Recent advances in stimuli-responsive theranostic systems with aggregation-induced emission characteristics

Jing-Jing Hu  |  Wenlian Jiang  |  Lizhen Yuan  |  Chong Duan  |  Qiming Yuan  |  Zi Long  |  Xiaoding Lou  |  Fan Xia

Engineering Research Center of Nano-Geomaterials of Ministry of Education, Faculty of Materials Science and Chemistry, China University of Geosciences, Wuhan, China

Correspondence Xiaoding Lou, Engineering Research Center of Nano-Geomaterials of Ministry of Education, Faculty of Materials Science and Chemistry, China University of Geosciences, Wuhan 430074, China. Email: louxiaoding@cug.edu.cn

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Abstract

Theranostic systems by integrating the tumor imaging and tumor therapeutic capabilities into one platform have attracted numerous attentions from worldwide researchers. Despite the great developments, their clinical application is still in the nascent stage, owing to the unsatisfied imaging quality and limited therapeutic efficacy. Fortunately, the emerging of aggregation-induced emission (AIE) molecules with unique fluorescence property offers an opportunity to solve the imaging problem. Besides, further utilizing the tumor microenvironments and external triggers to design the stimuli-responsive imaging-guided therapy could enhance the therapeutic efficacy and reduce the side effects. In this review, the advancements in stimuli-responsive theranostic systems with AIE characteristics are summarized. Theranostic systems are first classified according to their treatment modes, and then subdivided based on various stimuli, including pH, redox, enzyme, and light. In each section, the design strategies and application examples are introduced. At last, the current state of the art, limitations, as well as prospects are also discussed.

KEYWORDS

aggregation induced emission, imaging, stimuli-responsive, theranostics, tumor therapy

1 | INTRODUCTION

In the clinical term, from the discovery of disease to the recovery of health, diagnosis and treatment are two relatively independent processes, leading to the separate utilization of diagnostic contrast agents and therapeutic agents. In this circumstance, the long time interval between the two treatments will make it easy to miss the optimal treatment time. Besides, the side effects by the two injections will also increase the pain and risk of patients suffered. Theranostics, which integrates imaging and tumor therapy into one system, is able to offer early personalized diagnosis and subsequently precise treatment to enhance the therapeutic efficiency and reduce adverse effects.[1–3] Despite that scientists have been devoted into understanding the mechanisms of tumorigenesis, preventing the growth of tumor, and developing effective diagnosis and treatment methods for several decades, the increasing number of new cases and high mortality rate of tumor are still great challenges that should be faced.[4] The rapid proliferation of tumor cells, the change of metabolic pathway, and the uneven distribution of blood vessels result in the unique tumor microenvironment, such as being weakly acid, overexpressing some particular enzymes, presenting high oxidative stress, as well as developing antioxidant systems.[5,6] These factors could not only further induce the development and metastasis of tumor, but also seriously reduce the response of tumor tissue to conventional treatment. Therefore, reasonably utilizing the salient features in tumor microenvironment to exploit new tumor theranostic methods with safety and high efficiency has become a hot issue.

In recent years, the continuous development of biomedical field provides new ideas and possibilities for the construction of new diagnosis and treatment systems.[7,8] Based on the abundant physical and chemical properties of different materials, researchers have designed a series of theranostic systems combining the imaging agents and therapeutic agents (e.g., chemo-drugs, photosensitizers [PSs], siRNA) in a single formulation with good response to tumor microenvironment to achieve cancer detection, diagnosis, and treatment.[9,10] These theranostic systems can be small molecules, peptide or prodrug-based bioprobes, as well as imaging reagents with nanoscale drug delivery systems (DDSs). Compared to traditional diagnostics and medicines, these smart stimuli-responsive theranostic systems have their...
own advantages. For example, small molecules, peptide, or prodrug-based bioprobes have the exact structure before and after the reaction, which is easier to precisely assess the separate functions of each part and has a better clinical application prospect. As the imaging reagents contained DDSs, they could exhibit longer blood circulation, passive or active tumor tissue accumulation ability, controlled drug release or activation ability, and remarkably enhanced imaging quality, thus improving the accuracy of tumor diagnosis, the selectivity, and efficiency of treatment.

