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Review

Dynamic supraparticles for the treatment of age-related diseases

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ABSTRACT

Age-related diseases (ARDs) are arising as a major threat to public health in our fast-aging society. Current development of nanomedicine has sparked much optimism toward ARDs management by improving drug delivery and controlled drug release. However, effective treatments for ARDs, such as cancer and Alzheimer's diseases (AD), are still lacking, due to the complicated pathological features of ARDs including multifactorial pathogenesis, intricate disease microenvironment, and dynamic symptom manifestation. Recently, dynamic supraparticles (DS), which are reversibly self-assembled functional nanoparticles, have provided a novel strategy for combating ARDs. Besides the intrinsic advantages of nanomedicine including multifunctional and multitarget, DS are capable of dynamic structural reconfiguration upon certain stimulation, creating another layer of maneuverability that allows programmed response to the spatiotemporal alterations of ARDs during progression and treatment. In this review, we will overview the challenges faced by ARDs management, and discuss the unique opportunities brought by DS. Then, we will summarize the designed synthesis of DS for ARDs treatment. Finally, we will dissect the therapeutic targets in ARDs that can be exploited by DS, and present the encouraging advances in this field. Hopefully, this review will bridge our knowledge of the design principle of DS and ARDs management, which may inspire the future development of potent theranostic agents to improve the healthcare.

1. Introduction

Over the past century, the average life expectancy (at birth) worldwide has historically increased from approximately 30 years to above 70 years [1,2]. In addition to improved life qualities, a major contributing factor to our extended lifespan is the rapid development of modern medicine. However, as the majority can live up to their sixties and seventies while the birth rate continues to drop especially in industrialized countries, our society is growing old at a pace faster than ever before. It is estimated that, by 2050, 22 percent of the world population will be over 60 years of age, nearly twice the current number of 12 [3]. Consequently, many age-related diseases (ARDs) that commonly develop in aged individuals are becoming the primary threat to public health.

Among a variety of ARDs, perhaps the most notorious are cancers in various tissues, neurodegenerative diseases, and cardiocerebrovascular diseases. These ARDs erode the well-being of the patients and, inevitably, their families, by imposing tremendous economic and emotional burden. It is reported that in 2018 alone, the number of new cancer cases was estimated to reach 18.1 million globally, with new cancer deaths summing up to a staggering 9.6 million [4]. It is the same case for neurodegenerative disorders. In the United States, the approximate number of AD or other dementia patients has risen to 5.7 million in 2018, costing an estimated 277 billion dollars, and the number of AD will probably double to 13.8 million by 2050 [5]. To this end, these aged-related diseases are indeed causing tremendous public concern with high mortality/morbidity rate, which may one day go beyond what our society can sustain if we cannot find a solution.

During the past decades, enormous resources have been invested in the researches on the ARDs, but it is rather disappointing that a cure for them will still be out of reach in the short term. With regards to cancer interventions, radiotherapy and chemotherapy with general toxicity are struggling hard to balance the side effects against therapeutic effects, while targeted therapies and recently blooming immunotherapies are challenged by the tumor heterogeneity and hence are not effective for the majority of patients, although they have indeed benefited some subgroups [6–8]. It is even more devastating for the development of AD treatment, which is hampered by a series of failures in clinical

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trials testing new drugs based on our current knowledge of AD pathogenesis [9].

Recently, nanotechnology has opened a new frontier in combating ARDs. It attracted a lot of attention to the design and synthesis of nanomedicine against cancer, which displayed many advantages over conventional agents, including targeted drug delivery [10], enhanced bioavailability [11], reduced toxicity [12], functional diversity [13], etc. With rapid advances in controlled synthesis and fabrication, a large repertoire of nanoparticles of different elements, sizes, shapes, and surface properties, has been established for diverse biomedical applications. Among the confetti, dynamic supraparticles (DS), which refer to the reversible assembly of functional nanoparticles with changeable structures, are gaining increasing attention in recent years [14]. As an organic combination of different nanoparticles, DS combine the advantages of various nanomedicines as mentioned above. More importantly, DS can undergo structural transformations in response to given stimuli, conferring tunable properties such as size, morphology, and physical properties. Similar to the situation in the battlefield that flexible tactic formations lead to victory, DS with flexible configurations have a better chance to outmaneuver the variable biological environment and traverse all the pathophysiological barriers to fulfill its task. Functionalized with proper ligands, DS can be programmed to transform in response to certain alterations of ARDs, which endows them with self-adjustable capabilities for different purposes during disease progression and treatment, for example, size expansion for enhanced retention [15], and activatable magnetic resonance imaging (MRI) for therapy monitoring [16]. These characteristics make DS ideal theranostic agents to tackle refractory diseases. Indeed, many preclinical studies have already shown the impressive performance of DS in various ARDs, including cancer, neurodegenerative diseases, and stroke (Fig. 1).

In this review, we will describe the intrinsic challenges to ARDs treatment, and then give our perspectives on how the DS will meet the demand for effective management of ARDs. Next, we will summarize the designed synthesis and recent advances in fabricating dynamic stimuli-responsive DS for diagnostic and therapeutic purposes. Finally, we will discuss the problems remained to be addressed before the DS can translate from bench to beside. Hopefully, our work will provide a timely overview of the status quo of DS, and may inspire the future development of more powerful and smart agents to ultimately guarantee a healthy aging process.

2. Understanding the challenges of ARDs management

ARDs refer to those disorders with late onset predominantly in the elderly population. Typical diseases include type II diabetes, cancer, atherosclerosis, stroke, and neurodegenerative diseases represented by AD and Parkinson's diseases (PD). Indeed, the odds of developing any of these diseases increase exponentially with age [17]. Among all the ARDs, the most intimidating ones are cancer, neurodegenerative diseases, and stroke, which are labeled with high mortality/morbidity rates and great emotional and financial cost. Management of ARDs has always been challenging, mainly because of their unique epidemiology, intricate pathology, and elusive aetiology, as described below.

2.1. Epidemiology

As indicated by the terminology, ARDs mainly develop in aged individuals. This population has an overall decline in nearly all body functions concerning drug responses, including but not limited to gastrointestinal motor function, liver function, and renal function. In addition, elderly people generally have reduced moisture content and increased fat content in the body. These alterations have profound impacts on the bioavailability, distribution and elimination of drugs, which heightens the chance of adverse drug events (ADE), especially when comorbid diseases exist [18–20]. Old age is also associated with a decline in the capacity of cellular and tissue homeostasis maintenance and tissue regeneration [21,22], which renders the elderly less resilient to toxicity and invasive treatment such as surgery. Such health conditions narrow the therapeutic window of conventional agents that have low selectivity and duration, and limit surgical operations. In this

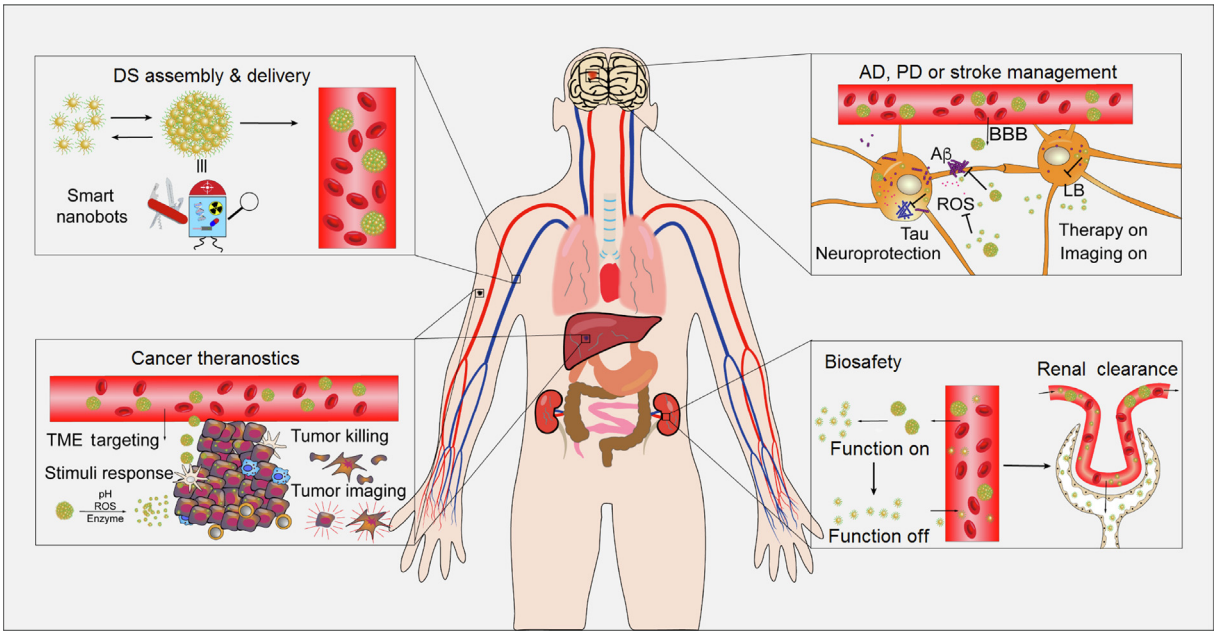

Fig. 1. (Color online) Schematic illustration of potential applications of intelligently designed dynamic supraparticles (DS) in combating various age-related diseases (ARDs). DS are reversibly assembled multifunctional nanobots that can undergo structural reconfigurations in response to the dynamic alterations of ARDs during disease progression and treatment, which have displayed many advantages in drug delivery, imaging performance, synergistic therapeutic effects, and safety profiles over conventional nanomedicines.

regard, an ideal therapy for ARDs should be potent, targeted, controllable, non-invasive, easy to excrete, and with low systemic toxicity, which is rather challenging for conventional therapeutic regimens to achieve in a dynamic biological environment. Most importantly, ARDs commonly have a rather long premanifestation period ranging from years to decades, especially neurodegenerative diseases which only develop evident symptoms at late stages of the diseases [17,23]. This situation often renders therapeutic interventions too late to be effective. Therefore, accurate early diagnosis is even more crucial than interventive therapeutics to ARDs management. A diversity of imaging techniques, such as positron emission tomography-computerized tomography (PET-CT) and magnetic resonance imaging (MRI), have already pushed the limit of accurate diagnosis of ARDs. However, the resolution and contrast of these diagnostic modalities are yet to be improved before they can detect the very initiative pathological alterations of ARDs. For example, MRI is widely used for tumor imaging because of its sensitivity and safety profiles as compared to other imaging tools, whose resolution can be further enhanced by contrasting agents that enrich in the abnormal disease sites. However, when the size of the tumor is extremely small, it is very difficult to discern tumor signals from other normal tissues, which necessitates even higher target-to-noise ratios. As to AD, PET and MRI are routinely used for examining amyloid (Aβ) [24] aggregates and thickness of different brain sections [25], respectively, which only display evident alterations at mid-to-late stages of AD, while earlier detection of amplification of soluble Aβ that may impart timely intervention remains to be solved. It is the same case for PD, which is diagnosed via motor behaviors in the late stages, while early detection of prodromal Lowy bodies (LBs) composed of aggregated α-synuclein is still inapplicable [26].

2.2. Pathology

One lesson from numerous failures in the battle against ARDs is that the pathology of many ARDs involves both cellular phenotypes and the disease microenvironment, which commonly display mutual dependency and form a co-evolution system [7,27]. A typical example is the paradigm shift in cancer treatment. Cancer in its nature is defined by uncontrolled cellular growth and division that ultimately lead to all clinical symptoms. Hence, the majority of anticancer agents are based on their activities to suppress tumor proliferation by disrupting DNA replication, growth signaling, *etc.* Nevertheless, the limited benefits of many available drugs prompt the reconsideration of the strategy merely focusing on cellular phenotypes. Instead, increasing attention has been focused on the tumor microenvironment (TME) characterized by hypoxia, acidity, and inflammation, etc. [28–30]. The hostile TME is initially shaped by the rapid growth of cancer cells in the absence of sufficient vascularization. Counterintuitively, the harsh microenvironment does not lead to self-destruction of cancer, but only accelerates its progression by promoting cancer cell evolution and metastasis. In addition, the TME also has major implications in the resistance of cancer cells to chemotherapy, radiotherapy, and immunotherapy (reviewed in Refs. [31–34]). As a result, increasing emphasis has been put on the therapeutic relevance of TME modification. It is the same case for the AD, whose progression is determined not only by the demise of neuronal cells, but also by the highly oxidative and inflammatory microenvironment that propagates neuronal damage [35–37]. To conclude, both the cellular phenotypes and the microenvironment are essential elements of the pathology of ARDs, which resembles the weeds and the nurturing soil. Hence, to eradicate ARDs, not only should we pull out the "weeds", but also transform the "soil" adaptable to their growth. Indeed, there are many attempts to modify the microenvironment of ARDs, e.g., vitamin C to ameliorate the oxidative stress in AD brains [38], and targeting-intratumoral-lactic-acidosis-transcatheter arterial chemoembolization (TILA-TACE) for cancer (NCT03314532). However, these treatments are either questioned for uncertain dose-effect relationships or limited by invasiveness, necessitating alternative approaches.

2.3. Pathogenesis

Although decades of researches have accumulated great amounts of knowledge on the mechanisms driving the development of ARDs, a clear picture of the pathogenic process is still underappreciated for most, if not all, of them, such as cancer, AD, and primary hypertension. Without a comprehensive understanding of the pathogenesis of ARDs, it is difficult to select the best targets for effective control of the disease progression. Perhaps the drawbacks in developing AD therapy are the most representative examples of our struggling to find a cure for ARDs without unveiling their pathogenic nature [9]. Based on the preclinical data showing the critical role of Aβ protein deposits in AD pathogenesis [39], huge amounts of resources have been put into developing drugs that can promote the clearance of Aβ aggregates from the brain, but only to end up with a series of failures in clinical trials. In fact, most ARDs are contributed by multiple factors that may be interdependent, redundant, or compensatory [40]. For instance, Aβ and tau protein both have well implicated roles in AD pathogenesis [41,42], which may explain that single-target therapies targeting only Aβ bring no benefits to the patients. Perhaps a reasonable approach to address this conundrum is to simply include multiple drugs into the therapeutic regimes for ARDs to generate synergistic effects, but the very unpredictable drug interactions among systemically administrated drugs in the elderly may lead to drug inhibition/induction or severe side effects, especially when other medicines are also used for comorbid diseases. Therefore, multitarget all-in-one agents may be much more favored for treating ARDs in future.

To this end, the major hurdles to ARDs management can be summarized into: (1) increased vulnerability and compromised resilience of the elderly patients; (2) presymptomatic diagnosis; (3) intricate disease microenvironment; (4) multifactorial pathogenesis. Moreover, in recent years increasing emphasis has been put on the heterogeneity among individuals that underlies substantially varying patient responses to treatments. Therefore, individualized therapy based on patient stratification and efficacy monitoring is of significant relevance, which will rely on highly informative imaging techniques. To fully change the gloomy picture of the ARDs management, all the challenges described may need to be addressed concurrently, which is clearly beyond the reach of conventional agents.

3. Designed synthesis of dynamic supraparticles for ARDs management

3.1. Dynamic supraparticles on the horizon

In the face of many challenges, nanomedicine appears to have a better chance to beat ARDs compared to conventional therapeutic agents. Indeed, various nanoagents with versatile functions have been developed for different ARDs. Some simple formulations like liposomes have already been clinically approved for their effectiveness in reducing side effects of small molecular anti-cancer drugs (e.g., DaunoXome®). Other nanoagents designed for targeted delivery, controlled drug release and imaging enhancement are also under active investigations and showing good promise, especially cancer and neurodegenerative diseases (reviewed in Refs. [10,13]). However, for these conventional nanomedicines with

fixed structures and functions, it is very challenging to handle the dynamic pathophysiological contexts such as blood circulation, tissue penetration, cell membrane traverse, heterogeneity, and in body clearance, to realize the expected superiority. Moreover, some of the requirements for optimal ARDs treatment agents are hard to reconcile for conventional nanomedicines. For instance, to acquire multifunctionality and better selectivity, the nanomaterial must be large enough to incorporate different functional modules and to avoid non-specific diffusion, albeit efficient clearance from the body favors smaller sizes. To address these problems, higher-order nanoarchitecture is introduced into nanomedicine, giving rise to DS reversibly assembled by functional nanoscale blocks, which may finally solve the dilemma and answer the call of effective ARDs management. As a dynamic combination of functional nanomaterials, DS not only inherit the intrinsic properties of individual nanoparticles, but also acquire additive advantages such as multifunctionality that allows multitarget and multimodal theranostics. Most importantly, the dynamic property of DS creates a new layer of flexibility, which renders DS more adaptive to the dynamic environment *in vivo* and thus provides a solution to reconcile the discrepant requirements (e.g., in size and shape) of different purposes. For those DS programmed to undergo structural transformations in response to the biological stimuli, the transformations provide an effective approach to achieve a qualitative improvement in the imaging and therapeutic utilities in ARDs management. For example, DS composed of iron oxide nanoparticles perform inverse MRI imaging with "black and white" contrast between normal and cancerous tissues [43], which is far beyond quantitative enhancement by conventional MRI contrast agents. In some cases, the activation or inactivation of functional modules within the DS, e.g., photosensitizer, is coupled to the structural transformation triggered by disease-specific stimuli, enabling remotely tunable interventions such as photodynamic (PDT) and photothermal therapy (PTT).

Despite being attractive, with great promise come great challenges in the fabrication of nanomaterials of such complexity. Indeed, we are still far from abstracting an equation to calculate the size, structure and other properties of the products from input materials, however, years of accumulation of empirical knowledge from hindsight has already made possible rational design and controlled synthesis of the DS.

