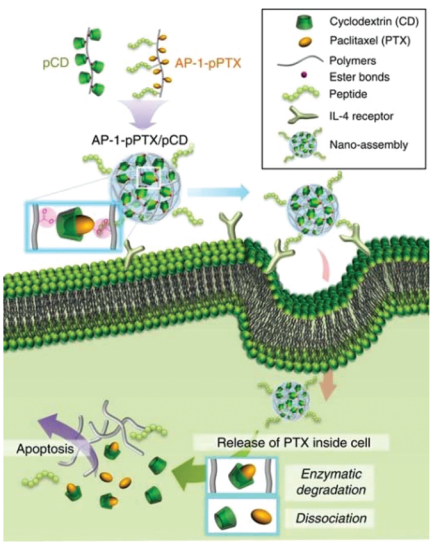

Fig. 7 Schematic illustration of nano-assembly-mediated PTX delivery.[66] Reproduced with permission, Copyright 2014 Nature Publishing Group.

survival rate in a mouse model. The strategy established in this study also provides new insight into the development of advanced anticancer drug delivery.

Yu Liu *et al.* constructed a supramolecular assembly capable of simultaneously responding to hyaluronidase and esterase.[67] The supramolecular assembly was composed of cationic cyclodextrin (EICD) and natural hyaluronic acid (HA), through charge combination. This combination could eventually form a negatively charged nanoparticle on the surface, which could be degraded by hyaluronidase and esterase. The cationic carboxylic ester pendants on HA supported hyaluronidase (HAase)-responsive sites and the EICD supported artificial carboxylic esterase-responsive sites. Hydrophobic anticancer drugs could be loaded into cationic cyclodextrins, which could be released into tumor tissue under the action of enzymes, because of the higher expression of hyaluronan receptors on tumor cells.[68]

Paula Diez *et al.* reported the design of a smart delivery system in which cargo delivery from capped mesoporous silica (MS) nanoparticles was controlled by an integrated enzyme-based "control unit" (Fig. 8).[69] When the concentration of glucose and ethyl butyrate in the environment increases, the carrier could release the drug. The key factor controlling the drug release was the glucose oxidase and esterase immobilized on the Au face. Glucose oxidase can convert glucose to gluconic acid, and lipase can cause lipid decomposition to form fatty acids, so the concentrations of glucose and butyrate increase, and the surroundings of the carrier become an acidic environment under the action of glucose oxidase and lipase. The acidic environment can promote benzimidazole protonation, and eventually lead to cyclodextrins being pulled away from benzimidazole. When the cyclodextrin is removed from the surface of the mesoporous silica, the drug can be successfully released. The drug carrier has very promising application prospects in the treatment of diabetes. The entire process of drug release is initiated by the glucose oxidase and esterase, but the final drug release is performed by pH control.

All biological and metabolic process are enzyme-related, so an enzyme-responsive system based on cyclodextrins has unique superiorities, such as substrate specificity and high selectivity under mild conditions. This responsive system can achieve enzyme-mediated drug release by biocatalytic action at the site of the tumor cells. However, there are still challenges that need to be addressed. The major challenge is the heterogeneous expression of a specific enzyme at different stages of one particular tumor and even in different tumors. Furthermore, it is not clear whether the carrier can respond successfully at a low concentration of the enzyme.

Multiply responsive system

When the drug carrier can respond to a variety of stimuli at the same time, its sensitivity to the surrounding environment will be further improved and eventually lead to more accurate drug delivery and release. Because of the coexistence of many kinds of stimulus signal in certain pathological conditions, in particular cases stimulus signals can be used in combination.

Xuezhong Du *et al.* fabricated a simultaneous and cascade-controlled release of two drugs from CD-gated mesoporous silica nanoparticle carriers through dual dynamic covalent bonds (Fig. 9).[70] The surface of mesoporous silica nanoparticles (MSN) was co-modified with disulfide-linked carbamoylphenylboronic acid moieties and amines. When one of the drugs was entrapped within the MSN cavity, CD was effectively attached to the surface of MSN by the boronate linkages to encapsulate the drug within the MSN cavity. Subsequently, the second type of drug was included within the CD cavities. In addition, the CD on the exterior surface of the MSN could reduce serum-associated negative effects and improve biocompatibility as well.[71] The simultaneous and cascade release of two drugs could well be solved through the use of CD-gated mesoporous silica nanoparticle carriers based on dual dynamic covalent bonds. Under acidic conditions, the boronate bonds are broken, and the drug in the cyclodextrin cavity

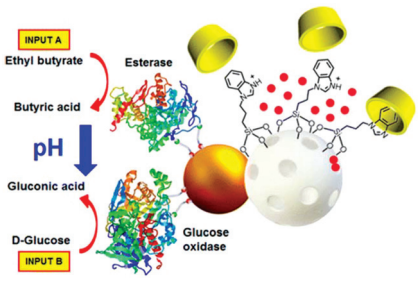

Fig. 8 The "control unit" (Au face) is functionalized with two effectors (enzymes) which control cargo delivery from the silica mesoporous face *via* interpretation of different chemical inputs (D-glucose, ethyl butyrate).[69] Reproduced with permission, Copyright 2014 American Chemical Society.