Among the existing imaging modalities, fluorescence imaging technique is appealing owing to its low cost, excellent resolution, simple operation, high safety, and superb sensitivity.\[11,12\] In comparison with the fluorescent inorganic materials (e.g., quantum dot), organic fluorescent dyes with obvious advantages, such as biodegradability, great biocompatibility, easy modification, as well as multifunctional possibility, have been widely applied in biomedical fields.\[13\] Physical encapsulating or covalent conjugating fluorescent agents is a straight strategy to construct fluorescent imaging-guided therapeutic systems.\[14\] These systems offer an opportunity to real-time, in situ, and noninvasive monitor biological processes in cells or organisms, such as tracking drug release, tracing the processes of translocation, monitoring the excretion of therapeutic agents, and predicting treatment responses.\[15–37\] However, conventional organic fluorescent molecules may suffer from obvious photobleaching and severe background interference. What is worse, when they aggregate because of the high concentration or hydrophobicity, their fluorescence would be quenched dramatically, known as the aggregation caused quenching (ACQ) effect, which greatly decreases signal-to-noise ratio and results in the disappointing bioimaging performance.\[18,19\]

Fortunately, in 2001, Tang et al. have reported a new class of fluorogens with aggregation-induced emission (AIEgens) characteristics. To be specific, different from conventional organic fluorescent molecules, AIEgens are weakly or nonfluorescent in solution but can emit remarkable fluorescence in the aggregate state because of the restriction of intramolecular motions, which could circumvent the ACQ phenomena.\[20,21\] Besides, AIEgens generally exhibit large Stokes shift, outstanding photostability, and great sensitivity, which ensures the application prospect of AIEgens in biosensing and bioimaging fields.\[22–24\] Based on the superiority of AIEgens, AIE molecules demonstrate high potential in constructing activatable “turn on” theranostic systems in vitro and in vivo, which would achieve the aim of monitoring of intracellular biological processes in real time.\[25–29\] In this circumstance, it will be really interesting to construct stimuli-responsive AIEgens-based theranostic systems for imaging-guided tumor therapy.

In this review, the recent research advances in stimuli-responsive AIEgen-based theranostic systems including small molecules, peptide or prodrug based bioprobes, and nanomaterials for bioimaging and therapy were summarized. According to the different treatment modes, theranostic systems were classified into chemotherapy, photodynamic therapy (PDT), gene therapy, surgical therapy, as well as combination therapy. In each section, the design strategy and application examples in vitro and in vivo were introduced on the basis of different internal and external stimuli (e.g., pH, redox, enzyme, light, and reactive oxygen species [ROS]). Finally, the current state-of-art, limitations, as well as perspective were also discussed. We aimed to outline the strategies and advantages of theranostic systems, and then provided outlook to design promising theranostic systems with AIE features in the future. We hope this review can inspire new ideas and boost incessant developments in this burgeoning field.

## 2. CHEMOTHERAPY-BASED THERANOSTIC SYSTEMS

Nowadays, despite the various emerging therapeutic modes, chemotherapy using anticancer drugs to kill the tumor cells is still the most common and the first-line treatment owing to their significant therapeutic efficiency.\[30\] Nevertheless, the short blood circulation time, nonspecific biodistribution, as well as the severe adverse effects on normal tissues still limit the developments of chemotherapy. To further optimize the therapeutic efficiency of chemotherapeutic drugs and relieve the pain of patients, researchers have tried to develop lots of smart DDSs.\[31–33\] Ideal and sophisticated DDSs should be equipped with the following capabilities. First, they can protect the drug molecules, enhance the drug stability, and prolong the blood circulation time. Then, they can be sensitive to the diverse stimuli to avoid unwanted drug leakage and realize on demand drug release at the desired site. What is more, to reveal the endocytosis or phagocytosis behaviors of DDS as well as the drug release destination, they would be able to real-time track the delivery process of drug. Thus, stimuli-responsive DDSs containing AIEgens can be a desirable strategy. In the past decades, various stimuli have been employed, including external stimuli (light, magnetic field, ultrasound, and temperature) and tumor unique characteristics (internal stimuli) (acid, redox, enzyme, hypoxia, etc.).\[34,35\]

Taking the common nanomicelle as a typical example, because of the hydrophobicity of AIEgens, it is easier to construct an amphipathic molecule and then form a nanocarrier with drug loading ability and real-time monitoring ability. After the particular stimulus triggering the structure transformation of the amphipathic molecule, the nanomicelle swell or disassemble, leading to the drug release. In this section, the produgs or DDSs for imaging-guided chemotherapy would be introduced according to the different stimuli types.