3.2. Building blocks

Both organic and inorganic nanoparticles can be used as building blocks to construct DS, as long as they can be directed to undergo dynamic assembly/disassembly. Since inorganic nanoparticles often afford additional functions due to their inherent physicochemical attributes, they are attracting growing interest from researchers. For instance, magnetic iron oxide nanoparticles (IONPs) are desirable probes for MRI because of their magnetism [44]. Notably, they can generate different contrast signals in monodispersed or aggregated states, which renders them ideal ingredients for image-enhancing DS [45]. Pt nanoparticles can exert anticancer activities by releasing bivalent Pt ions (Pt^{2+}) in the acidic environment [46], while gold nanoparticles (AuNPs) have characteristic surface plasma resonance properties applicable for colorimetric biosensing [47]. Moreover, upconversion and downconversion nanoparticles (UCNPs and DCNPs, respectively) are also commonly used for DS synthesis. They are rare-earth-doped nanocrystals that can convert infrared or ultraviolet radiations to visible emissions. Because of the unique Stokes and anti-Stokes optical properties of UCNPs or DCNPs, they are widely applied in bioimaging and light-triggered drug delivery systems [48–50]. Besides the basic functional nanoparticles, the ligand materials play an equally essential role in the fabrication of DS. Various kinds of ligands are employed to coat the nanoparticles for their colloidal stability and, more importantly, their self-assembly into DS through e.g., electrostatic forces, hydrophobic interactions, hydrogen bond, and so on. In contrast to the block nanoparticles, the ligands are mostly organic polymers or macromolecules with diverse functional groups, which therefore provide the nanoparticles and the assembled DS with functionalities suitable for biomedical applications. Another major purpose of the ligands is to endow the DS with dynamic properties. In this scenario, they are serving not only as glues to stick nanoparticles together, but also as sensors of environmental parameters such as acidity, redox status, the content of certain proteins, heat, magnetic field, etc. (reviewed in Ref. [14]). Once activated by the environmental alterations, the ligands will undergo drastic changes such as charge inversion and hydrophobicity/hydrophilicity swift due to excessive protonation or bond cleavage of their functional groups, which will impact nanoparticle interactions and lead to dynamic structural and functional reconfigurations of the DS. The functional nanoparticles and the surface ligands form the skeleton of DS, which is permissive for further engineerings such as drug loading and surface modification.

3.3. Formation mechanisms of diverse DS

The tenets of chemical design of DS can be explained at different levels such as changes in entropy/enthalpy equilibrium, which can be found in other comprehensive reviews [14,51]. Here we will focus right on the "visible" alterations or reconfigurations of the ligands that define the DS as dynamic, with an emphasis on biological relevance. We will introduce these principles from four aspects, including dynamic physical interaction, dynamic bond formation, dynamic protein-ligand binding, and dynamic DNA strand annealing/displacement. A summary of representative examples is provided in Table 1.

3.3.1. Dynamic physical interaction

Given DS are fabricated for biomedical applications, it is conceivable that the dynamic reconfiguration of DS will mainly occur in the aqueous phase. In this scenario, various types of physical interactions that determine the aggregation states of matter in water have been considered for DS construction.

Among many, the hydrophobic interaction, which is widely used for the formation of micelles and liposomes, represents a natural and highly accessible force that have been frequently employed for DS fabrication. In fact, most uniform nanoparticles are synthesized in organic solvents, thus possessing hydrophobic surface ligands (e.g., oleic acid or oleylamine) on their surface that make them poorly dispersible in aqueous solution. For the hydrophobic nanoparticle building blocks, the hydrophobic interactions between the nanoparticle surface ligands and the hydrophobic head (hydrocarbon chains) of amphipathic polymers is a productive approach to assist DS formation [67]. In this process, biocompatible stimulus-responsive amphipathic polymers encapsulate the nanoparticle blocks, generating DS with micelle-like structures. While the hydrophobic parts of amphipathic polymers drive DS assembly, their hydrophilic segments (e.g., ethylene glycol) that stretch into the aqueous solution play a crucial role in stabilizing the ordered DS architecture (Fig. 2). Since most of the amphipathic polymeric ligands for DS formation can be imparted with stimuli-responsive functional groups, which ensured the structural dynamics of DS. Upon stimulation, the alteration of the responsive groups (e.g., protonation) will break the hydrophobic/hydrophilic balance of the DS, leading to structural changes in these sophisticated architectures. To conclude, there are three advantages in this system: first, the relatively long polymer chains existed on the particle surface maintain the steric stabilization of

Table 1
Fabrication of DS based on different formation mechanisms.

Substrate/stimulus	Ligands	Nanoparticle component	Assembly/ disassembly mechanism	Application	Ref.
Imidazole/pH	Poly(N-(N′,N′-diisopropylaminoethyl) aspartamide) (PAsp(DIP))	Quantum dots	Dynamic physical interaction	Drug release and fluorescent imaging	[52]
Poly(e-caprolactone)/ Temperature	Poly(ethylene glycol)-bpoly(e-caprolactone) (PEG-b-PCL)	Gold nanoparticles	Dynamic physical interaction	PA imaging and enhanced PTT efficacy	[53]
Imidazole/pH	Polypeptide (octadecylaminep(API-Asp)10) and maleimide functionalized pluronic F127	Platinum nanoclusters	Dynamic physical interaction	Enhance tumor therapeutic effect	[54]
Imidazole/pH	Poly(ethylene glycol)-poly(β-benzyl-L-aspartate) with 1-(3-aminopropyl) imidazole (API) and 3-phenyl-1-propylamine	Upconversion nanoparticles	Dynamic physical interaction	Enhanced cellular internalization and PDT effect	[55]
Imidazole/pH	Poly(ethylene glycol)-poly(β-benzyl-Laspartate) (PEG-PBLA) with 1-(3-aminopropyl) imidazole (API) and API with 3-phenyl-1-propylamine (PPA)	Iron oxide nanoparticles	Dynamic physical interaction	Dual modal imaging and PDT effects	[56]
Disulfde bonds/GSH	Poly(ethylene glycol)-b-((2,5-bis[(4-Carboxylicpiperidylamino) thiophenyl]-croconine)-co-4-vinyl pyridine (PEG-PCRVP)	Gold nanorods	Dynamic physical interaction	Drug release and enhanced PA signal	[57]
Peptide/Enzyme	Poly(ethylene glycol)-Pro-Leu-Gly-Met-Trp-Ser-Arg-OH	Iron oxide nanoparticles	Dynamic chemical bond formation/cleavage	Enhanced MRI signals	[58]
Peptide/Enzyme	Ala-Ala-Asn-Cys-Asp and 2-cyano-6-amino-benzothiazol	Gold nanoparticles	Dynamic chemical bond formation/cleavage	Size increase and enhanced tumor site retention	[15]
Hydrazone bonds/pH	Aldehyde and hydrazine	Iron oxide nanoparticles	Dynamic chemical bond formation/cleavage	Enhanced T1-MRI signals	[59]
Disulfide bonds/GSH	Tetrasulfide	Mesoporous silica nanoparticles	Dynamic chemical bond formation/cleavage	Depletion of GSH and drug delivery	[60]
Sulfhydryl/ROS	Amino acids	Downconversion nanoparticles	Dynamic chemical bond formation/cleavage	Enhanced retention and imaging signals	[61]
O-nitrobenzyl groups/UV	Polyacrylamide-poly(ethylene glycol) with o-nitrobenzyl groups	Upconversion nanoparticles	Dynamic chemical bond formation/cleavage	Controlled Drug delivery	[62]
Antibody/antigen	Antibody and streptavidin/biotin	Gold nanoparticles	Dynamic protein-ligand binding	Amyloid protein aggregate detection	[63]
Peptide/Enzyme	Pro-Leu-Gly-Met-Trp-Ser-Arg-GPLGVRGC and neutravidin/biotin	Iron oxide nanoparticles	Dynamic protein-ligand binding	Enhanced MRI signals	[64]
DNA/temperature	Surface ligands (5′AAAAAAAAAACCTATCGACCATGCT-3′, 5′-TAACAACGATCCCTCAAAAAAAAAA-3′); linker (5′-GGATAGCTGGTACGATTGCTTGCTTATGCTTATGCTTGTGCTTATTGTTGCTAGGGAG-3′ and complementary 5′-ACGAACGAATACG-3′/5′-AATACGAACACGA-3′)	Gold nanoparticles	Dynamic DNA strand annealing/ displacement	On command drug release	[65]
i-motif DNA/pH	Surface ligands (5′-alkyneCGACGACGACGA-3′); linker (5′-CCCTAACCCTAACCCTAACCCTATACTTCGCGTCGTCG-3′ and partially complementary 5′-GGGTTAGGTAGCACTGCTCGTTTCGTCGTCGTCG-3′)	Iron oxide nanoparticles	Dynamic DNA strand annealing/ displacement	Enhanced imaging contrast via T2 to T1 transition	[43]
Anti-miR21/miR21	AS1411 aptamer conjugated to anti-miR21	Quantum dots	Dynamic DNA strand annealing/ displacement	Targeted delivery, controlled drug release and synergism	[66]

DS; second, polymer chains can be easily functionalized with a variety of stimulus-responsive groups, enabling the structure change under multifarious stimuli; third, utilization of the micelle-like DS structure allows encapsulation of hydrophobic drugs to enhance the therapy effects.

Based on above principles, Shuai group [52] designed a novel multifunctional micelle composed of quantum dots (QDs) and PTX for tumor-targeted intracellular drug release and fluorescent imaging. In this assembly, poly(*N*-(*N*′, *N*′-diisopropylaminoethyl) aspartamide) (PAsp(DIP)) in polymeric chains underwent a hydrophobic/hydrophilic transition in the acidic lysosomes of cancer cells, triggering drug release for cancer killing. Huang et al. [53] reported a biodegradable gold nanovesicles (BGVs) composed of a disulfide bond (S–S)-terminated amphiphilic block copolymer (BCP) and AuNPs for tumor photoacoustic (PA) imaging and photothermal therapy (PTT). The self-assembly process was driven by the balance of hydrophilic/hydrophobic interactions of amphiphilic BCPs and AuNPs, enabling dense packing of AuNPs with on-demand interparticle orientations. The majority of BGVs collapsed when the temperature was over 70 °C, indicating that BGVs could degrade into isolated AuNPs in response to temperature alterations. Recently, our group designed a platinum (Pt) nanocluster DS (Pt-NA) assembled with the help of pH-sensitive polymer [54]. The synthetic pH-sensitive polymers consisted of Pluronic

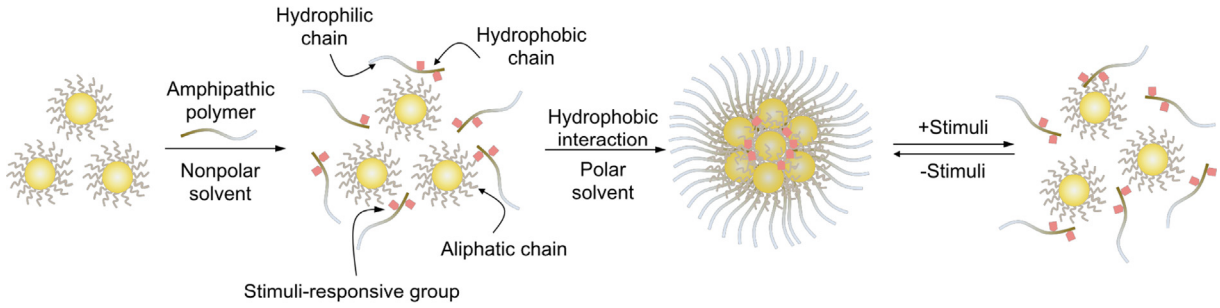

Fig. 2. (Color online) Schematic illustration of the Dynamic supraparticles formation based on hydrophobicity/hydrophilicity shift.

F127 and octadecylamine-*p*(API-Asp)$_{10}$ with ionizable imidazole groups on the side chains. Once these DS accumulated in the tumor site with the help of hepatocellular carcinoma (HCC)-targeting peptide, the imidazole groups would be protonated by the acidic tumor microenvironment, forming a positively charged particle surface that facilitates rapid cellular uptake. After transportation to the lysosome, further acidification would eventually break the hydrophilic-lipophilic balance and induce the disassembly of Pt nanoclusters, resulting in increased Pt ion release rate inside HCC cells for effective cell killing (Fig. 3).

3.3.2. Dynamic chemical bond formation/cleavage

In addition to the physical interaction-mediated assembly/disassembly of DS, the use of chemical bonds to crosslink the functional nanoparticle moieties is another method for DS construction (Fig. 4). This strategy involves formation of chemical bonds between two chemical groups, or breakage of chemical bonds by physicochemical perturbation or enzymes. Generally, for biomedical application, three principles must be considered for assembling DS with chemical bonds, including biocompatibility, sensitivity, and specificity. Biocompatibility means that the formation (when applied *in situ*) or breakage of chemical bonds must take place under mild conditions within the pathophysiological range, while sensitivity and specificity are key to the controlled *in vivo* dynamics of DS. Following these rules, a copper-free click-chemistry-based *in situ* assembly of IONPs was reported by Dong and co-workers [58]. They synthesized two sets of IONPs with azide and alkyne on the surface, respectively, both of which were further modified with polyethylene glycol (PEG) chain linked to a CXCR4-targeting cyclopeptide via a substrate of matrix metalloproteinases (MMPs). When the IONPs reached MMP-enriched tumors, the cleavage of the MMP substrate exposed the azide

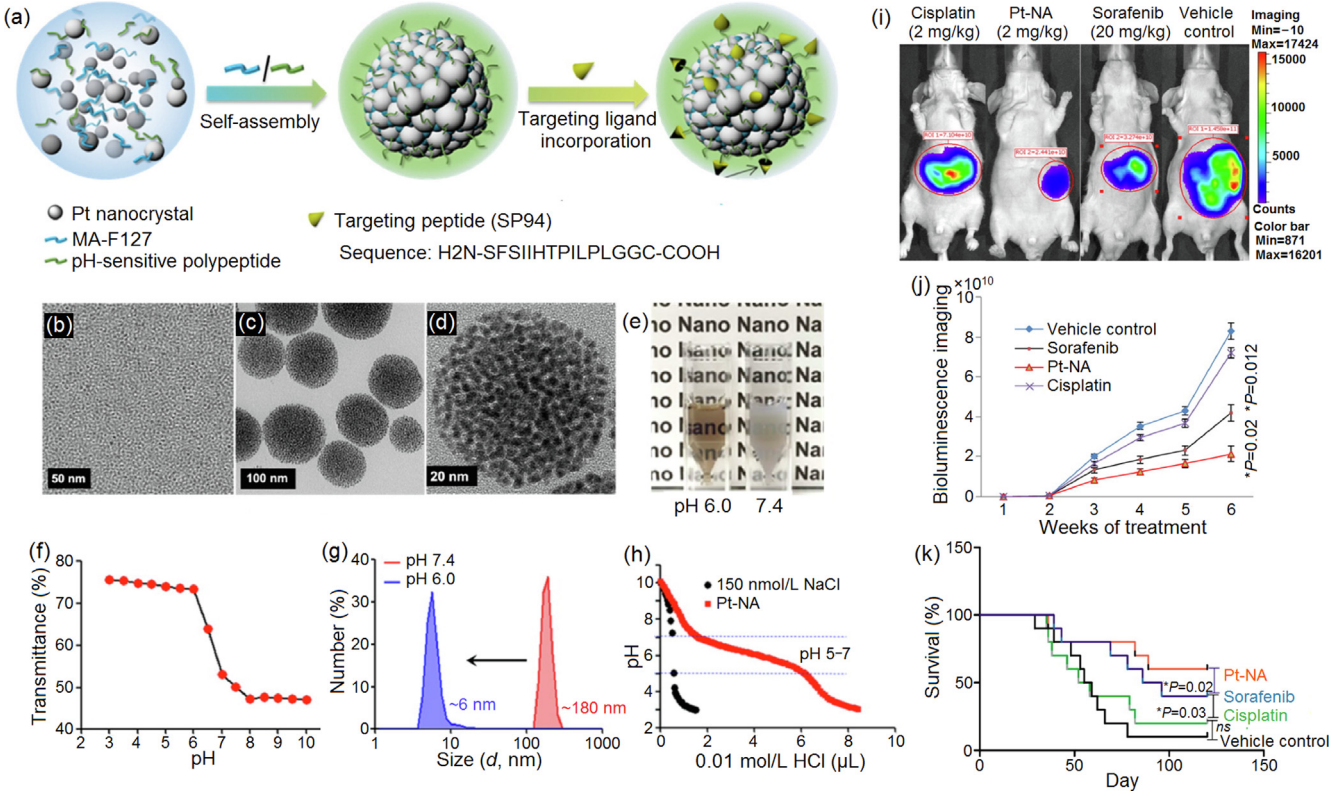

Fig. 3. (Color online) Fabrication of dynamic supraparticles with amphipathic polymer ligands. (a) Schematic diagram of the assembly of dynamic Pt suparparticles (Pt-DS). (b) TEM image of the synthesized Pt nanoclusters. (c) TEM image of Pt-DS. (d) The magnified TEM image of Pt-DS. (e) Photographs of Pt-DS at different pH. (f) Transmittance of a suspension of Pt-DS at different pH. (g) DLS size measurement of Pt-DS at different pH. (h) pH profile of Pt-DS by acid-base titration. (i, j) Representative images (i) and quantitative analysis (j) of bioluminescence signals of hepatocellular carcinoma bearing mice on a weekly basis. (k) Survival of mice in the four treatment groups. Reprinted with permission from Ref. [54]. Copyright (2016) American Chemical Society.