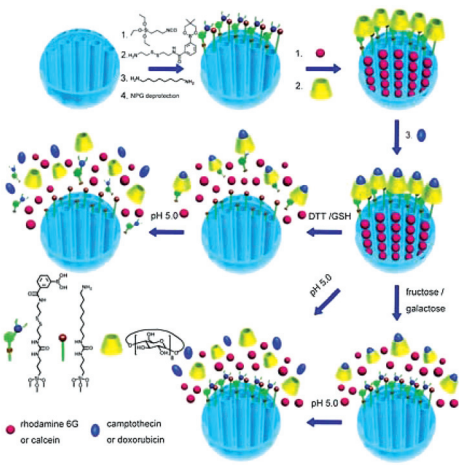

Fig. 9 Schematic illustration of the construction of CD-gated MSN vehicles functionalized with dual drug loading for simultaneous and cascade release in targeted combination drug therapy.[70] Reproduced with permission, Copyright 2015 The Royal Society of Chemistry.

can be protonated, leading to a reduced binding capacity; in turn, this leads to both drugs being simultaneously released from the MSN pores and the CD cavity. Similarly, upon exposure to the reducing agents glutathione (GSH) or dithiothreitol (DTT), the drug entrapped in the MSN pores could be released by the cleavage of the disulfide bonds, and the other drug could subsequently be rapidly released from the CD cavities under acidic conditions. The cyclodextrin-gated mesoporous silica nano carrier with dual drug loading provides new insights into the development of a smart platform for combination drug release (both simultaneous and cascade release).

Similarly, Xianzheng Zhang *et al.* fabricated a novel type of cellular-uptake-shielding multifunctional cyclodextrin-based mesoporous silica nanoparticle carrier for tumor-triggered targeted drug delivery to cancer tissue.[72] In brief, after the anticancer drug DOX was entrapped in the MSN pores, CD was effectively grafted onto the surface of MSN through disulfide linkages to encapsulate the DOX within the MSN pores. Then an adamantine-modified guest polymer (including adamantane, a tumor-targeting peptide RGD motif, a matrix metalloproteinase-sensitive short peptide PLGVR, and a polyanion protection polymer PASP) was introduced onto the surface of the nanoparticles *via* the host–guest interaction of adamantane and CD. Once the drug carrier had accessed the cancer tissue, the protective PASP layer could be removed through the cleavage of the PLGVR peptide *via* matrix metalloproteinase to expose the targeting RGD motif. Then, after reaching the tumor cells, the gating CD could be readily dissociated from the surface of MSN by the cleavage of the disulfide bonds owing to the high concentration of glutathione (GSH) inside the tumor cell. Subsequently, the anticancer drug DOX entrapped in the MSN pores could be rapidly released to achieve the desired anti-cancer activities. Consequently, CD-based smart drug carriers could be of great interest and demonstrate great potential for their promising applications in cancer therapy.

Despite the advantageous versatility of multiple-response drug carriers, they often appear to be too complicated in comparison with a single-response drug carrier. To ascertain the viability of multiple-response drug carriers, evidence of the regulation of the response to each stimulus signal would be needed both *in vitro* and *in vivo*.

Conclusion and future perspectives

The specific distribution of drugs to target tissues or organs is the main bottleneck in drug research. One of the foundations of precision medical research is to carry out individualized drug delivery. Accurate delivery can use the minimum dose of the drug to achieve the highest biological efficacy with the lowest side effects. Biological stimuli-responsive carriers are able to trigger drug release at the right time and place, and this is just one of the objectives of precision medicine. It is difficult to construct this kind of drug carrier by general synthetic chemistry, because of its complex structure. However, the advent of supramolecular self-assembly of cyclodextrin provides us with a convenient, fast, and flexible way to construct biological stimuli-responsive carriers.