### 2.1 pH-Responsive

pH gradient has been recognized as one of the most representative characteristics of tumor location.\[36–38\] Being different from the normal tissues, tumor tissues are more inclined to conduct aerobic glycolysis to turn the glucose into lactate, which is the well-known Warburg effect. The accumulated lactic acid through the secretion of tumor cells results in the mildly acidic tumor microenvironment (pH 6.8), which presents a difference in acidity to the normal tissues (pH 7.2–7.4). Besides, after the internalization of materials by tumor cells, a rapid endosomal acidification occurs because of the vacuolar proton ATPase-mediated proton influx, eventually inducing the late endosomes and lysosomes are with pH < 6.0. Therefore, taking the advantages of the pH gradient between physiological environment,
FIGURE 1  Schematic representation of the pH-responsive imaging guided chemotherapy based on the self-assembly of AIEgens-conjugated polymers. By inserting the hydrazone bonds, the drug-loaded nanoparticles could achieve pH-triggered theranostics.

By inserting the hydrazone bonds, the drug-loaded nanoparticles could achieve pH-triggered theranostics of the low pH in lysosomes. Eventually, the blue signal of TPE distributed in cytoplasm and the red fluorescence of DOX was observed in cell nucleus, where it executed anticancer function. Thanks to the switch on and off of two fluorescent signals, it is easier to monitor the process of cellular uptake, pH-responsive drug release, as well as the final subcellular locations of drugs and carriers. Following this work, the same group has constructed a similar self-indicating nanoprodrug (THyD NPs) with TPE and DOX through the acid-labile hydrazone bond, which is broken up in lysosome acid environments. By reason of the different interactions between TPE and DOX, THyD NPs demonstrated a slower, sustained release of the drug in comparison to the above TD NPs. However, limited by the short excitation and emission wavelength in these systems, it is hard to realize the ideal self-indicating effect in in vivo, considering the light penetration ability and the location of deep tumor tissues.

As mentioned above, hydrophobic AIEgens can not only possess remarkable fluorescent signal when aggregate to monitor the location of nanocarriers but also be grafted to hydrophilic polymers through sensitive linkers to form micelles via self-assembly to encapsulate therapeutic drugs and achieve drugs release at the desired site (Figure 1). For instance, in 2015, Cheng’s group has simply conjugated TPE to amphiphilic polymer (MPEG) through the hydrazone bonds to self-assemble into micelles (MPEG-hyd-TPE) for bioimaging and cancer treatment. Then, the polymeric micelles were used to encapsulate hydrophobic DOX. The results demonstrated that TPE was easily cleaved from polymer under endosomal pH (5.0), resulting in the burst of the low pH in lysosomes. Eventually, the blue signal of TPE distributed in cytoplasm and the red fluorescence of DOX was observed in cell nucleus, where it executed anticancer function. Thanks to the switch on and off of two fluorescent signals, it is easier to monitor the process of cellular uptake, pH-responsive drug release, as well as the final subcellular locations of drugs and carriers. Following this work, the same group has constructed a similar self-indicating nanoprodrug (THyD NPs) with TPE and DOX through the acid-labile hydrazone bond, which is broken up in lysosome acid environments. By reason of the different interactions between TPE and DOX, THyD NPs demonstrated a slower, sustained release of the drug in comparison to the above TD NPs. However, limited by the short excitation and emission wavelength in these systems, it is hard to realize the ideal self-indicating effect in in vivo, considering the light penetration ability and the location of deep tumor tissues.