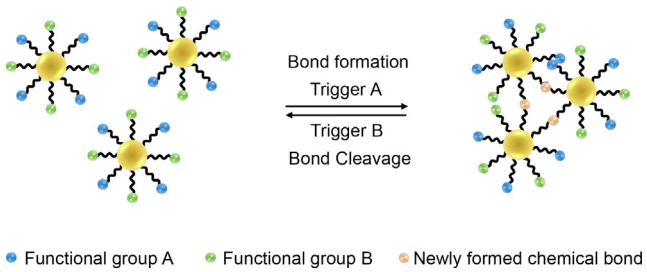

Fig. 4. (Color online) Schematic illustration of the dynamic supraparticles formation based on dynamic chemical bond formation/cleavage. Yellow sphere represent nanoparticles. Solid lines ended with green or blue dots denote surface ligands containing functional groups that can form reversible chemical bonds.

and alkyne groups, initiating biorthogonal click reaction to direct IONPs self-assembly. This dynamic bond cleavage/formation process caused a decrease of T2-weighted MRI signal of the tumor, which could be used for diagnosis. Similarly, Gao and co-workers [15] demonstrated another kind of click chemistry-based assembly of AuNPs. Two groups of AuNPs were firstly synthesized, one co-modified with SH-PEG-R8-RGD and SH-PEG-Ala-Ala-Asn-Cys-Asp (AK) (AuNP-AK-R), and the other one with SH-PEG-R8-RGD and 2-cyano-6-amino-benzothiazol (CABT) (AuNP-CABT-R). After further modification with RGD peptide and octaarginine, the two groups of AuNPs could effectively accumulate inside $\alpha_v\beta_3$-expressing tumor cells, where they were activated by tumor specific legumain to self-assemble via click cycloaddition reaction. Such dynamic *in situ* DS assembly resulted in an increased size and promoted the retention in the tumor sites.

The above two examples are typical *in situ* DS assembly *via* the formation of chemical bonds, which is normally used for imaging purposes. In fact, dynamic chemical bond breakage can also endow DS with therapeutic functionalities such as on-demand drug release. For example, Lu et al. [60] developed a biodegradable GSH-sensitive DS using mesoporous organosilica with a cross-linked tetrasulfide-based framework, which was further modified with PEI to incorporate oligo DNA (GDMON-P). The high intracellular GSH level could cleave S-S bonds and destroy the overall structure, initiating payload release. Apart from biological stimuli, some physical energy sources are also applied to induce breakage of certain chemical bonds. Zhao and co-workers [62] developed a cross-linked system based on photosensitive o-nitrobenzyl groups and upconversion nanoparticles (UCNPs). The UCNPs would generate UV light upon NIR irradiation to cleave the o-nitrobenzyl groups and dismantle the whole backbone for drug release.

Notably, the abovementioned DS mostly involve macromolecular ligands such as PEG to improve the water dispersibility of nanoparticles, which inevitably increase steric hindrance that may affect the bond formation/cleavage reaction and impede the interaction between nanoparticles and certain stimuli. Therefore, there is a practical need for small molecular ligands that can facilitate interparticle interaction and stimuli accessibility, and thus better reversibility. Unfortunately, small molecular ligands are much less exploited for nanoparticle assembly, mainly ascribed to the instability in the aqueous phase or a lack of specificity. However, two recent studies have shown the promise of small molecules as ligands to instruct DS assembly/disassembly *via* chemical bond formation/breakage. Zhao et al. [61] reported a type of DS composed of GSH-modified fluorescent downconversion nanoparticle (DCNP@GSH). When encountering ROS, these DCNP@GSH could crosslink with each other through the formation of sulfide bonds and self-assemble into large nanocomposites with a dramatic size increase, facilitating accumulation in lesions and imaging of ROS with amplified signal-to-noise ratios (SNR). Using the small molecular ligand citric acid, our group designed a pH-sensitive DS composed of extremely small-sized iron oxide nanoparticles (ESIONs) that were cross-linked by aldehyde derivative ligands (Fig. 5) [59]. At neutral pH (~7.4), the DS structure was robust due to the linking hydrazone bonds. In the acidic TME, the hydrazone bonds were hydrolyzed, leading to disassembly of hydrophilic ESIONs and dramatically amplified T1 MR signal. Furthermore, the assembly/disassembly behaviors are highly reversible even after six complete cycles, demonstrating excellent structural reversibility.

3.3.3. Dynamic protein-ligand binding/dissociation

The interactions between natural biomacromolecules also provide a clue to the construction of DS. Particularly, the specific interactions among some proteins/peptides [68] and their corresponding ligands (i.e., antibodies and antigens [69,70], streptavidin and biotin [71]) in the living body can be used as the driving force for the nanoparticle assembly. Because the protein-ligand interactions in most cases are specific and sensitive, this strategy can be used to endow the DS with many functions such as biosensing and on-demand reconfiguration. The surface modification of nanoparticles with certain proteins and/or ligands is a critical step for protein-ligand interaction-directed assembly of DS. It can be achieved through the anchoring of specific proteins or ligands to the inorganic nanoparticles via the archetypal interaction of nanoparticle surface with specific functional groups in the side chains of amino acids, for example, AuNPs with –SH of cysteines, and iron oxide nanoparticles with –COOH of aspartic acids [72]. In general, two groups of nanoparticles are coated with either proteins or the corresponding ligands, which then self-assemble via protein-ligand binding (Fig. 6). In some cases, specific proteins are used to trigger the aggregation of nanoparticles when one molecule of the protein can interact with multiple ligands on different nanoparticles, *vice versa.*

Currently, the streptavidin and biotin are the most widely applied pair of protein and ligand for instructing nanoparticle assembly due to their high specificity and strong binding affinity. For instance, Wang et al. [63] designed a dot-blot immunoassay system for sensitive detection of Aβ protein in AD (Fig. 7). Firstly, they immobilized antibodies against the C terminal of Aβ on the nitrocellulose (NC) membrane that can capture the soluble Aβ peptide, and then synthesized two groups of AuNPs, one with streptavidin attached on the surface (SA-AuNPs), and the other one with biotin and antibodies against the N terminal of the Aβ peptide (Ab16-AuNPs). These two groups of AuNPs were added to the NC membrane, which consequently aggregated via both antibody-antigen and streptavidin-biotin interactions, causing a drastic change in the light scattering intensity that could quantitively indicate the content of Aβ peptide. Likewise, Bhatia and co-workers [64] attached neutravidin and biotin to the superparamagnetic iron oxide NPs, respectively. The modified iron oxide NPs were further linked with PEG chains through GPLGVRGC (the substrate of MMP2) to prevent the specific binding of neutravidin and biotin. Upon MMP-2 addition, the GPLGVRGC together with the steric repulsion exerted by PEG chains were proteolytically removed, permitting neutravidin, biotin association and subsequent nanoparticle self-assembly, which amplified the T2 MRI signal and facilitated the detection of the tumor.

3.3.4. Dynamic DNA strand annealing and displacement

DNA is the genetic material of all living organisms ranging from the tiniest virus to the largest mammals. It is the masterpiece of nature that writes all the stories of life with only four letters, A (adenine), T (thymine), G (guanine), and C (cytosine). In its natural form, DNA presents as the double helix structure consisting of two sets of antiparallel single-strand DNA (ssDNA) that have complementary nucleotide sequences. The formation of the DNA duplex

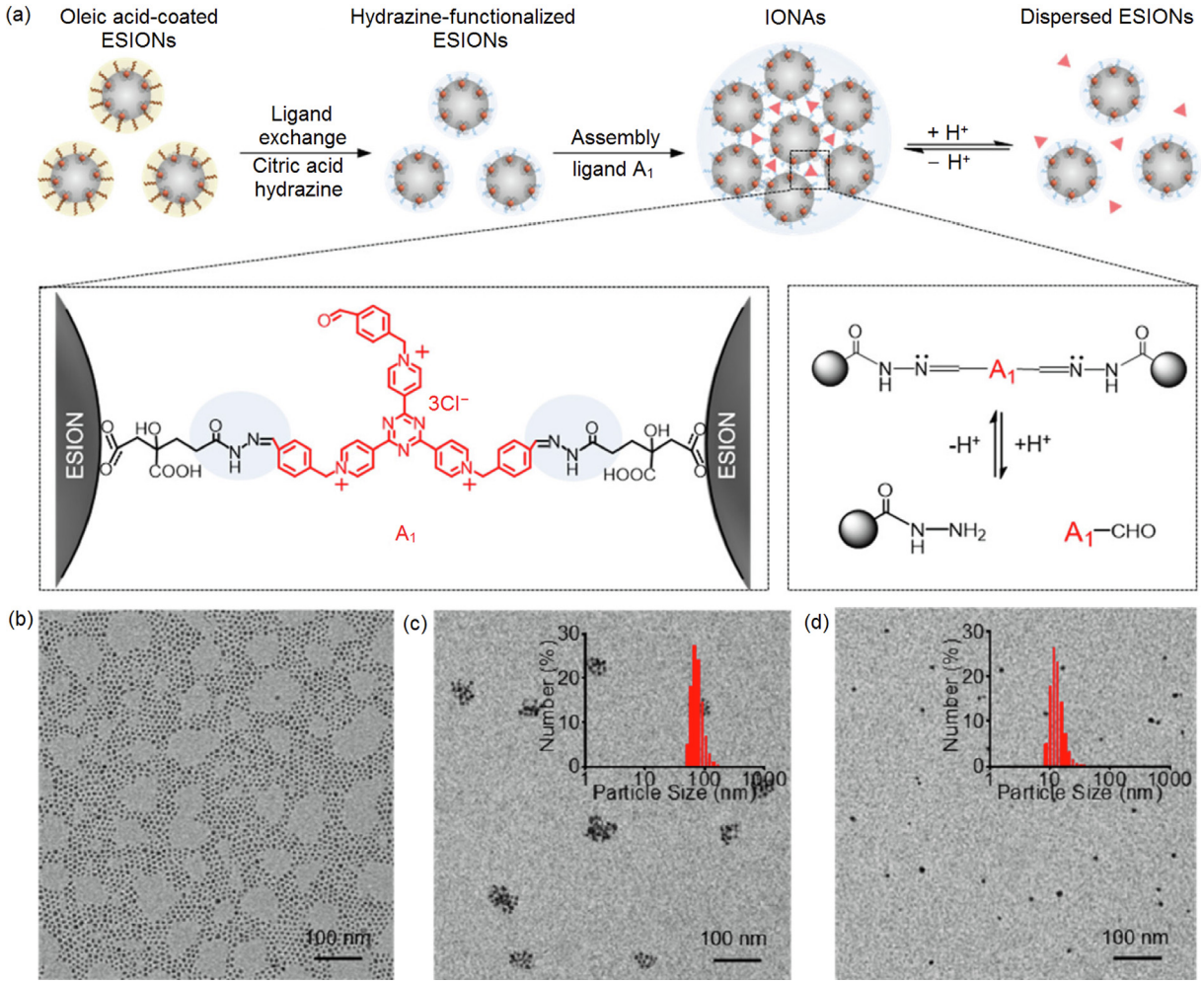

Fig. 5. (Color online) Fabrication of iron oxide-based dynamic supraparticles (DS) using pH-sensitive small molecular ligands. (a) Schematic illustration of the fabrication of pH-sensitive hydrazonecrosslinked iron oxide-based DS. (b–d) TEM images of extremely small iron oxide nanoparticles (ESIONs) (b) and their assembly at pH 7.4 (c) and pH 5.5 (d). Inset: corresponding hydrodynamic size of the DS under different pH values. Reprinted with permission from Ref. [59]. Copyright (2019) American Chemical Society.

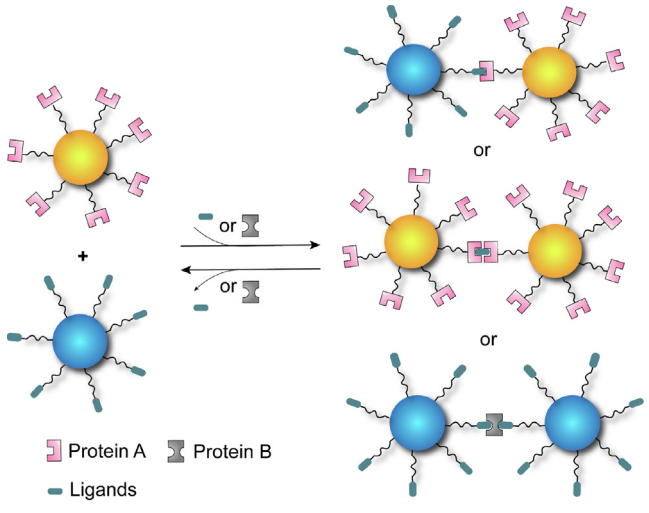

Fig. 6. (Color online) Schematic illustration of the dynamic supraparticles formation based on dynamic protein-ligand binding/dissociation. Spheres of different colors depict two different groups of nanoparticles. Curved lines linked to the surface of the spheres illustrate surface modifications with proteins or ligands containing specific binding areas. Free proteins or ligands can be used to mediate the assembly of functionalized nanoparticles.

is governed by the stringent base pairing mechanism, in which A forms two hydrogen bonds with T, whereas G forms three with C. It should be noted that double strand DNA (dsDNA) does not necessarily contain two ssDNA molecules, since it can also form within one strand of ssDNA with self-complementary sequences. The stability of dsDNA is determined by many factors that can affect the total number and distribution of hydrogen bonds between ssDNA, e.g., the length of the strands, the number of paired bases, G/C content, and nucleotide sequences. In addition to the properties of DNA *per se*, context parameters including pH, temperature and salt concentration also have huge influences on the formation of hydrogen bonds, and thus the stability of dsDNA. Given this tunable "stickness" and the natural nanoscale size, DNA is an ideal ligand for constructing DS. Moreover, the sequence specificity endows DNA with great addressability, which holds great potential to truly realize DS fabrication by design.

In general, DNA molecules mediate assembly or disassembly of nanoparticles through the combination of two actions, strand annealing and displacement. Typically, artificial ssDNA molecules with chemically modified ends are anchored on two sets of nanoparticles *via* covalent binding or streptavidin/biotin binding. When the nanoparticles are mixed together, the surface ssDNA molecules with complementary sequences will anneal to form dsDNA, linking different nanoparticles together to form the supraparticles. In some cases, a third strand of ssDNA is used to connect

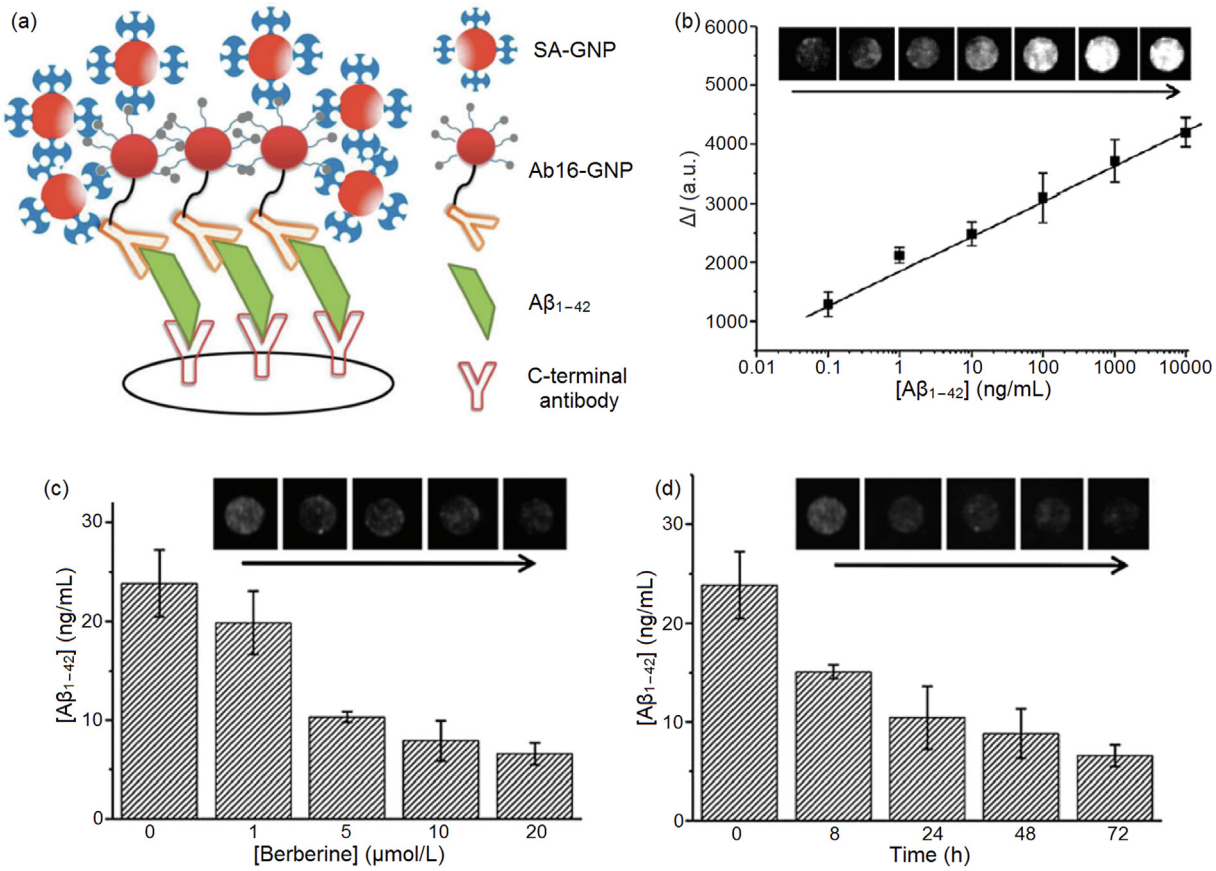

Fig. 7. (Color online) Gold nanoparticle assembly triggered by antibody-antigen recognition and streptavidin-biotin binding in a dot-blot system for detection of soluble Aβ protein. (a) Schematic illustration of the dot-blot immune detection of Aβ. (b) The light scattering intensity as a function of the Aβ peptide concentration. Aβ peptide content determined by gold nanoparticle-based dot-blot assay using lysates of SHG-44 cells treated by Berberine (an inhibitor for Aβ precursor processing enzyme) at different doses (c) or for different times (d). Reprinted with permission from [63]. Copyright (2012) Royal Society of Chemistry.

nanoparticle by annealing with the ssDNA ligands on both groups of nanoparticles. The stability of DNA-based assembly depends on the stability of the linking dsDNA regions, which is determined by the number of hydrogen bonds as mentioned earlier. Therefore, variations in any of the factors that affect the overall number of hydrogen bonds between ssDNA on the nanoparticles can cause strand displacement, and consequently trigger disassembly of the dsDNA-linked supraparticles. Based on this principle, many strategies have been devised to realize the dynamic transformation of DNA-based DS, which can be summarized into: (1) temperature responsive; (2) pH responsive; (3) competitive binding (Fig. 8).