The biosafety of biological stimuli-responsive carriers, to a certain extent, is the premise and guarantee for the realization of precision therapies. As previously mentioned, the structure of biological stimuli-responsive carriers is very complex, as they are often combinations of a variety of materials. It is necessary to ensure that the carrier not only has a smart effect, but that it also has biocompatibility, which will be the main challenge for the design of biological stimuli-responsive carriers.

The endogenous responses of smart drug carriers can, according to the changes in the tumor or local inflammation tissue microenvironment, trigger drug release, realizing specificity of drug distribution to the tissue or organ, and thus improving drug efficacy. But we should be fully aware that the organs and tissues of the body are extremely complex, and the internal environmental factors are varied. Whether the biological stimuli-responsive carriers can eliminate all kinds of interference factors, so as to realize the control of drug release in time, place, and dose is one of the main challenges to be faced.

In summary, we are still at the stage of basic research and we should not be surprised that there is still a long way to go before these results can be translated to the stage of clinical research. We are confident that with further exploration of the design and construction of cyclodextrin-based biological response smart drug carriers, some of the main bottlenecks will gradually be overcome. Then the capabilities of smart and precise medicine will advance dramatically, and we shall truly have entered the era of smart medicine.

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References

1 C. Toumey, *Nat. Nanotechnol.*, 2017, **12**, 1–1.
2 F. S. Collins and H. Varmus, *N. Engl. J. Med.*, 2015, **372**, 793–795.
3 J. Shi, P. W. Kantoff, R. Wooster and O. C. Farokhzad, *Nat. Rev. Cancer*, 2016, **17**, 20.
4 Y. Min, J. M. Caster, M. J. Eblan and A. Z. Wang, *Chem. Rev.*, 2015, **115**, 11147–11190.
5 L. Tang and J. Cheng, *Nano Today*, 2013, **8**, 290–312.
6 R. Tong, L. Tang, L. Ma, C. Tu, R. Baumgartner and J. Cheng, *Chem. Soc. Rev.*, 2014, **43**, 6982–7012.
7 S. Mura, J. Nicolas and P. Couvreur, *Nat. Mater.*, 2013, **12**, 991–1003.
8 M. Karimi, A. Ghasemi, P. Sahandi Zangabad, R. Rahighi, S. M. Moosavi Basri, H. Mirshekari, M. Amiri, Z. Shafaei