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release of DOX for chemotherapy. At the same time, the strong blue fluorescence of TPE molecules aggregates has been used to directly observe the intracellular distribution of the nanocarriers. Similarly, TPE was also conjugated to other hydrophilic polymers, like dextran, [46] zwitterionic copolymer (poly(MPC-co-FPEMA)), [47] tadpole-shaped polymers PEG-POSS [48] through pH-cleavable hydrazone bonds to monitor intracellular drug delivery. Despite developments of lots of pH-responsive polymeric micelles, the choice of acid-labile bonds is still restricted to hydrazone bonds considering the pH variation in tumor cells. Moreover, the advantages of different hydrophilic polymers seem not that clear.

Apart from inserting acid-cleavable linkers to build micelles, there are several other pH-responsive examples that could also achieve the same goal. For example, Hu et al. reported dual-mode imaging theranostic nanovectors by synthesizing an azide-terminated diblock copolymer and further functionalizing the DOTA(Gd) or benzaldehyde. [49] Then, tetrakis[4-(2-mercaptoethyl)phenyl]ethylene (TPE-4SH) was used to conduct cross-linking reactions with GMA residues in the diblock copolymer to co-assemble into a micelle and at the same time, the TPE fluorescence could be turned on attributing to the restriction of intramolecular rotation. The surface-modified pH-LiP peptides could form an α-helical structure to enhance target ability under mildly acidic conditions. Moreover, the loaded anticancer hydrophobic drug camptothecin (CPT) can be released under weakly acidic pH because of the protonation of diisopropylamino (DPA) parts inducing the swelling of the micelles. Yu et al. utilized the high drug loading capacity and pH-dependent drug release of hollow mesoporous silica nanospheres and further anchored the 1,2-bis[4-(bromomethyl)-phenyl]-1,2-diphenylethene to achieve the simultaneously “on demand” drug release and cell imaging. [50]

### 2.2 Reduction-responsive

Owing to the metabolic aberrations, genetic and microenvironment-related alterations, tumor cells usually exhibit relatively high ROS level compared to normal cells, which could result in severe oxidative damage [51,52]. To alleviate the aggressive attacks from ROS, tumor cells usually evolve antioxidant system. [53,54] Hence, taking advantages of the intracellular reduction substances and reduction-sensitive linkers are a common tactic to achieve redox-triggered imaging-guided chemotherapy or controllable drug release (Figure 2A).

As an example, Pt(IV) prodrugs, which can be activated by intracellular reduction to cisplatin (Pt(II) complex) can be both used as a redox-responsive linker and an anticancer drug after activation (Figure 2B). For example, Liu et al. have conjugated a tetrabenzylenethene pyridinium (PyTPE) unit and a peptide sequence with hydrophilic part and targetting cyclic tripeptide part to the two sides of Pt(IV) prodrugs (PyTPE-Pt-D5-cRGD). [55] After being uptaken by the tumor cells, the Pt(IV) prodrugs respond to the intracellular reduction and be activated. In the meantime, owing to the departure of hydrophilic peptide, the nonfluorescent PyTPE with AIE features aggregates and emits a bright fluorescence. The “turn on” fluorescence could indicate the prodrug activation in turn. The theranostic system can track the delivery process of Pt(IV) prodrugs and real-timely monitor the Pt(II) drug activation in situ. Following this work, the same group further modify the structure to track the dual drugs (DOX and cisplatin) as shown in Figure 2B. [56] The theranostic system (cRGD-TPE-Pt-DOX) was composed of a targeting peptide, a TPE unit, DOX, and a Pt(IV) prodrug as the linker. Initially, the fluorescence of TPE was quenched because of the energy transfer to DOX as mentioned in Section 2.1. At this point, the red fluorescence of DOX was used to track the theranostic system. After the redox stimulating, the released DOX and activated cisplatin led to the aggregation of TPE, the blue fluorescence of which was recovered and indicated the dual drugs activation. The co-administration of dual drug displayed a synergistic anticancer effect.