(i) Temperature responsive. The thermostability is an intrinsic parameter of dsDNA, which is mainly determined by the DNA length and the G/C content [73]. Theoretically, all DNA molecules of various lengths and sequences can be employed to construct temperature-responsive DS, provided the temperature is high enough to melt the interparticle dsDNA. However, for biomedical applications, there are actually fewer options. In principle, an ideal DNA ligand for assembly of DS should remain very stable under physiological temperatures while respond promptly to a modest temperature change, which requires rational design of the DNA length and sequence. In addition, because the variation of the body temperatures is rather small, an external source of heat is generally required. Therefore, the typical working model of temperature-responsive DS is as follows: first, two groups of nanoparticles self-assemble via annealing of the complementary ssDNA ligands attached on their surfaces. After delivery to the target site, the DS will be heated via, for example, near-infrared (NIR) mediated photothermal effects, triggering DNA displacement and the desired transformation.

(ii) pH responsive. Given the natural pH gradient present in body, e.g., tissue fluid (~pH 7.4)—early endosome (~pH 6.5)—late endosome (~pH 5.5)—lysosome (~pH 4.5) [74], it is very attractive to use pH, in particular acidic pH, as the stimulus for DNA-based DS. Although acidic pH can directly destabilize the DNA duplex by impairing hydrogen bond formation *via* depurination, it only occurs under extreme conditions (below pH 2) with very low efficiency [75]. An alternative approach to endow the DS with pH responsiveness is to incorporate the so-called "i-motif" into the sequence of ligand ssDNA. The i-motif structure is initially solved by Gehring et al. for the d(TCCCCC) DNA oligomers [76]. It is a four-stranded DNA structure folded in C-rich regions at acidic pH, where two parallel-stranded DNA duplexes are conjugated antiparallelly by intercalated base pairing of C and protonated C^+. There is a multitude of i-motif sequences that have been investigated, some even have additional functions other than quadruplex formation (reviewed in Ref. [77]). Because of the sensitivity of i-motif DNA to pH values, it is extensively employed in fabricating pH-responsive nanomaterials, including DS. Basically, ssDNA containing i-motif sequence is attached to the surface of one group of nanoparticles, and the other group of nanoparticles is coated by partially complementary ssDNA. When the two groups of nanoparticles are assembled at neutral pH, i-motif sequence-containing ssDNA preferentially anneals with its complementary strand on the other group of nanoparticles, which directs DS assembly. While at acidic pH, the C bases become protonated,

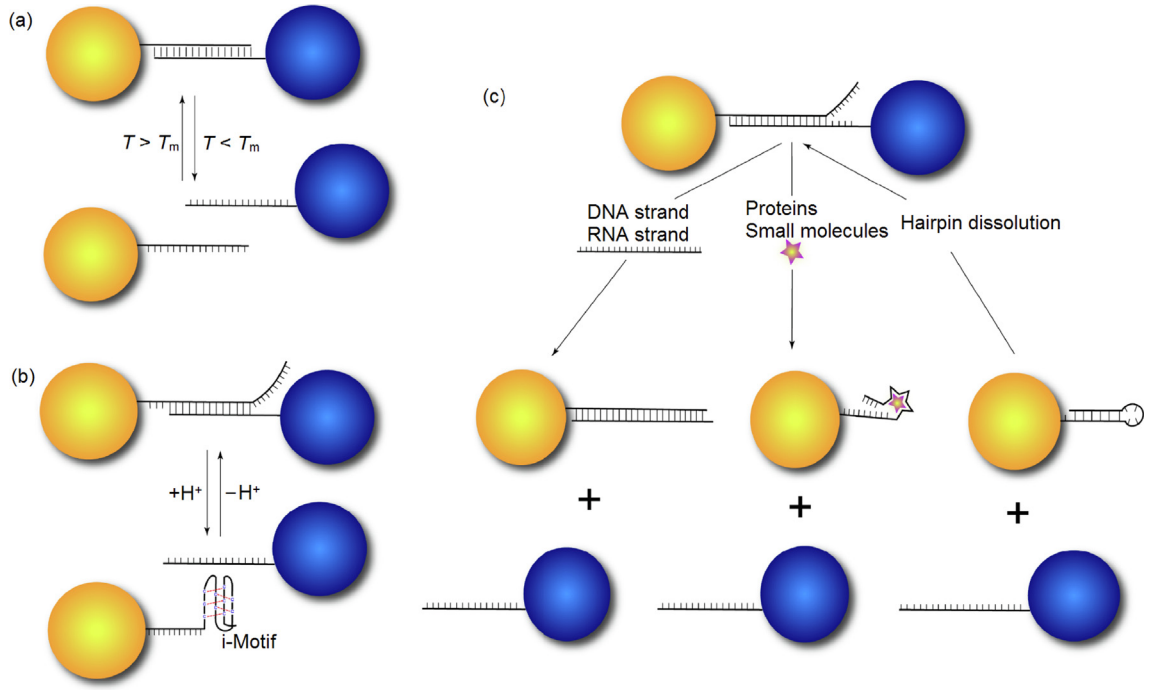

Fig. 8. (Color online) Schematic illusion of the dynamic supraparticles formation based on DNA strand annealing and displacement. Dynamic assembly/disassembly triggered by temperature alteration (a), pH change (b), and competitive binding (c). Spheres represent nanoparticles of different groups. Solid lines with perpendicular ticks depict DNA ligands.

facilitating self-folding of i-motifs that in turn interrupts the linker dsDNA, leading to DS disassembly. This process is highly dynamic with the pH, providing an elegant mechanism for designing pH-switchable nanomaterials. For example, our group fabricated a pH-responsive IONP DS for inverse contrast enhancement of MRI of early stage small HCCs (Fig. 9) [43]. Firstly, iron oxide nanoclusters (USIONCs) were modified with ssDNA ligands, which then annealed with the complementary ssDNA overhangs flanking the i-motif sequence-containing dsDNA linkers, mediating DS assembly. This structure was stable under normal pH and served as T2 contrast agents. While in the acidic tumor microenvironment, the reconfiguration of i-motif DNA would trigger the disassembly of DS, resulting in a T2 to T1 conversion and contrast enhancement.

(iii) Competitive binding. The competitive binding strategy follows the "Winners Take All" principle. In this scenario, when a third strand of ssDNA has a longer complementary sequence to one of the two strands of a partially annealed duplex DNA molecule, it will displace the other one strand of the original duplex, forming a new dsDNA region with higher stability. It should be noted that the competitor is not necessarily a DNA molecule. In fact, small molecules, or biomacromolecules such as proteins, can also compete with an ssDNA molecule for binding with another ssDNA molecule that, in this case, is called an aptamer [78]. During the process of competitive binding, the connective interparticle dsDNA is disrupted, leading to nanoparticle dissociation and disassembly of the DS. The competitive binding can be implemented not only among strands, but also within the self-complementary strand. For example, when the ssDNA ligand containing self-complementary sequence is attached to the surface of one group of nanoparticles, it may fold into the hairpin structure with a duplex stem. When a competitor sequence is added, it will anneal with the complementary sequence of the ligand ssDNA, dismantling the hairpin structure to expose a sticky end that can be used to mediate nanoparticle assembly [79]. In the context of *in vivo* applications, an RNA molecule is most likely to be the competitive key to unlock a DNA duplex for on-demand drug release or other purposes [66], given that DNA is localized within the cell nucleus and inaccessible at most time.

Here we only enlisted the simplest models of DNA-based fabrication of DS. In practical applications, surface ligands are usually duplex DNA with ssDNA overhangs instead of a pure single strand. Moreover, the efficiency and accuracy of these assembly/disassembly processes remain to be determined. In the future, by combinational use of the strategies, such as concurrent heating and competitive binding, perhaps we can step further into the programmable synthesis of DS.

4. Applications of DS in ARDs

Based on the principles discussed, a large arsenal of DS is established for biomedical applications including the treatment of ARDs, which have displayed superior performance in therapy, imaging and adaptability to the biological environment [55,56,80,81]. From hindsight, the successful design and synthesis of DS for concrete diseases rely not only on the appropriate usage of block nanoparticles and ligands, but also on the right stimulus that can efficiently and specifically trigger the transformation of DS at diseased sites. Generally, ARDs-targeting DS ligands prefer endogenous biological stimuli enriched in the diseased regions rather than exogenous ones such as light and magnetic field, which heavily depend on accurate detection of the disease sites in the first place or otherwise can lead to substantial toxicities. However, in some multifunctional DS, the response to a biological stimulus in diseased sites can condition further application of external stimuli for synergistic therapeutic effects. Because ARDs are commonly characterized by a unique microenvironment distinct from normal tissues, many researchers are using disease-specific microenvironment factors as the trigger for DS ligands, which are less heterogeneous within the lesions or among different individuals, endowing DS with more consistent performance. A potential advantage of microenvironment factor-responsive ligands is that they may concomitantly change the disease microenvironment by depleting the

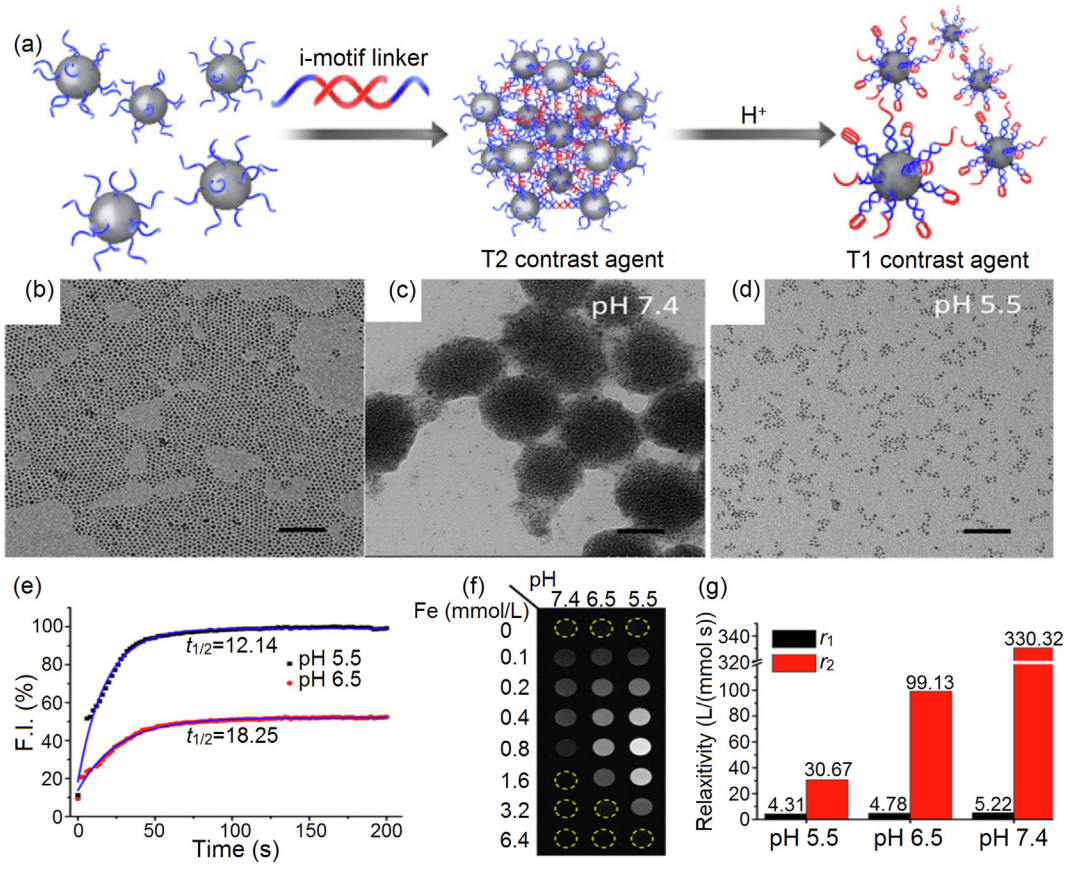

Fig. 9. (Color online) Fabrication of dynamic supraparticles based on pH-dependent i-motif DNA. (a) Schematic diagram of the i-motif DNA-directed pH-responsive iron oxide nanocluster assemblies (RIA). TEM images of ultra-small superparamagnetic iron oxide (USPIO) (b), RIAs in PBS (c), and RIA in MES (d). (e) Kinetics of RIA disassembly at different pH. (f, g) T1 weighed imaging of phantoms and relaxivity data of RIAs under different pH values. Reprinted with permission from [43]. Copyright (2018) American Chemical Society.

stimuli pool or interfering with *in vivo* functions of the stimuli. Given some biological stimuli have major implications in the progression of ARDs, such as acidity in cancer [82] and reactive oxygen species (ROS) in AD [36], their consumption by the responsive ligands in the DS may further enhance the therapeutic effects. In these considerations, a thorough understanding of the biological part is as important as the material part for rational design of DS for optimal ARDs control.

In the following sections, we will summarize the most often employed biological stimuli for activating DS, with introductions on their formation mechanisms and biological roles in the progression of ARDs, which may facilitate the rational design of appropriate responsive ligands for high performance theranostics. Then, the purpose-driven applications of DS will be reviewed that address the challenges in ARDs management. Although most of the DS introduced are fabricated for cancer, neurodegenerative diseases, and stroke, the promises displayed, such as early diagnosis and excellent biosafety, are encouraging for future development of more diverse DS for a wider range of ARDs.

4.1. DS for cancer management

Cancer is a malicious disease with age as a major risk factor apart from hereditary genetic mutations [83]. During disease progression, cancer acquires abnormal phenotypes that have provided therapeutic targets for therapies. DS employ the distinctive phenotypes of cancer as well as their microenvironmental biological stimuli to trigger the transformation for specific missions. With rational design, DS respond to a broad range of biomolecules or biochemical entities enriched in the tumor cells or the TME, which makes them adaptive to the heterogeneous landscape of cancer.

4.1.1. Enzyme-responsive DS

As cancer cells undergo active biological processes such as DNA replication, cell division, or even cell death after therapeutic intervention, many of the protein enzymes have elevated expression or enhanced activities. For example, telomerase is reactivated in many types of cancer to escape replicative senescence [84]. The proprotein convertase furin is upregulated in a wide range of cancers such as neck squamous cell carcinoma and breast cancer [85]. When cells undergo apoptosis after treatments, the activities of different caspases are mounted [86]. Apart from intracellular enzymes, some cell membrane-anchored or secreted enzymes are found to enrich in tumor sites, such as secreted phospholipase A2 (sPLA2) in prostate cancer [87].

Another major group of enzymes that have aberrant activities in cancer are extracellular matrix (ECM) remodeling enzymes [88], which are also frequently used as biological stimuli for DS. For example, the family of matrix metalloproteinases (MMPs), which have substrate specificities for nearly all the ECM components [89]. Several MMPs have been well implicated in the progression and metastasis of cancers. MMP2 and MMP9 are upregulated in hypoxia breast and colon cancers in a HIF-dependent manner [90,91]. They catalyze the degradation of type IV collagens, the main constitute of the basement membrane, thus facilitating the epithelium mesenchyme transition (EMT) and cancer metastasis [92]. MMP1 that acts on type I collagens also plays an important role in promoting cancer metastasis by hydrolyzing the type I

collagens in the interstitial/stromal matrix [93]. Its elevated expression is correlated with aggressive cancer phenotype and poor survival [94]. Besides, MMP7, the smallest member of MMPs, has been extensively shown to foster tumor aggravation and can serve as a biomarker for prognosis of multiple types of cancer such as pancreatic ductal adenocarcinoma and renal cell carcinoma [95,96]. There have been attempts in clinical trials to use MMP inhibitors as a complement treatment to mitigate tumor invasion and improve therapeutic response, most of which failed due to a lack of therapeutic response or severe adverse effects [97,98]. Other enzymes that are enriched in the tumor ECM have also been identified. The fibroblast activation protein (FAP) is a transmembrane gelatinase highly expressed in most solid tumors by tumor stromal fibroblasts [99], while hyaluronidases (HAases) are found to be upregulated along with its substrate hyaluronic acid in the ECM of prostate [100], colorectal cancer [101], lung carcinoma [102], etc.

Some enzymes, e.g., cysteine cathepsins, that work both intracellularly and extracellularly are also applicable as the trigger for DS. Plenty of studies have shown that cysteine cathepsins are highly expressed during the progression of various tumors, which correlate well with poor patient outcomes (well-reviewed in Ref. [103]). Cysteine cathepsins predominantly function in the acidic environment of endosomes and lysosomes to degrade proteins [103]. However, some cathepsins can be secreted into the ECM, such as cathepsins B and L, which favors tumor cell migration and invasion [104].

Collectively, all the enzymes that are enriched in cancer provide us with diverse biological stimuli to activate DS for dynamic cancer therapy and response monitoring, when we incorporate their substrate moieties into the surface ligands of the DS. Notably, enzymatic activities can lead to various consequences, including bond cleavage (e.g., MMP [58]), length reduction or elongation (e.g., telomerase [105]), binding affinity (e.g., kinases [106]), which allows the enzyme-activating DS to undergo different types of transformations for specific purposes. With regards to *in vivo* applications, we must take into consideration the tumor-type specificity, enzyme localization and abundance, catalytic efficiency, and biological consequences of their consumption by ligands. Based on these principles, many enzyme-responsive DS have been constructed for sensitive tumor imaging and selective cancer killing.