Pishabad, A. Aslani, M. Bozorgomid, D. Ghosh, A. Beyzavi, A. Vaseghi, A. R. Aref, L. Haghani, S. Bahrami and M. R. Hamblin, *Chem. Soc. Rev.*, 2016, **45**, 1457–1501.
9 R. Liao, M. Liu, X. Liao and B. Yang, *Prog. Chem.*, 2015, **27**, 79–90.
10 C. He, D. Liu and W. Lin, *Chem. Rev.*, 2015, **115**, 11079–11108.
11 W. Zhang, Y. M. Zhang, S. H. Li, Y. L. Cui, J. Yu and Y. Liu, *Angew. Chem., Int. Ed.*, 2016, **55**, 11624–11628.
12 Z. Dan, H. Cao, X. He, L. Zeng, L. Zou, Q. Shen and Z. Zhang, *Int. J. Pharm.*, 2015, **483**, 63–68.
13 B. G. Mathapa and V. N. Paunov, *Biomater. Sci.*, 2014, **2**, 212–219.
14 Y. Kang, K. Guo, B.-J. Li and S. Zhang, *Chem. Commun.*, 2014, **50**, 11083–11092.
15 G. M. Whitesides and B. Grzybowski, *Science*, 2002, **295**, 2418–2421.
16 X. Ma and Y. Zhao, *Chem. Rev.*, 2014, **115**, 341–352.
17 I. Antoniuk, V. Wintgens, G. Volet, T. T. Nielsen and C. Amiel, *Carbohydr. Polym.*, 2015, **133**, 473–481.
18 R. Serra-Gómez, C. A. Dreiss, J. González-Benito and G. González-Gaitano, *Langmuir*, 2016, **32**, 6398–6408.
19 G. Yu, K. Jie and F. Huang, *Chem. Rev.*, 2015, **115**, 7240–7303.
20 C. Stoffelen and J. Huskens, *Small*, 2015, **12**, 96–119.
21 K. Ariga, H. Ito, J. P. Hill and H. Tsukube, *Chem. Soc. Rev.*, 2012, **41**, 5800–5835.
22 Yu Liu, B. Han, S. Sun, T. Wada and Y. Inoue, *J. Org. Chem.*, 1999, **64**, 1487–1493.
23 Q. D. Hu, G. P. Tang and P. K. Chu, *Acc. Chem. Res.*, 2014, **47**, 2017–2025.
24 Y. Chen and Y. Liu, *Adv. Mater.*, 2015, **27**, 5403–5409.
25 S. Yi, B. Yang and R. Liao, *Int. J. Polym. Anal. Charact.*, 2017, **22**, 247–255.
26 L. Yin, S. Xu, Z. Feng, H. Deng, J. Zhang, H. Gao, L. Deng, H. Tang and A. Dong, *Biomater. Sci.*, 2017, **5**, 698–706.
27 H. Wei and C.-Y. Yu, *Biomater. Sci.*, 2015, **3**, 1050–1060.
28 M. E. Davis, J. E. Zuckerman, C. H. J. Choi, D. Seligson, A. Tolcher, C. A. Alabi, Y. Yen, J. D. Heidel and A. Ribas, *Nature*, 2010, **464**, 1067–1070.
29 S. Svenson, M. Wolfgang, J. Hwang, J. Ryan and S. Eliasof, *J. Controlled Release*, 2011, **153**, 49–55.
30 S. Zimmer, A. Grebe, S. S. Bakke, N. Bode, B. Halvorsen, T. Ulas, M. Skjelland, D. De Nardo, L. I. Labzin, A. Kerksiek, C. Hempel, M. T. Heneka, V. Hawxhurst, M. L. Fitzgerald, J. Trebicka, I. Björkhem, J.-Å. Gustafsson, M. Westerterp, A. R. Tall, S. D. Wright, T. Espevik, J. L. Schultze, G. Nickenig, D. Lütjohann and E. Latz, *Sci. Transl. Med.*, 2016, **8**, 333RA50.
31 Y. Yang, Y.-M. Zhang, D. Li, H.-L. Sun, H.-X. Fan and Y. Liu, *Bioconjugate Chem.*, 2016, **27**, 2834–2838.
32 P. Lv, C. Zhou, Y. Zhao, X. Liao and B. Yang, *Carbohydr. Polym.*, 2017, **168**, 103–111.
33 A. Feng, Q. Yan, H. Zhang, L. Peng and J. Yuan, *Chem. Commun.*, 2014, **50**, 4740–4742.
34 H. Meng, M. Xue, T. Xia, Y.-L. Zhao, F. Tamanoi, J. F. Stoddart, J. I. Zink and A. E. Nel, *J. Am. Chem. Soc.*, 2010, **132**, 12690–12697.
35 I. Antoniuk and C. Amiel, *J. Pharm. Sci.*, 2016, **105**, 2570–2588.
36 A. Kulkarni, R. VerHeul, K. DeFrees, C. J. Collins, R. A. Schuldt, A. Vlahu and D. H. Thompson, *Biomater. Sci.*, 2013, **1**, 1029–1033.
37 G. González-Gaitano, J. Ramon Isasi, I. Vélaz and A. Zornoza, *Curr. Pharm. Des.*, 2017, **23**, 411–432.
38 M. Kanamala, W. R. Wilson, M. Yang, B. D. Palmer and Z. Wu, *Biomaterials*, 2016, **85**, 152–167.
39 F. O. Yousef, M. B. Zughul and A. A. Badwan, *J. Inclusion Phenom. Macrocyclic Chem.*, 2007, **57**, 519–523.
40 M. Xue, X. Zhong, Z. Shaposhnik, Y. Qu, F. Tamanoi, X. Duan and J. I. Zink, *J. Am. Chem. Soc.*, 2011, **133**, 8798–8801.
41 Z. Zhang, J. Ding, X. Chen, C. Xiao, C. He, X. Zhuang, L. Chen and X. Chen, *Polym. Chem.*, 2013, **4**, 3265–3271.
42 Z. Zhang, Q. Lv, X. Gao, L. Chen, Y. Cao, S. Yu, C. He and X. Chen, *ACS Appl. Mater. Interfaces*, 2015, **7**, 8404–8411.
43 J. Liu, L. Zhong, J. Zhang, T. Luo, J. Zhou, X. Zhao and K. Cai, *Biomaterials*, 2016, **83**, 51–65.
44 S. H. Pun and M. E. Davis, *Bioconjugate Chem.*, 2002, **13**, 630–639.
45 L. Feng, K. Li, X. Shi, M. Gao, J. Liu and Z. Liu, *Adv. Healthcare Mater.*, 2014, **3**, 1261–1271.
46 B. Yang, X. Dong, Q. Lei, R. Zhuo, J. Feng and X. Zhang, *ACS Appl. Mater. Interfaces*, 2015, **7**, 22084–22094.
47 R. Liao, S. Yi, M. Liu, W. Jin and B. Yang, *ChemBioChem*, 2015, **16**, 1622–1628.
48 R. Liao, Y. Zhao, X. Liao, M. Liu, C. Gao, Y. Jian and Y. Bo, *Polym. Adv. Technol.*, 2015, **26**, 487–494.
49 M. Huo, J. Yuan, L. Tao and Y. Wei, *Polym. Chem.*, 2014, **5**, 1519–1528.
50 H. Wang, L. Tang, C. Tu, Z. Song, Q. Yin, L. Yin, Z. Zhang and J. Cheng, *Biomacromolecules*, 2013, **14**, 3706–3712.
51 M. B. Toledano, *Nat. Rev. Mol. Cell Biol.*, 2007, **8**, 813–824.
52 C. Liu, X. Zhu, X. Wang, D. Miao, X. Liang, C. Wang, L. Pang, H. Sun, D. Kong and J. Yang, *Biomater. Sci.*, 2016, **4**, 255–257.
53 Q. Deng, X. Li, L. Zhu, H. He, D. Chen, Y. Chen and L. Yin, *Biomater. Sci.*, 2017, **5**, 1174–1182.
54 C. Nathan, *J. Clin. Invest.*, 2003, **111**, 769–778.
55 M. C. Haigis and B. A. Yankner, *Mol. Cell*, 2010, **40**, 333–344.
56 B. Newland, P. Wolff, D. Zhou, W. Wang, H. Zhang, A. Rosser, W. Wang and C. Werner, *Biomater. Sci.*, 2016, **4**, 400–404.
57 S. Feng, Y. Hu, S. Peng, S. Han, H. Tao, Q. Zhang, X. Xu, J. Zhang and H. Hu, *Biomaterials*, 2016, **105**, 167–184.
58 D. Zhang, Y. Wei, K. Chen, X. Zhang, X. Xu, Q. Shi, S. Han, X. Chen, H. Gong, X. Li and J. Zhang, *Adv. Healthcare Mater.*, 2015, **4**, 69–76.
59 R. Liu, Y. Zhang and P. Feng, *J. Am. Chem. Soc.*, 2009, **131**, 15128–15129.