It is found that the glutathione (GSH) concentration was 2–10 mM in the cytosol, which is almost 1000 times higher than that in the extracellular environment (2-10 μM). [57]-[59] Another widely used redox-sensitive linker is the disulfide bond, which could be cleaved in the GSH-rich cytoplasm through the thiol-disulfide exchange reaction (Figure 2C). Just like pH-sensitive systems, researchers have also conjugated TPE to amphiphilic polymer through disulfide bonds to self-assemble into micelles. [60,61] To achieve on-demand drug delivery and in situ evaluation of drug release, Zhao et al. covalently conjugated the chemotherapeutic drug curcumin (Cur) to a block copolymer through a disulfide bond and chemically linked TPE to the polymer through an amide bond. [62] The TPE and Cur can also serve as an energy transfer pair. Upon the supplement of GSH, disulfide bonds were cut, leading to the released Cur, the disassembled micelle, as well as the enhancement of both TPE and Cur fluorescence. Taking advantages of united fluorescence resonance energy transfer (FRET) and AIE, they have designed redox-responsive micelles to probe intracellular Cur release in situ.

### 2.3 Enzyme-responsive

Along with the occurrence and development of the diseases, some particular enzymes would be overexpressed in tumor tissues and cells. [63,64] For instance, matrix metalloproteinases (MMPs) can degrade the extracellular matrix and play a crucial role in the tumor progression, angiogenesis, or metastasis. In the past decades, researchers have discovered several MMP-responsive peptide sequences (PLGVRG, PLGLAG, etc.) that could be cleaved efficiently by the enzyme. [65-68] Thus, designing enzyme-responsive theranostic systems by bringing in peptide substrates as the linkers between AIE molecules and other functional parts is a fascinating strategy.

In 2016, our group has designed an efficient cell-permeable and MMP-2 enzyme-responsive prodrug (DOX-FCPPs-PyTPE, DFP) as shown in Figure 3A. [69] MMP-2 responding peptide sequence was used as linker to the arginine-rich motif modified PyTPE and DOX. Without MMP-2, only the fluorescence from DOX in highly water-soluble DFP can be observed. After being cleaved by extracellular enzyme, DFP was divided into two parts. The cell penetrating peptides conjugated with DOX could rapidly enter the tumor cells and exhibit their therapeutic effect. The residues with PyTPE would aggregate via hydrophobic interaction and emit strong yellow fluorescence, which would enable the
real-timely tracking of the drug delivery procedure in living cells.

As reported, cathepsin B (CB) in premalignant lesions and malignant tumors is usually overexpressed and secreted out of cells.\[^{70,71}\] The GFLG peptide sequence could be selectively cleaved by CB.\[^{72,73}\] To overcome one of the biggest problems in chemotherapy—tumor multidrug resistance (MDR), Zhang et al. have synthesized a transformable chimeric peptide that can self-assemble on tumor cell membrane and encapsulate cells (Figure 3B).\[^{74}\] The transformable chimeric peptide (CTGP) was composed of cell membrane targeted 16-carbon alkyl chain, TPE as fluorescence imaging domain, GGGH sequence for hydrogen bonding interactions, CB-responsive sequence, as well as hydrophilic polyethylene glycol (PEG). CTGP can self-assemble into micelles and encapsulate DOX (CTGP@DOX). After being stimulated by CB, CTGP@DOX was dissociated. Because of the hydrogen bonding interactions and hydrophilic-hydrophobic conversion, the spherical nanoparticles transformed to nanofibers and adhered to the cell membrane for restricting DOX efflux and cell encapsulation. The TPE probe could track nanofibers in real time and monitor DOX release through the FRET between TPE and DOX.