Li at el. [107] designed smart self-assembled DS as a vehicle for the hydrophobic drug paclitaxel (PTX). The PTX prodrug was linked to the PEGylated peptide dendrimer via a cathepsin B-sensitive linker glycyl-phenyl-alanyl-leucyl-glycine (GFLG). This assembly demonstrated excellent stability during circulation and significantly decreased nonspecific cellular uptake by the mononuclear phagocyte system (MPS). After entry into the tumor cells, the DS underwent rapid PTX release and disassembly of the structure due to the cathepsin B-catalyzed degradation of the linker moiety, which achieved potent cytotoxicity against breast cancer cells *in vivo* while minimizing side effects to normal tissues. In addition to enzyme-responsive disassembly of DS for therapeutic purposes, enzyme-triggered *in situ* DS assembly is currently more involved in imaging utility to indicate the dynamic changes during treatment or variations in TME. For example, Yuan et al. [16] synthesized a small molecule Ac-Asp-Glu-Val-Asp-Cys(StBu)-Lys-CBT(1) that could undergo Casp3/7-controlled condensation. They modified the USPIO with this ligand through covalent binding, rendering them Casp3/7 responsive. Both *in vitro* and *in vivo* results showed that the functionalized nanoparticles aggregated apparently and thus enhanced T2 MRI in apoptotic tumor cells, which could be applied for therapeutic effects monitoring (Fig. 10). Similarly, Zhou et al. [45] engineered a hyaluronidase (HAase)-responsive *in situ* DS assembly for high contrast tumor imaging. They coated USPIO with hyaluronic acid as the surface ligands, which could be degraded by the HAase enriched in TME, leading to self-assembly of USPIO supraparticles. This structural reconfiguration caused a T1 to T2 MRI pattern transition and enhanced the contrast between inner and peripheral regions of the tumor.

4.1.2. Tumor acidity-responsive DS

Cancer is characterized by an acidic TME resulting from anaerobic glycolysis producing acidic lactate and excessive protons [29,82], which are extruded to the extracellular space by a variety of transporters, eventually leading to a pH gradient with the intracellular pH (pH_i) alkaline (7.12–7.65) and the extracellular pH (pH_e) acidic (6.2–6.9). In addition, the poor vascularization of tumors leads to insufficient clearance of lactate and other acidic metabolic products, further contributing to the decreased pH value in the TME. The extracellular acidity is reported to play cell type-dependent roles in cancer cell survival in the harsh microenvironment. It promotes angiogenesis via regulating VEGF and IL-8 expressions [108,109], and facilitates metastasis through disrupting adherence junctions [110] and activating secreted proteases to dismantle the ECM, i.e., MMPs [111] and cathepsins [112]. TME acidity also has major contributions to tumor resistance to chemotherapy by neutralizing weak alkaline drugs [34], as well as to immunotherapies by exhausting T lymphocytes via lactate [113,114].

To this end, the TME acidity appears to be not only an ideal stimulus to switch on the DS, but also an attractive target for manipulating sensitivity of cancer cells to different therapies. It should be noted that the acidity in the endosomes and lysosomes within cancer cells is also excellent triggers for DS. This natural pH gradient from blood to TME and to intracellular endosomes/lysosomes provides a chance for multistage dynamic reconfiguration, which can be exploited to program DS behaviors at different stages after administration. In this consideration, our group has constructed tumo pH-responsive magnetic nanogrenades (PMNs) for ultrasensitive "turn-on" imaging and therapy of small tumors (Fig. 11) [56]. The PMN supraparticles were assembled by IONPs with the help assisted with the chlorin e6 grafted, imidazole modified poly(ethylene glycol)-poly(β-benzyl-*L*-aspartate) based pH-sensitive polymeric ligands. When the PMNs reached the acidic TME, the imidazole group was protonated, leading to a charge reverse of the ligands from negative to positive, which facilitated cellular uptake. After internalization into the lysosomes, PMN ligands were further protonated and underwent a hydrophilic shift to trigger DS disassembly, activating MRI and fluorescent signaling for highly sensitive detection of very small tumors. This structural transformation also switched on the photodynamic function, enabling imaging-guided precise killing of the heterogeneous tumor cells.

Recently, our group reported bismuth subcarbonate nanotubes (BNTs) that can disassemble into extremely small (~1.5 nm) bismuth subcarbonate nanoclusters (BNCs) in response to acidic pH (Fig. 12) [80]. This type of DS exhibited enhanced tumor targeting ability compared to commonly used small-sized metallic nanomaterials based on their anisotropic tubular shape, and could load additional chemotherapeutic drugs due to their hollow structures. After i.v. injection into the mice, the BNCs efficiently enriched in the acidic tumor sites and then disassembled on demand for CT imaging and exert synergistic radio-chemotherapeutic effects, and then disassembled on demand to be readily eliminated from the body *via* renal clearance to ensure minimal safety risk.

As mentioned, the TME have highly dynamic topography, which may cause inconsistent performances of DS, particularly of drug-loading DS, at different sites or times during progression and treatment. Therefore, on top of the pH-responsive tumor targeting, our group incorporated a photodynamic module and synthesized

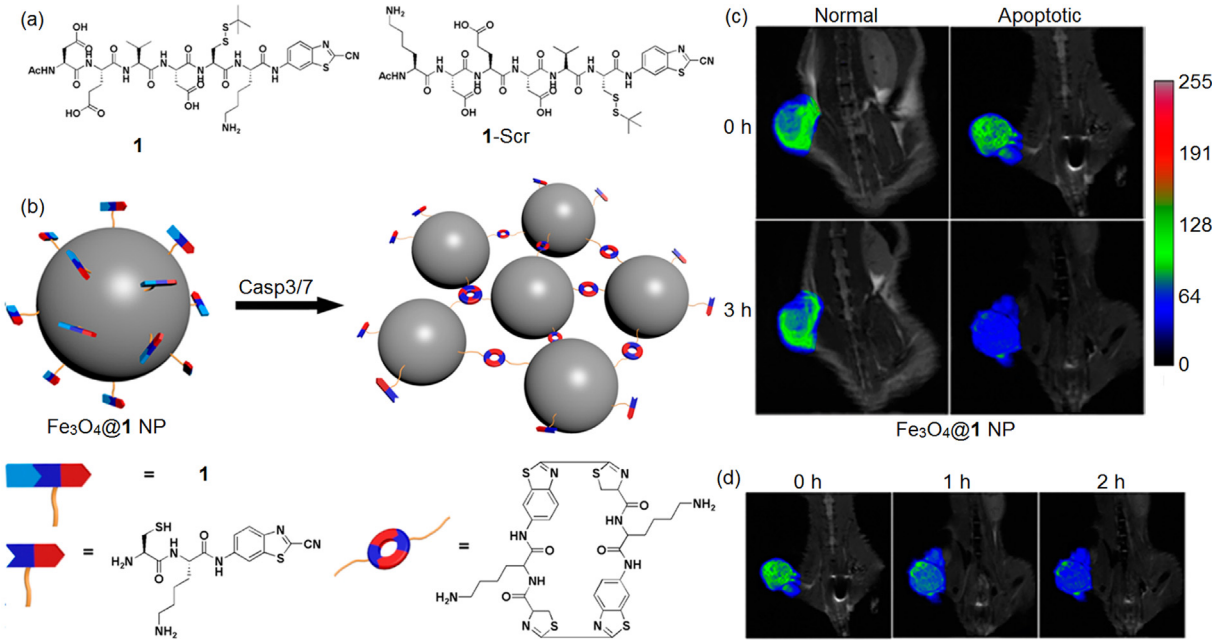

Fig. 10. (Color online) Caspase-responsive dynamic supraparticles for *in vivo* detection of apoptotic cells. (a) Chemical structures of the surface ligands. (b) Schematic illustration of the assembly of DS triggered by Casp3/7. (c) *In vivo* T2-weighted coronal MRI after injection of 1-functionalized USPIO to saline or DOX-treated mice. (d) Time-course T2-weighted coronal MRI after injection of 1-functionalized USPIO into DOX-treated mice. Reprinted with permission from Ref. [16]. Copyright (2016) American Chemical Society.

tumor acidity-activated photodynamic nanoagents (PPNs) (Fig. 13) [55]. This multifunctional DS is reversibly assembled by UCNPs in the presence of pH-sensitive polymers. After arrival at the tumor sites of acidic pH, the DS underwent charge reversal that facilitated cellular internalization into endosomes/lysosomes, where the assemblies fell apart, reactivating the self-quenched photosensitizers for highly efficient NIR-mediated photodynamic therapy. Because of the superior tissue penetration capacity of NIR, this strategy offered a promising solution to treat deep seated tumors.

4.1.3. Oxidative stress-responsive DS

Oxidative stress is a biomarker of the aging process, which is constitutively high in nearly all ARDs including cancer [115]. It is the result of rampant production of ROS and insufficient antioxidant activities. The ROS as a collective includes superoxide radical anions ($O_2^{\cdot-}$), hydroxyl radicals ($^{\cdot}OH$), peroxides (H_2O_2), and other non-radical species. The endogenous ROS can be traced back to two major sources: mitochondria metabolisms and NADPH oxidases (NOXs) [116]. In cancer cells, the mitochondria electron transport chain (ETC) lacks the electron receptor O_2 because cancer cells are addicted to anaerobic glycolysis [117], which permits electron leakage and subsequent formation of superoxide anions ($O_2^{\cdot-}$), the precursor of hydrogen peroxide (H_2O_2). NOXs are plasma membrane-anchored enzymes that produce $O_2^{\cdot-}$ by using NADPH and O_2, which contributes to both intracellular and extracellular oxidative stress. The H_2O_2 molecules are relatively stable and have good membrane permeability. They are either converted into water by peroxiredoxins (PRXs), glutathione peroxidase (GPXs) and catalase (CAT) at different sites, or degraded into the more cytotoxic $^{\cdot}OH$ radicals in Fenton reaction [118]. Another important environmental factor contributing to the oxidative stress in cancer is the hypoxia condition in TME resulting from insufficient blood supply, which further exacerbates ETC dysregulation and ROS generation [119]. Although there is a net increase in the ROS levels, it does not reflect that the antioxidant capacity is compromised in cancer cells. In fact, many canonical antioxidant pathways are enhanced as a result of the adaptation to the sustained oxidative stress, leading to a highly reductive intracellular environment indicated by high-level glutathione (GSH) [120,121].

The excessive ROS and high-level reductive GSH in the tumor sites, they are both desirable biological stimuli for DS. There are many functional groups possessing redox responsiveness, such as boronic esters/acids and thioketals for ROS, and disulfide bond for GSH. By employing ligands containing these moieties, both ROS and GSH-responsive DS have been successfully designed for therapeutic and imaging purposes. For example, Chen et al. [122] constructed an H_2O_2-reactive nanoassembly for tumor-selective PDT. In the core of the assembly, photosensitizer and H_2O_2 degrading CAT were encapsulated, with black hole quencher installed in the polymeric shell to sequester the activity of the photosensitizer. Upon exposure to high level of H_2O_2 in the tumor cells, CAT could catalyze production of large amounts of O_2 from H_2O_2 that diffused into the core, causing disintegration of the shell and thus activation of the photosensitizer for tumor-specific PDT. Notably, the generated O_2 could sustain the PDT process, which significantly enhanced the therapeutic potency. Interestingly, to increase the responsiveness of DS, external source of ROS can be applied. Yue et al. [123] constructed a ROS-responsive nanoparticle assembly for cancer treatment by synergistic chemotherapy and photodynamic therapy. In their system, a novel block copolymer, TL-CPT-PEG_{1K}-TPP was synthesized by functionalizing PEG with triphenylphosphonium and camptothecin (CPT) conjugated to a thioketal linker (TL). The amphiphilic copolymer then self-assembled with 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)] (DSPE-PEG) into the final product encapsulating the photosensitizer Zinc phthalocyanine (ZnPc). Because of the presence of triphenylphosphine cations on the surface, the obtained DS (ZnPc/CPT-TPPNPs) effectively targeted mitochondria, the major source of endogenous ROS. Upon laser irradiation, ZnPc further increased the ROS level, concurrently activating chemotherapy and PDT, which conferred prominent anticancer effects without causing evident toxicities. Hu et al. [124] fabricated GSH-responsive hyaluronic acid (HA)-based nanocomposites (PFH@HSC) conjugated with photosensitizer

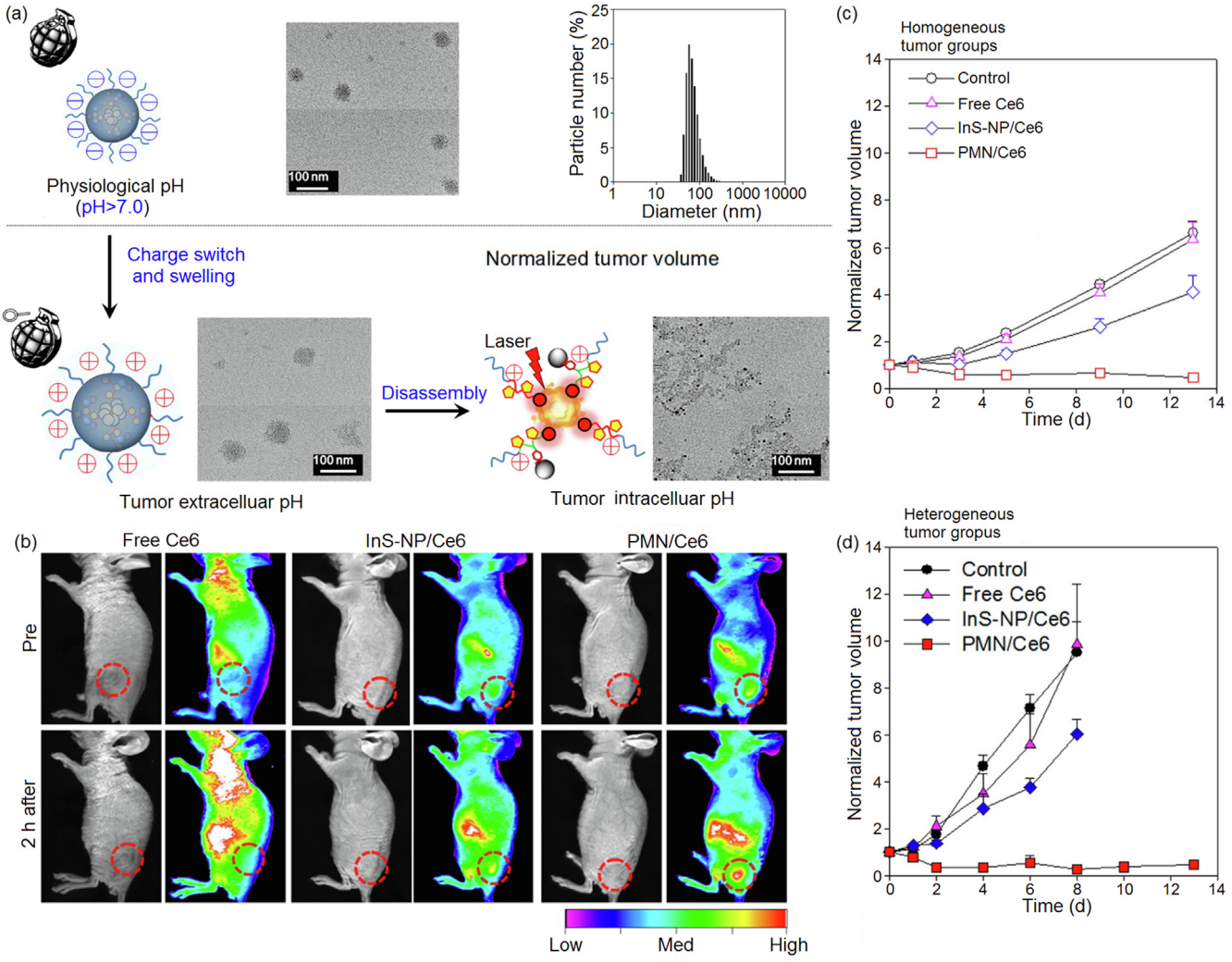

Fig. 11. (Color online) pH-responsive magnetic nanogrenades for early detection of small tumors and photodynamic therapy of heterogeneous cancer. (a) pH-responsive structural reconfiguration of PMNs with corresponding TEM images. (b) Live NIR imaging of tumor-bearing nude mice before and after i.v. injection of indicated agents. Growth curves of homogeneous HCT116 tumors (c) or heterogeneous tumors (d) under different treatments. Reprinted with permission from Ref. [56]. Copyright (2014) American Chemical Society.

chlorin e6 (Ce6) through sulfide bond. They also encapsulated oxygen carrier perfluorohexane (PFH) inside the core. The abundant GSH inside tumor cells would disintegrate the structure of PFH@HSC to release Ce6 and PFH, recovering the fluorescence and phototoxicity of Ce6. Meanwhile, the oxygen supply offered by PFH could relieve tumor hypoxia and enhanced PDT effects, which holds great promise for hypoxia tumor treatment.

In addition to therapeutic purposes, redox-responsive DS designed for tumor imaging and therapy monitoring has also been fabricated. For instance, Yang and co-workers [125] constructed a disulfide-linked ligand to direct self-assembly iron oxide nanoparticles under physiological conditions. The resultant assembly was further capped by cyanine 5.5-labeled human serum albumin (HSA-Cy5.5) through electronic interaction to form a multifunctional redox-responsive DS (RMNs-HSA-Cy5.5). After tumor cell endocytosis, HSA-Cy5.5 and iron oxide nanoparticles would be released after breakage of the disulfide bond, turning on NIRF and T2-weighted dual-model imaging for accurate diagnosis of tumors. Recently, Wang et al. [126] reported redox-responsive sulfide-crosslinking polymeric magnetosomes (PolyMags) (Fig. 14). When loaded with chemotherapeutic drug doxorubicin (DOX), Plolymags realized targeted drug delivery to the GSH abundant tumor sites, which was accompanied by disassembly of the iron oxide nanoparticles that activated T2 MRI of the tumor. In addition, DOX dissociation from the nanoparticle surface would increase the access of iron oxide nanoparticles to bulky water and thus caused a change in MRI signal intensity, providing an approach for monitoring the dynamic process of drug release. Moreover, PolyMags could activate photothermal therapy upon MRI-guided laser irradiation for synergistic tumor treatment.