60 L. Jia, L. Xu, J. Yang, Q. Chao, Q. Li, Y. Zhang, X. Jiang, G. Wang, W. Zheng and W. Lin, *ACS Appl. Mater. Interfaces*, 2016, **8**, 14200–14210.
61 T. Sun, L. Shu, J. Shen, C. Ruan, Z. Zhao and C. Jiang, *RSC Adv.*, 2016, **6**, 52189–52200.
62 J. Du, L. A. Lane and S. Nie, *J. Controlled Release*, 2015, **219**, 205–214.
63 H. Wang, M. Xu, M. Xiong and J. Cheng, *Chem. Commun.*, 2015, **51**, 4807–4810.
64 M. Egeblad and Z. Werb, *Nat. Rev. Cancer*, 2002, **2**, 161–174.
65 K. Kai, V. Plaks and Z. Werb, *Cell*, 2010, **141**, 52–67.
66 R. Namgung, L. Y. Mi, J. Kim, Y. Jang, B. H. Lee, I. S. Kim, P. Sokkar, Y. M. Rhee, A. S. Hoffman and W. J. Kim, *Nat. Commun.*, 2014, **5**, 3702.
67 Y. Liu, P. Hu, Y. Chen and J. J. Li, *Chemistry – Asian J.*, 2015, **11**, 505–511.
68 Y. Zhang, Y. Cao, Y. Yang, J. Chen and Y. Liu, *Chem. Commun.*, 2014, **50**, 13066–13069.
69 P. Diez, A. Sanchez, M. Gamella, P. Martinez-Ruiz, E. Aznar, C. De La Torre, J. R. Murguia, R. Martinez-Manez, R. Villalonga and J. M. Pingarroon, *J. Am. Chem. Soc.*, 2014, **136**, 9116–9123.
70 S. Zhou, H. Sha, X. Ke, B. Liu, X. Wang and X. Du, *Chem. Commun.*, 2015, **51**, 7203–7206.
71 B. Yang, H. Jia, X. Wang, S. Chen, X. Zhang, R. Zhuo and J. Feng, *Adv. Healthcare Mater.*, 2014, **3**, 596–608.
72 J. Zhang, Z. F. Yuan, Y. Wang, W. H. Chen, G. F. Luo, S. X. Cheng, R. X. Zhuo and X. Z. Zhang, *J. Am. Chem. Soc.*, 2013, **135**, 5068–5073.