### 2.4 | ROS-responsive

It has been reported that tumor cells and inflammatory cells have a higher level of H$_2$O$_2$ (5-1000 × 10$^{-6}$ M) than that in healthy cells (0.001-0.7 × 10$^{-6}$ M).\[^{53-77}\] In the same way,
employing the concentration difference to construct H$_2$O$_2$-sensitive benzil containing amphiphilic polymer, which could self-assemble into drug-loaded micelles, could achieve ROS-triggered theranostics.$^{[78]}$ Besides, in 2018, our group developed a ROS-responsive system, which could not only achieve selective imaging but also inhibit the growth of inflammatory cells.$^{[79]}$ Tyrosine-containing TPE (TT) fluorescent probe was composed of one TPE and two tyrosine moieties. TPE acted as an intermediate emission core, and two tyrosine moieties were connected to the both ends of the core. Due to the existence of tyrosine, this probe possessed good hydrophilicity and stability, resulting in weak fluorescence intensity in aqueous solution. Owing to the assistance from abnormal level of H$_2$O$_2$ and myeloperoxidase (MPO) in inflammatory cells, TT molecules cross-linked with each other to form TT oligomer along with the activation of AIE process. The results from confocal laser scanning microscopy (CLSM) and cell viability experiments proved that TT fluorescent probe could selectively reacted in inflammatory cells instead of normal cells due to the differentiation of intracellular H$_2$O$_2$ and MPO levels.

The produced singlet oxygen during photodynamic reactions could also be used to construct ROS-responsive theranostic systems.$^{[80-83]}$ Liu’s group developed a light-responsive micellar nanoparticle, which encapsulated anticancer drug DOX to overcome the drug resistance of cancer cells (denoted as AIE-NPs/DOX).$^{[84]}$ The micellar nanoparticles were self-assembled by amphiphilic polymer TPETP-TK-PEG, where an intermediate thioketal (TK) linker bonded PEG and an AIEgen PS (TPETP). Upon the white light irradiation, the produced ROS could damage the endo-/lysosomal membrane and cleave the TK linker, triggering drug release and enhancing DOX accumulation and retention in tumor cells. The effect of enhanced DOX accumulation and retention in cancer cells could overcome drug resistance in DOX-resistant MDA-MB-231 cancer cells.

2.5 | Multiple stimuli-responsive

Compared with single stimulus, dual or multiple stimuli-responsive theranostic systems have attracted widespread attention because of their higher sensitivity. Taking the complexity of tumor microenvironments into consideration, the design of carriers that can respond to two or more stimuli at the same time can make up the weakness of a single
environmental responsive system, achieving comprehensive and superior imaging and treatment of diseases.

In 2015, Ji et al. have reported an enzyme and redox dual responsive gemcitabine (GEM) produg (TPE-GEM-RGD) for targeted imaging-guided chemotherapy. As shown in Figure 4A, the TPE-GEM-RGD theranostic system contained following several parts: (a) a hydrophilic peptide (five ASP, D5) to increase the hydrophilicity of system and a targeting peptide (RGD); (b) GEM, which was linked to peptide through GSH-responsive disulfide bonds; (c) TPE, which was linked to D5 through the CB-responsive GFLG peptide sequence. After entering the tumor cells, the high concentration of GSH led the release of GEM for selective killing of pancreatic cancer cells. At the same time, overexpressed CB cleaved GFLG peptide, resulting in the TPE aggregation and the light up of intracellular fluorescence. In Section 2.2, Liu et al. have proposed the reduction-responsive imaging-guided cisplatin therapy. These theranostic systems were able to utilize the “turn on” AIE fluorescence to indicate the cisplatin location and activation, but they are not able to quantify the therapeutic efficacy in situ. To further noninvasively estimate the treatment outcome in situ, Liu and co-workers have adopted the activation of caspase-3 during drug-induced apoptosis pathway to specifically cleave DEVD peptide sequence and incorporate the apoptosis sensor into the redox-responsive Pt(IV) prodrug systems. In brief, they functionalized two axial positions of Pt(IV) produg with a targeting peptide (cRGD) and an apoptosis sensor that is composed of AIE molecules (tetraphenylsilole [TPS] fluorophore) and a DEVD peptide. The theranostic system is water soluble and nonfluorescent before entering the tumor cells. After the Pt(IV) produg was reduced intracellularly, the Pt(II) drug can induce the cell apoptosis and activate caspase-3 enzyme to cleave the DEVD peptide sequence. Then, the hydrophobic TPS residue aggregated, leading to the restriction of intramolecular rotations of the phenyl rings and the fluorescence enhancement ultimately. Similarly, Wang and co-workers have conjugated the TPE molecule through a DEVD peptide sequence to the surface of drug-loaded nanomicelle (STD-NM) to achieve pH-triggered tumor targeting, efficient drug delivery, as well as enzyme-responsive precise evaluation of treatment efficacy. As shown in Figure 4B, STD-NM20R exhibited a stronger fluorescence signals than other control groups. And 10 hours after injection, the tumor to normal tissue signal ratio could reach a maximum value in STD-NM20R group, demonstrating the effective tumor accumulation ability. It is worth mentioning, besides the in vitro data, the observed two-photon fluorescence signals (Ex: 720 nm, Em: 420–500 nm) in ex vivo imaging results proved that STD-NM could achieve the efficient therapy, caspase-3 generation, as well as the “turn on” fluorescence in the living systems.