4.1.4. Others

Besides enzymes, pH and redox, some other biological stimuli specific for tumors have also been used as the trigger to activate DS. Since cancer cells have altered gene expression patterns, they often overexpress certain mRNA or miRNA, which can be used as the key to switch the on/off of DS. The principle is simple: RNA has the same base paring mechanism as does DNA, therefore it can hybrid with a DNA or RNA molecule of the complementary sequence to induce reconfiguration of the DS. For instance, Zhang et al. [66] fabricated a mesoporous silica-coated quantum dots assembly (MSQDs) carrying the chemotherapeutic DOX. The assembly was capped with the nucleolin aptamer AS1411 with flanking anti-miR-21 sequences, which could hybrid with the complementary anchor-DNA surrounding the pores. Therefore, the chemotherapy drug DOX was sealed inside the nanoparticle

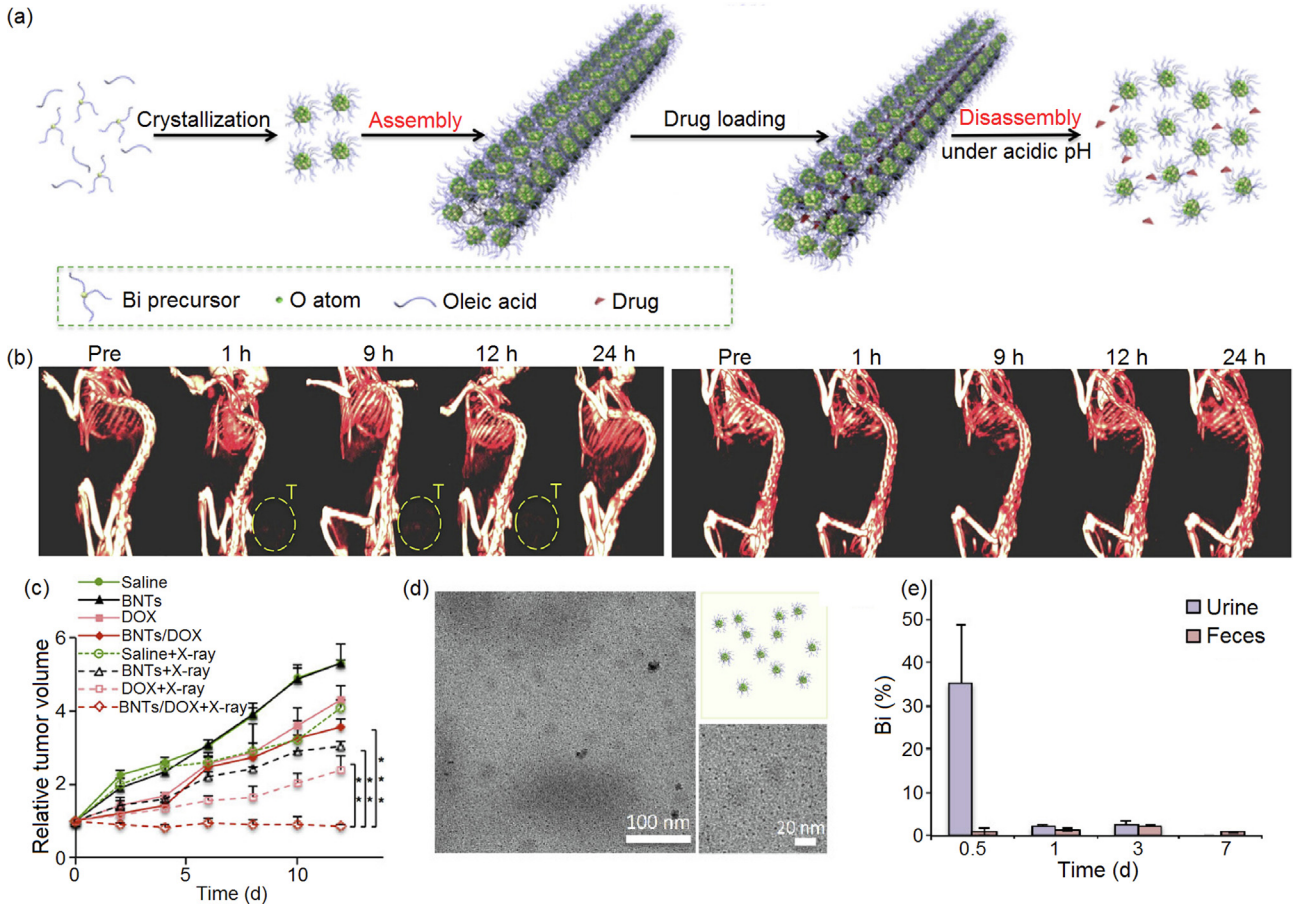

Fig. 12. (Color online) pH-responsive bismuth subcarbonate nanotubes (BNTs) for synergistic radiochemotherapy and renal clearance. (a) Schematic illustration of the fabrication of BNTs. (b) Reconstructed 3D CT images of mice at different times after injection of (left) BNTs and (right) BNCs; yellow dashed circles denote tumor. (c) Relative tumor growth curve of the nude mice after indicated treatments. (d) TEM images of the urine of a tumor-bearing mouse at 3 h post BNTs injection. (e) The content of Bi in urine and feces of tumor-bearing mice on different days post injection. Reprinted with permission from Ref. [80]. Copyright (2018) American Chemical Society.

assembly to prevent non-specific toxicity to normal tissues. When the assembly arrived at the TME, AS1411 could facilitate active targeting by binding to the cancer cell specific cell membrane protein nucleolin. After endocytosis and endosome escape, the overexpressed miR-21 in cancer cells would competitively bind to the anti-miR-21 sequence, thus unlocking the DNA hybrid cap to release DOX for cancer killing. Meanwhile, the pro-tumoral miR-21 would be depleted in this process, enhancing the therapeutic performance of MSQDs.

Overall, different types of DS responsive to the dynamic TME have been fabricated for cancer treatment. No matter enzyme-responsive, acidity-responsive or oxidative stress-responsive, DS have proven to be superior in both imaging and therapy of cancer. Currently, most DS only use the TME features as a trigger for activating the designed functions, whether they concomitantly modify the TME, e.g., TME acidity, and how this potential effect contributes to their therapeutic performance remain to be determined by quantitative profiling of those TME parameters after interventions. Future researchers may directly add some sensor modules into the DS for real-time monitoring of the TME dynamics during treatment.

4.2. DS for treating neurodegenerative diseases

Neurodegenerative are notorious for their devastating consequences, including disorders of motor functions, loss of memory and cognitive functions, and eventually morbidity/mortality. Neurodegenerative diseases are financially and mentally costing, imposing great family and social burdens [5,127]. There are a variety of neurodegenerative diseases that threaten a healthy aging process. Considering the prevalence and severity, AD and PD are among the top of the list. Effective treatments for these diseases are in urgent need, with only a handful of drugs clinically approved to control the symptoms or promote recovery. For AD and PD, currently available drugs are nearly doomed with ineffectiveness at the late stages, leaving the patients in a completely helpless state [27,40]. The failures of the development of disease-modifying drugs for neurodegenerative diseases can be ascribed to many factors. Apart from the common challenges faced by ARDs management, some unique problems have further added to the complexity of neurodegenerative disease management. First, drug delivery to the brain is obscured by the natural barrier, blood brain barrier (BBB). Second, unlike cancer treatment that is essentially killing of malignant cells, the treatment of neurodegenerative diseases is centered around the protection of the neural cells that are still working to maintain the cognitive functions. Therefore, selectivity and safety are of primary importance to neurodegenerative disease management. Third, it is increasingly appreciated that multitarget therapy is essential for preventing neural cell death in multifactorial neurodegenerative diseases, which is largely different from the cancer treatment in which single target agents can be effective by triggering cell death. Fourth, the therapeutic response of treatments for neurodegenerative diseases, for example, improvement of cognitive functions, is difficult to evaluate in a

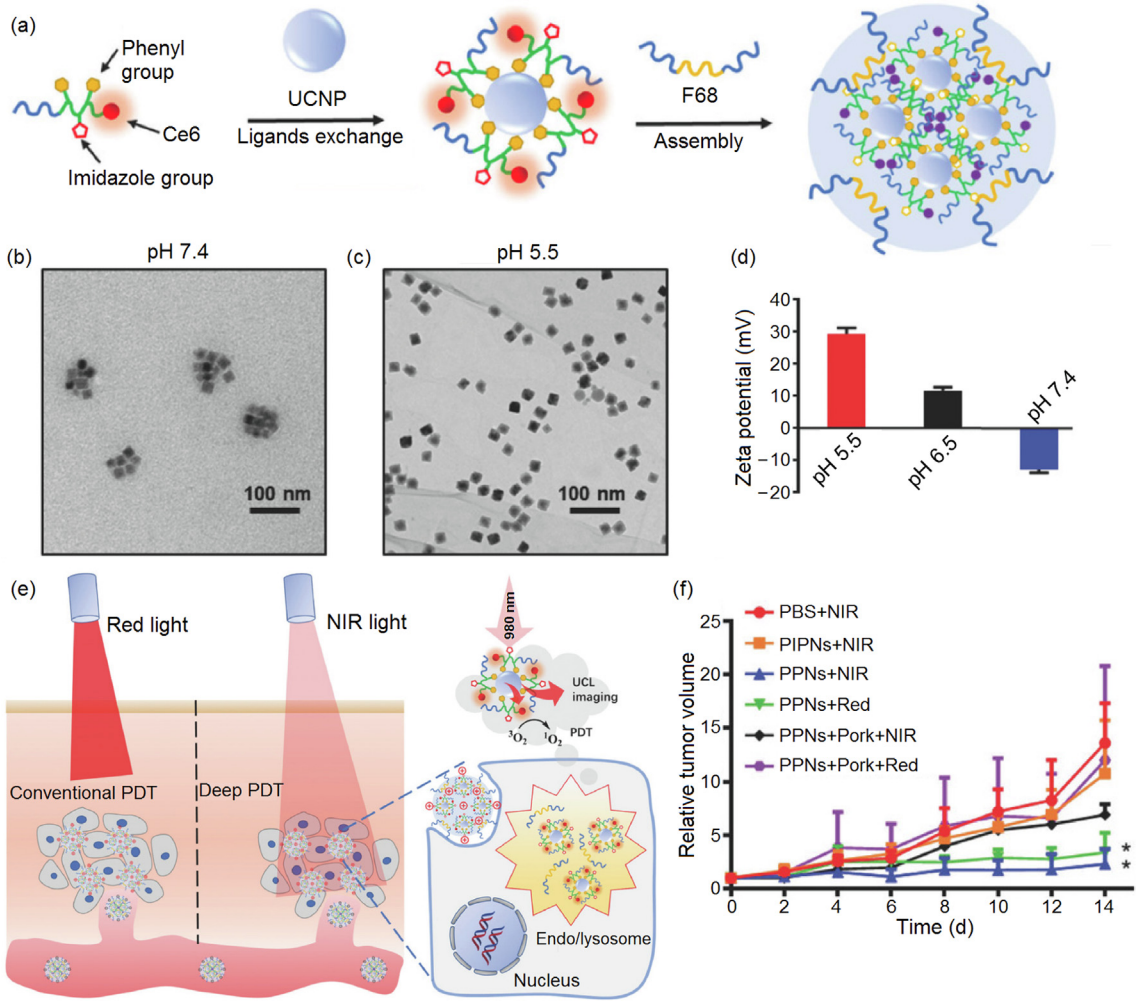

Fig. 13. (Color online) pH-responsive UCNP-based DS for effective killing of deep tumors. (a) Schematic illustration of the design of the pH responsive UCNPs assembly. (b, c) TEM images of the pH dependent assembly (b) and disassembly (c) of UCNPs. (d) Reverse of the Zeta potential from neutral pH to acidic pH. (e) Schematic illustration of the mechanism of effective killing of deep tumors via PPNs. (f) Tumor growth curves of deep tumor-bearing mice under indicated treatments. Reprinted with permission from Ref. [55]. Copyright (2018) WILEY-VCH.

short period. In this regard, therapy monitoring at the pathological level is especially helpful to timely adjust treatment regimens.

In fact, there are extensive studies to address these challenges using nanotechnologies, but for long only as a carrier to improve the penetration of physiological barriers, e.g., BBB, and thus to improve bioavailability (reviewed in Refs. [128,129]). Recently, DS have shown promise to bring new hope to the therapy of neurodegenerative diseases with all their advantages, especially multifunctionalities and targeted activation. Perhaps because of the technical challenges of establishing disease relevant animal models for neurodegenerative diseases, and of the safety concerns about nanomaterials in the brain, this field is largely underexplored compared to applications of DS in cancer. In the following sections we will introduce the pioneering studies that are aimed to improve the outcomes of neurodegenerative diseases with rationally designed DS. Although these DS are not as sophisticated as their counterparts for cancer theranostics, they may inspire future researches to fabricate better and smarter DS. Since AD and PD share many common pathological features such as protein aggregation and oxidative stress, the development of DS to treat them may follow similar principles. Here we will focus on AD as an example to show how DS can and should be designed to bring substantial benefits to patients.

4.2.1. Oxidative stress-responsive DS for AD management

Similar to cancer, high-level oxidative stress also prevails in AD brains. The increased oxidative stress causes oxidative damage to various macromolecules, including proteins, lipids, nucleic acids (reviewed in Refs. [35,36]), imperiling the normal activities and survival of neurons. Particularly, many studies using redox proteomics have demonstrated that in AD brains a number of glucose metabolism proteins bear oxidative damage [130,131], which leads to a series of consequences, including mitochondrial dysfunction, energy starvation, loss of calcium homeostasis, and eventually synaptic disruption and neuronal cell death. Oxidative stress in AD brains is closely related to the interaction between Aβ and metal ions, and mitochondrial dysfunction. The human brain has high concentrations of metals including iron, copper and zinc [132], which play dynamic roles in both neuroprotective and neurotoxic pathways [133–135]. During AD development, the interaction between metals and Aβ promotes Aβ oligomerization and aggregation, meanwhile generating oxidative (Cu^{+}) ions [136], which catalyze Fenton reaction to produce excessive ·OH using H_2O_2 derived from aged mitochondria. More recently, oxidative stress has been shown to directly contribute to Aβ aggregation by stimulating γ-secretase that cleaves the amyloid precursor protein (APP) into Aβ [137]. To this end, oxidative stress seems to be a

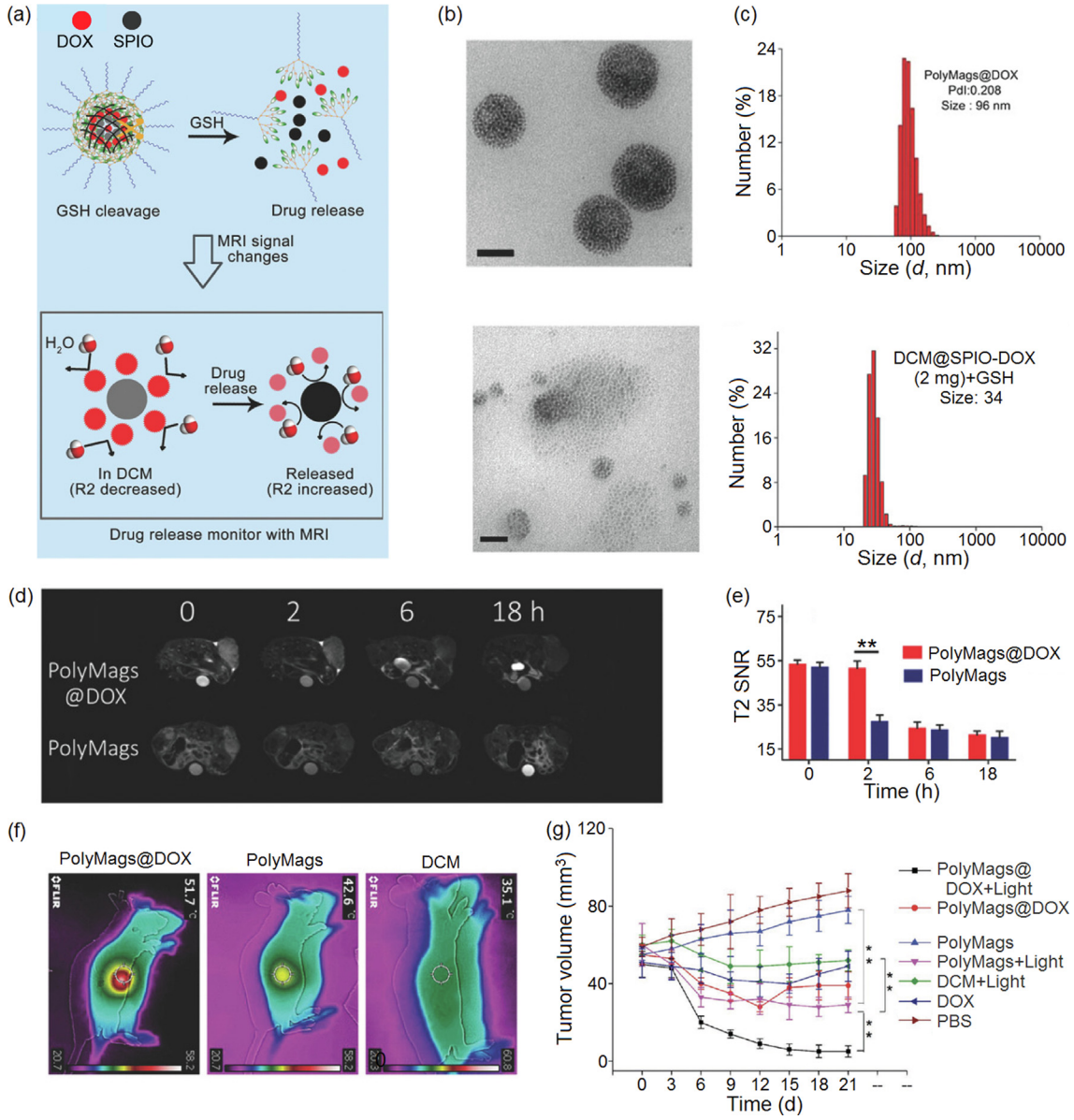

Fig. 14. (Color online) GSH-responsive dynamic supraparticle for drug-release monitoring and bimodal tumor therapy. (a) Schematic illustration of the GSH-responsive multifunctional polymeric magnetosomes (PolyMags). (b) TEM images indicating the size of PolyMags before (upper panel) and after (lower panel) exposure to GSH. (c) Quantification of (b) by DLS. (d) T2-weighed MRI of the breast tumor-bearing nude mice using PolyMags or PolyMags with DOX loaded. (e) T2 SNR corresponding to (d). (f) Representative thermal images of MCF7 xenograft-carrying mice at 12 h after light irradiation following different treatments. (g) Tumor growth curve of tumor-bearing mice treated as indicated. Reprinted with permission from Ref. [126]. Copyright (2018) WILEY-VCH.

reasonable target for AD intervention. Indeed, antioxidant therapies such as Vitamin C or E have been used as supplements for AD treatment in epidemiological trials, but failed to benefit the patients [38], probably because of the blurred dose-effect relationship and poor bioavailability. Currently, metal chelators with putative antioxidant properties are also examined in preclinical animal models for their ability to improve cognitive deficits in AD, which have generated optimistic results. However, these metal chelators lack selectivity and can disrupt the physiological functions of metal ions in healthy neuronal cells, which may cause sever adverse effects.

To improve the selectivity of metal chelators toward oxidatively stressed neuronal cells, Geng et al. [138] designed DS using mesoporous silica nanoparticles (MSNs) that could be activated by ROS. The MSN was functionalized with 3-carboxyphenylboronic acid (BA-MSN), a derivative of arylboronic acids. The copper ion (Cu^{2+}) chelator clioquinol (CQ) was then encapsulated into the system, after which the pores were capped with the glycoprotein IgG, which was natural nanoparticles that could form ester bonds with boronic acid (MSN-CQ-IgG). It was shown *in vitro* that MSN-CQ-IgG specifically reacted with H_2O_2 to release CQ after removal of the IgG caps by oxidation-dependent breakage of the boronate easter bonds. The MSN-CQ-IgG assembly effectively decreased Cu^{2+}-induced deposition of Aβ in the presence of H_2O_2 in a biochemistry assay. Concordantly, this assembly exerted marked protective effects on rat pheochromocytoma PC12 cells insulted by Aβ40-Cu^{2+}. Li et al. [139] used the similar strategy to achieve selective and controlled release of metal chelators in oxidatively stressed neurons. Instead of antibodies, they used glucose-functionalized cerium nanoparticles (G-CeNP) to cap the pores of BA-MSN (Fig. 15). The *cis*-diol moiety of glucose could form the arylboronic ester bond with the BA on the surface of MSN

(MSN-G-CeO2), thus sequestering the encapsulated metal chelators CQ. Upon exposure to high-level H_2O_2, the arylboronic esters underwent breakage, triggering the release of the payloads. Meanwhile, CeNPs had durable ROS-scavenging activities that conferred synergistic effects with the metal chelator CQ in suppressing Aβ aggregation and its toxicity toward PC12 cells. Currently, none of these nanoparticle assemblies have been examined in disease-relevant animal models for their capability to improve cognitive performance in AD. Future studies will definitely need to address it and, perhaps, endow the nanoparticle assemblies with more functions, for example, by incorporating Aβ and/or tau antibodies to boost immune clearance of those plaques.

4.2.2. Multifunctional DS for synergistic AD management

AD is a multifactorial disease resulting from the intricate interactions between genetic and environmental factors. Apart from the oxidative stress in the abnormal microenvironment and the Aβ aggregates, another two proteins, tau and Apolipoprotein E4 (apoE4), also play central roles in AD etiology. Tau is normally localized to the axons and may facilitate excitatory neurotransmission. During AD, it relocates to the neuronal soma and dendrites and become hyperphosphorylated, which promotes aggregation of tau into the neurofibrillary tangles (NFTs) [42]. The apoE4 expression is also a major risk factor of AD, which contributes to tau aggregation in both Aβ-dependent and -independent ways [37]. Although all above factors have been shown to foster AD, a common pathway is still missing that provides a coherent understanding of how exactly they interact and lead to AD. Moreover, the pathological consequences of abnormal protein aggregation are further exacerbated by the compromised waste disposal mechanisms. Normally, cellular garbage including damaged or aggregated proteins and damaged organelles are cleared by autophagy and the ubiquitin-proteasome system (UPS). In AD brains, neither of these two mechanisms work properly [140–142], leading to accumulation of dysfunctional mitochondria and protein aggregation including Aβ plaques and tau fibrils. Based on current

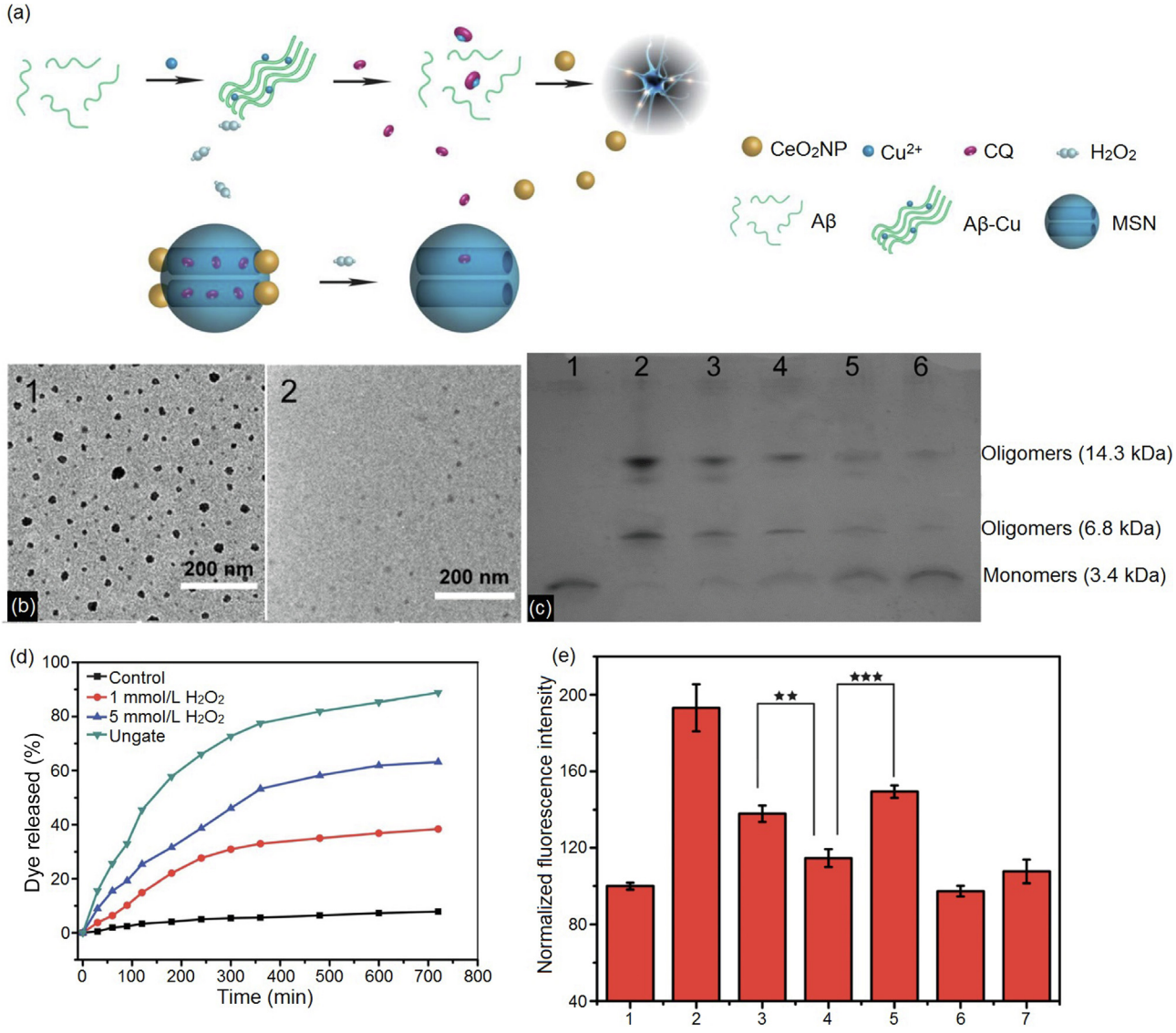

Fig. 15. (Color online) ROS-responsive supraparticle for controlled drug release and oxidative stress amelioration. (a) Schematic illustration of the design of metal chelator CQ-loaded mesoporous silica nanoparticles (MSNs) caged with cerium oxide nanoparticles (MSN-CQ-G-CeO_2). (b) TEM images of Cu^{2+}-induced Aβ aggregates in the absence (1) or presence (2) of MSN-GCeO_2 supplemented with H_2O_2. (c) Biochemical study of dissolution of Aβ aggregates after incubation (1) without any chelators, (2) with MSN-CQ-G-CeO2NP in the presence of H_2O_2, (3) with CQ and (4) with G-CeO2NP (5) with MSN-CQ-G-CeO2NP in the absence of H_2O_2. (d) Release of a model dye from capped or uncapped MSNs in the presence of H_2O_2 of different concentrations. (e) ROS level of PC12 cells that are untreated (1) or challenged for 48 h with (2) Aβ-Cu^{2+}, (3) Aβ-Cu^{2+} plus CQ, (4) Aβ-Cu^{2+} plus MSN-CQ-G-CeO_2NP, (5) Aβ-Cu^{2+} plus G-CeO_2NP, (6) Aβ, (7) Cu^{2+}. Reprinted with permission from Ref. [139]. Copyright (2013) American Chemical Society.

knowledge of the pathogenesis of AD, many strategies have been developed to target the key factors in AD development, including Aβ, tau, and autophagy. Although some have displayed evident disease-modifying effects in preclinical models, they failed to generate evident benefits or were yet to be tested in clinical trials. From hindsight, preclinical studies commonly use AD animal models established by manipulation of single factors, such as Aβ overexpression [143], which is far from the complex pathogenesis of AD in humans. Therefore, single target agents that are effective for single pathogenesis animal models have very minor chance to be effective for multifactorial AD in human patients. Taken together, to achieve a better outcome, we must have the mindset that AD must be managed in a more systemic manner.

To address the multifactorial pathogenesis of AD, our group constructed a methylene blue (MB) loaded nanocomposite (CeNC/IONC/MSN-T807) for multitarget AD treatment (Fig. 16) [144]. Ultra-small ceria nanocrystals (CeNCs) and iron oxide nanocrystals (IONCs) were assembled onto the surface of MSNs through nucleophilic substitution reaction, methylene blue (MB) was loaded into the pores of MSNs and a tau tracer Amino-T807 was grafted onto the surface of MSNs via macrocyclic chelator 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA). The ultra-small CeNCs, with ROS scavenging ability, could ameliorate mitochondrial oxidative stress-induced damage in AD. MB, a tau aggregation inhibitor could be released in neurons to prevent hyperphosphorylated tau aggregation. Such a combination generated synergistic effects and effectively protected neurons from damage caused by hyperphosphorylated tau aggregation and high-level of ROS. In addition, the IONCs were used as MRI agents, and the NOTA labeled with ^{68}Ga as PET agents, which provided bimodal imaging for dynamic monitoring of therapy response. Although promising, this multifunctional DS definitely requires further optimization to reduce its invasiveness, probably by allowing systemic administration.

4.3. DS for stroke treatment

A stroke is caused by the restriction of blood flow to a certain region of the brain that leads to deprivation of oxygen and nutrients. Since neuronal cells are highly metabolic and energy-consuming, they can undergo cell death within minutes, leading to symptoms ranging from mild ones such as headache and numbness of the face, to severe ones including permanent disability and death [145,146]. Currently stroke is the second leading cause of death worldwide [147], which can be ascribed to the sudden onset and vulnerability of neuronal cells. There are two types of stroke, ischemic stroke and hemorrhagic stroke, the former of which accounts for 80% of all cases. Ischemic stroke mainly occurs in elderly people because comorbid diseases such as atherosclerosis and type II diabetes predispose them to thrombi (blood clot). The microenvironment of cerebral ischemia contains a core region that undergoes complete oxygen deprivation, and a partially ischemic penumbra surrounding the core. In the core area, persistent energy starvation directly leads to rapid neuron death via necrosis, which in turn elicits inflammation by releasing danger-associated-molecular-patterns (DAMPs) [148]. While for the neurons in the ischemic

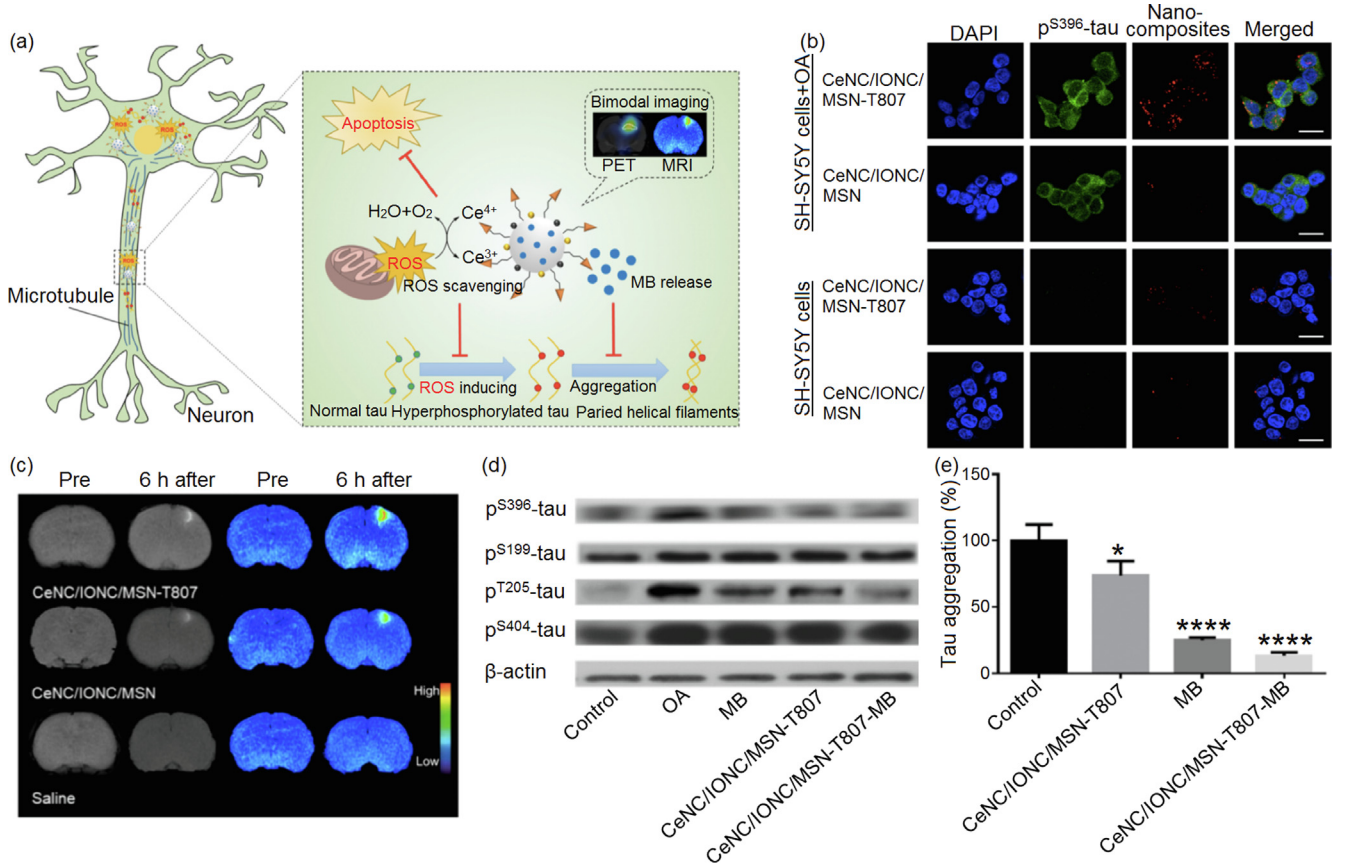

Fig. 16. (Color online) Multifunctional nanocomposites for imaging and therapy of AD. (a) Schematic illustration of the mechanism of action of multifunctional nanocomposites for AD management. (c) Confocal fluorescence images of p-tau tageted retention of the nanocomposites in OAtreated SH-SY5Y cells after 4 h incubation. (c) *In vivo* T1-weighted MR images of the brain of OA-treated rats after administration of different nanocomposites or saline for 6 h (left) and corresponding color mapped images (right). (d) Western blot analysis of tau hyperphosphorylation in SH-SY5Y cells challenged by OA for 12 h followed by another 12-htreatments as indicated. (f) Quantification of tau aggregation by ThS fluorescence. Reprinted with permission from Ref. [144]. Copyright (2018) American Chemical Society.

penumbra, injuries mainly occur during the reperfusion stage in which the oxygen burst and the calcium dysregulation incur intensive oxidative stress that damages a wide range of biomolecules and further exacerbates inflammation [148,149]. Two types of standard care for acute ischemic stroke are, intravenous injection of recombinant tissue plasminogen activator to dissolve the thrombi, and endovascular surgery to physically remove the blood clot. These two treatment regimens either have prominent systemic side effects [150] or are too invasive, which demonstrate inferior efficacy in elderly patients [151,152]. Many potential neuroprotective agents targeting oxidative stress and calcium homeostasis have been tested in preclinical studies and achieved applaudable results, but their therapeutic effects in clinical trials are in doubt. This discrepancy is likely to be caused by that the young animal used in experimental settings cannot model the elderly patient population, and that the sudden onset and delayed treatment in human patients are not addressed in most *in vivo* studies. From all the lessons of setbacks in treatment and the pathophysiological features of stroke, it may be appropriate to recapitulate that effective stroke control relies on: (1) efficient delivery through the BBB and targeting to the ischemic region; (2) constant therapy monitoring and microenvironment management; (3) post stroke neuron regeneration. Driven by these demands, some recent pioneering studies have constructed DS systems to improve the therapy of ischemic stroke, which achieved encouraging efficacies.

4.3.1. Oxidative stress-responsive DS for stroke treatment

As mentioned, ischemic stroke is largely exacerbated by the oxidative stress after oxygen deprivation. Therefore, it is an important target for therapeutic interventions. Meanwhile, high-level ROS is also a characteristic marker of ischemic regions of the brain, which can be used as a stimulus to trigger contrast imaging and/or on-demand drug release. Most recently, Rajkovic et al. [153] have fabricated ROS-responsive nanoparticles using poly(propylene sulfide) (PPS) (PPS-NPs) (Fig. 17). The PPS chains were abundant in terminal thiols, which were used to label fluorophores and crosslinking the PPS chains. The nanoparticles were coated with Pluronic F127 to increase its circulation time. Because of the disrupted BBB and leaky blood vessels at the inflammatory sites of ischemia, the nanosized particles can effectively accumulate in the region. In reaction to the high-level ROS, the PEG cap swelled and detached from the nanoparticle core, exposing more ROS-reactive groups for ROS scavenging. These nanoparticles

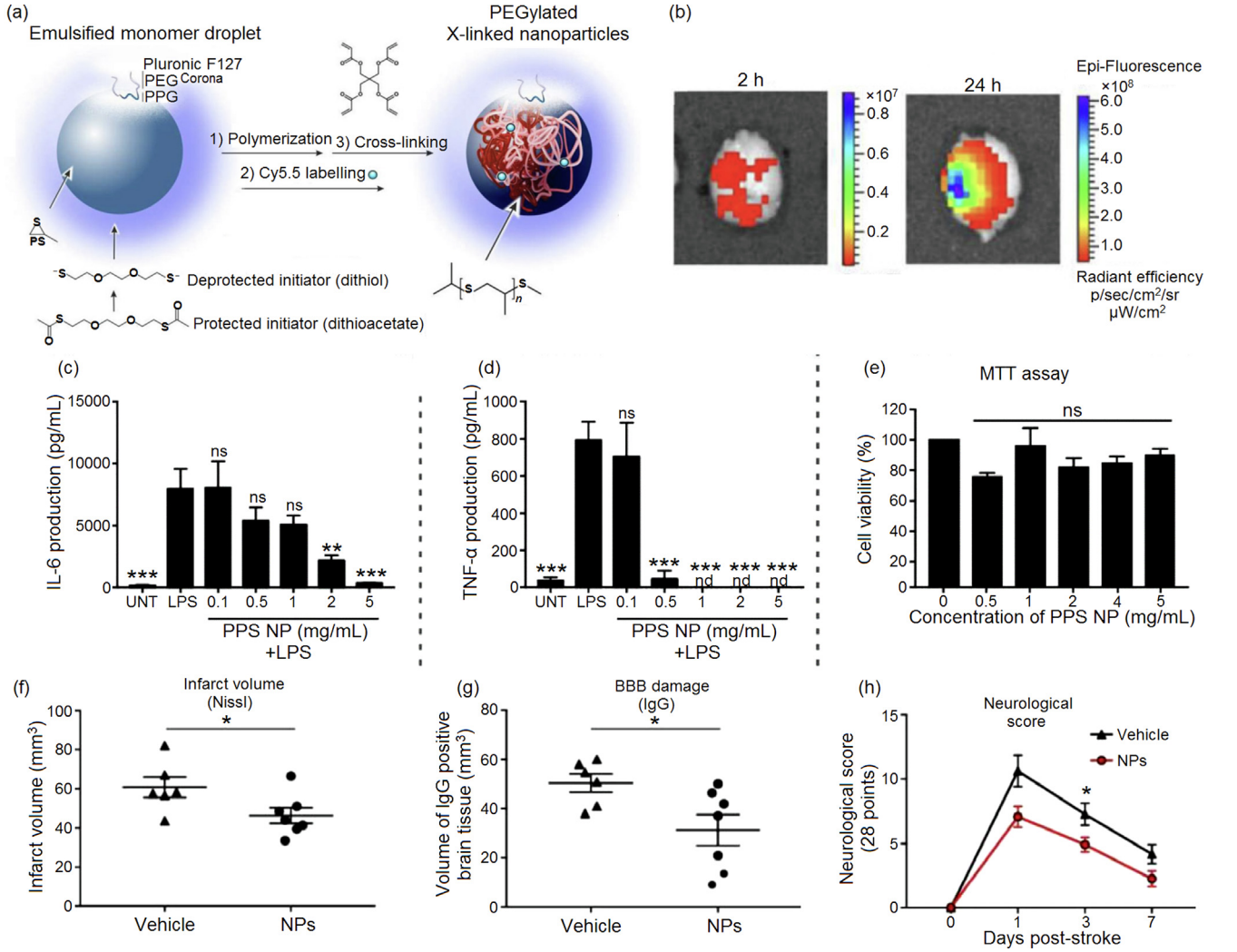

Fig. 17. (Color online) ROS-responsive poly(propylene sulfide) nanoparticle (PPS-NPs) for targeted delivery and controlled drug release to treat ischemic stroke. (a) Schematic drawing of the fabrication of PPS-NPs. (b) Representative *ex vivo* fluorescence images of enrichment of PPNs at the ischemic area. (c)–(e) PPN-NPs suppress secretion of pro-inflammatory cytokines by LPS challenged murine primary mixed glial cells without causing discernable cytotoxicity. (f)–(h) PPN-NPs treatment decreases infarct size and BBB damage, and improves the neurological score. Reprinted with permission from Ref. [153]. Copyright (2019) WILEY-VCH.

significantly mitigated the inflammation in the ischemic region and promoted recovery of neurological functions. This work indicates that stimuli-responsive nanoparticles can achieve targeted delivery in concert with therapeutic effects, and it will be interesting to further upgrade this system by incorporating imaging modules and/or synergistic agents.

4.3.2. DS-engineered stem cells for post stroke recovery

Post stroke recovery directly determines the outcomes of stroke patients, which depends on the protection and regeneration of neurons in the affected region. Conventional therapies are mostly targeting calcium channels or ROS to reduce the damage to neurons. These drugs demonstrate very limited efficacies in clinical practice, mainly due to their narrow therapeutic windows and insufficient targeting to the ischemic brain. In recent years stem cell therapy is emerging as a revolutionary approach for addressing the longstanding obstacle. By genetic engineering, stem cells can express the desired homing receptors and a wide range of neurotropic factors, making them super-carriers. Among different stem cells, mesenchymal stem cells are much preferred because they are abundant in adults and easy to extract. A major challenge in MSC engineering is the genetic transfection, which is currently based on liposomes or virus. However, the application of these methodologies is dimmed by the low transfection efficiency or safety concerns.

To solve the problems, our group designed a magnetosome-like 1D ferrimagnetic iron oxide nanochains (MFIONs) as a non-viral and magnetic field-independent gene transfection approach for MSCs-based therapy (Fig. 18) [154]. The MFIONs self-assembled from ferrimagnetic iron oxide nanocubes (FIONs) via permanent magnetic dipole interactions, and the pDNA was then combined with MFIONs through electrostatic interactions with the assistance of PEI. The assembled MFIONs could upregulate the expression of homing-related chemokine receptor CXCR4, which facilitated targeting to the ischemic brain. Importantly, they effectively delivered genetic materials into MSCs without the need of an external magnetic field. Furthermore, the ferromagnetic nanocubes exhibited high r2 relativity that allowed sensitive and non-invasive monitoring of the engineered MSCs via MRI. These advantages of MFIONs synergistically enhanced the therapeutic performance of engineered MSCs, which significantly promoted the post-stroke recovery in a mouse ischemic stroke model.

5. Concluding remarks and perspectives

As the global population is growing old, many ARDs that our ancestors did not evolve to adapt to are becoming major challenges to modern society, creating great public concerns that are further fueled by the limited progress made in effective treatment, especially for those featuring high incidence of morbidity and mortality, which are represented by cancer, neurodegenerative diseases, and stroke. The ARDs are extremely difficult to tackle in many aspects including the “fragile” patients they affect, the poor early diagnosis, the complicated microenvironment, and the multifactorial pathogenesis, etc. Dynamic nanoparticle assemblies (dynamic supraparticles) are complexs of two or more nanoparticles that are reversibly bonded. Based on the plenty sources of nanoparticles, ligands, and their diverse ways of combination, DS can be designed to be highly intelligent with self-adaptive capabilities. Here we may see nanoparticles as artificial atoms and the ligands as chemical bonds formed between the atoms. In this scenario, the fabrication of nanomolecular DS is analogous to designing artificial small molecules of desired properties by combining appropriate atoms with the right chemical bonds. Hopefully, smart DS may hold the potential to change the status quo of ARDs management by tackling dynamic diseases with dynamic drugs. which may complete all missions in one dose, including controlled drug release, multistage operation, multitarget therapy, ultrasensitive imaging, real-time therapy monitoring and so on.

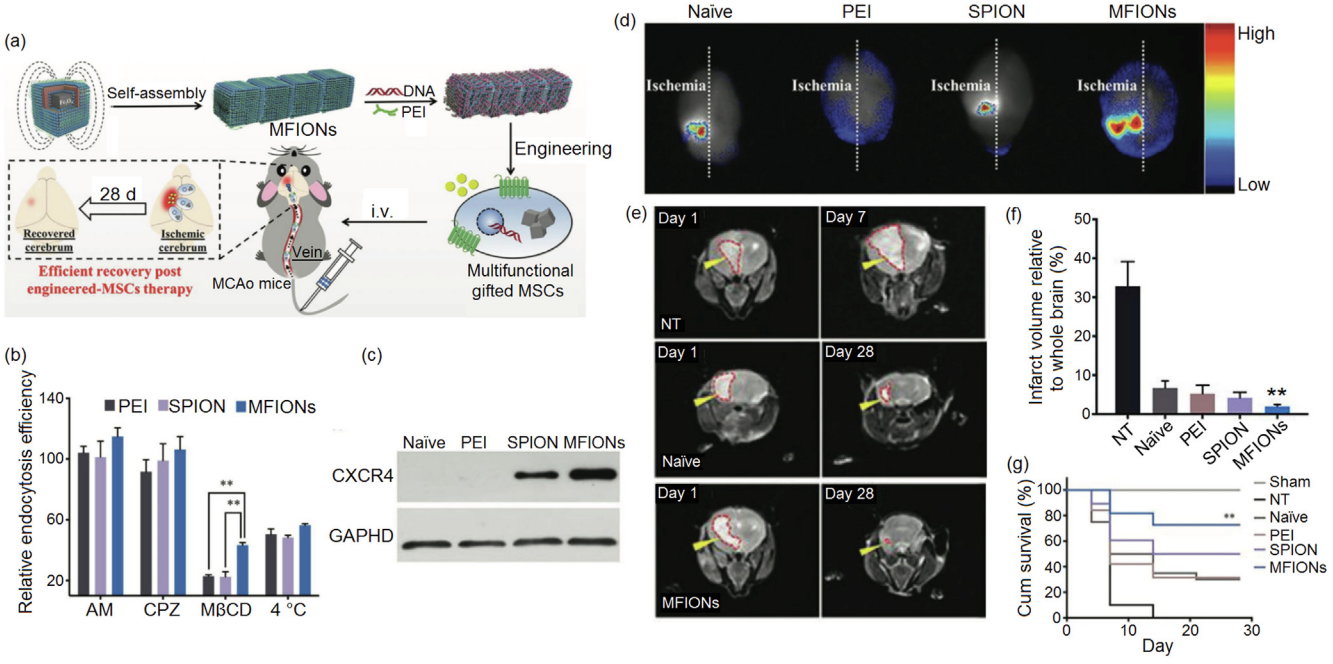

Fig. 18. (Color online) Ferrimagnetic iron oxide nanochains (MFIONs) for stem cell engineering to promote ischemic stroke recovery. (a) Schematic diagram of MFIONs-mediated MSC engineering for post-stroke recovery. (b) Cellular internalization efficiency of MFIONs upon treatment with inhibitors for different endocytic pathways (c) CXCR4 expression of MSCs after indicated treatments. (d) Fluorescent imaging of the GFP-expressing MSCs engineered by indicated methods after systemic administration. (e) MRI signals of the ischemic cerebrum after systemic administration of MFION-engineered MSCs. (f) Relative infarct volume after treatment with differently engineered MSCs. (g) Kaplan-Meier survival curves of ischemic stroke mice with various treatments. Reprinted with permission from Ref. [154]. Copyright (2019) WILEY-VCH.

Nevertheless, with all the advantages that DS may provide, it will still be a long-term shot to overcome ARDs. In fact, current DS fabricated are only prototypes containing limited functional modules to partially address the challenges in ARDs, the evolution of DS to a more powerful level and their translation are still facing several major hurdles.

First, the mechanisms governing the properties of obtained DS are largely unknown, which renders it difficult to implement stringent control over the synthesis process, leading to ununiform compositions and sizes of the product, as well as batch-to-batch variations. In fact, current fabrication methodologies are mostly based on empirical knowledge, as a result, it is rather challenging to truly acquire DS by design, and integration of new components and functional modules will require a lot of tentative labor. One promising solution to this problem is to resort to microfluidics. In sophisticatedly designed microfluidic devices, experimental setups and conditions can be more accurately controlled, leading to high reproducibility and minimal product variations. Importantly, by parallelizing reactions in the microfluidic devices, microfluidic approaches can achieve high throughput synthesis [155,156]. In addition, because the complexity of the DS in configurations and interparticle interactions, the long-term stability of DS is another concern that needs to be tackled. It will be necessary for future studies to gain a mechanistic insight into the key parameters that determine the quality of the output DS, and to establish close interdiscipline collaborations, such that scale-up synthesis can be possible.

Second, the nano-bio interactions are complex. Currently, the responsive model is mainly built on the direct interaction between the designed DS and the simplified disease microenvironment. However, the DS administrated into the body will take a much more tortuous path to finally arrive at the target disease sites. They will inevitably encounter numerous biomolecules, especially serum proteins that form a corona coating the nanoparticles, which may cause deviations of the actual performance of the designed DS. Moreover, immune cells, particularly macrophages, are very active in handling nanoparticles, whether they tackle different components of the DS (especially *in situ* assembled DS) with bias and how they affect the overall biodistribution further add to the complexity of their in-body behaviors. Finally, when the DS arrive at the disease microenvironment or enter the target cells, whether they will act exactly the same as they do in much more controlled laboratory conditions is also unclear, especially when it is difficult to trace all the functional blocks of the DS for quantitative validation. All these factors render it thorny to establish a model to predict the behaviors of DS in body, new methodologies that fit the evaluation of DS are urgently needed.

Third, the safety concerns. An important caveat in the current toxicity evaluation approaches for DS is that only acute adversary effects are recorded while long-term and chronic toxicity are usually ignored, for example, whether DS or their constitutive units retained in the body can cause chronic inflammation. Even for the acute toxicity examination, only major organs are paid attention to, such as brains, hearts, and livers, but apparently nanoparticles can end up in other tissues such as connective tissues that spread across the body. Moreover, toxicities are normally examined at the histological level, while cellular level retention is constantly ignored, which is of particular importance to macrophages that are indeed taking up something they are unable to break down as natural foreign substances. This is especially a concern for DS encompassing inorganic materials.

Fourth, the disease relevance of current animal models is in doubt. For example, AD and cancer primarily occur at old age, however, most animal models of AD and cancer, if not all, are established on young animals, which are clearly not the case in clinical settings. In fact, this discrepancy may partly explain the plenty of failures in clinical trials testing new drugs that work effectively in preclinical models, and may also lead to similar disasters for DS provided they reach to clinical trials for patients that are mainly elderly. The commonly used single factor-induced animal models also underestimated the multifactorial pathological of ARDs, underlying the inconsistence between preclinical and clinical studies. Moreover, it will never be too prudent to consider the heterogeneity among patients and within the diseased sites, which is not inspected in current homogenic animal models.

To conclude, smart DS are advantages over conventional medicine in many aspects, and represent a new frontier that may turn the tide in our battle against ARDs. Since the final purpose of the development of any drug is to benefit the human patients, future studies will have to face and address all the remaining problems to push this field from proof-of-principle stage to practical applications. To fully exploit the potential of DS, we encourage future researchers to look deeper into the mechanisms guaranteeing controlled chemical synthesis of DS, and into the very initiative pathogenesis of those ARDs, such that we can envisage super smart DS like "magic bullets" and "autonomous nanorobots" that can patrol our body and serve as the rapid reaction force to eliminate the threat in the first place, which may eventually revolutionize medical care of ARDs and all other human diseases by shifting from therapy to prevention.

Conflict of interest

The authors declare that they have no conflict of interests.

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Author contributions

Daishun Ling and Fangyuan Li designed, instructed, and polished the manuscript. Hongwei Liao, Zeyu Liang, Nan Wang, Min Wei and Ying Chen wrote the manuscript. All authors edited it.

Appendix A. Supplementary data

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