In the majority of advancements as mentioned above, the AIE fluorescence of systems is responsive to stimuli and dynamic to achieve the imaging. Besides, the AIE molecules with excellent photostability are able to be used for long-term tracking of stimuli-responsive systems. Wang et al. have synthesized multifunctional polymeric micelles with AIE feature for tumor therapy and imaging. Acidic environment could trigger the charge-conversion and induce enhancement of cellular uptake amount, and then acid and redox dual lead to the DOX release. In vitro and in vivo experiments demonstrated a high-quality two-photon bioimaging by AIE active fluorophores. Recently, Li’s group has designed a dual redox/enzyme-responsive NO-releasing theranostic nanosystem (QM-NPQ@PDHNs), which utilized the unique anticancer effects of high concentration of NO to overcome the limitation of traditional chemotherapeutic drugs. QM-NPQ@PDHNs used the GSH-responsive hybrid shells to simultaneously encapsulate glutathione S-transferases \( \pi \) (GST\( \pi \))-selective produg (NPQ) for NO release and AIE fluorogen (QM-2) for monitoring its biodistribution. The biodegradation of GSH-responsive shells was able to release NPQ, which could subsequently generate NO in response to GST\( \pi \). Recently, Ding et al. have reported a supramolecular AIE nanodots for imaging-guided chemotherapy. The supramolecular AIE dots were constructed through the host-guest interaction between \( \alpha \)-cyclodextrins (\( \alpha \)-CD) and poly(ethylene glycol) (PEG). \( \alpha \)-CD was functionalized by anticancer drug GEM via a disulfide bond and AIE lumogen, separately. PEG was further linked with a designed peptide sequence (mPEG2000-R8-PLGLAG-EK6). This theranostic system could achieve the charge conversion for enhanced cell internalization under the overexpression of MMP-2 and subsequently GSH-responsive GEM release. The activation of far-red/near-infrared (FR/NIR) emissive AIE lumogen could enable the high-quality in vivo fluorescence imaging and prove the efficient accumulation of theranostic system in tumor tissues.

### 3 PDT-BASED THERANOSTIC SYSTEMS

PDT is using light with particular wavelength to excite PSs to generate cytotoxic ROS, especially singlet oxygen (\( ^1 \text{O}_2 \)) for suppressing tumor cells. Owing to the noninvasiveness, selectivity, and biocompatibility, PDT has attracted numerous attentions in the past decades. In contrast with traditional chemotherapeutic drugs, PSs themselves are non-toxic or minimal toxic. Only satisfied with PSs accumulation and light irradiation at the same time, the photodynamic reaction can occur, which endows PDT with high intrinsic selectivity. More importantly, the fluorescence of PSs has the opportunity to realize imaging-guided PDT. However, besides some familiar drawbacks, such as short excitation wavelength, restricted structural stability, and limited tissue accumulation, most PSs are hydrophobic and would suffer from the ACQ effect. The aggregation of PSs would induce the attenuated fluorescence and insufficient ROS generation, resulting in disappointing bioimaging quality and PDT effect. Inspired by the discovery of AIE phenomena, scientists have synthesized a series of PSs based on the AIE fluorogens, which could simultaneously serve as PSs and imaging agents when restricting their intramolecular rotation. The general design principles of an effective AIEgen-based PS could include three parts: choosing a proper molecular skeleton for D-A system, incorporating the strong donor and acceptor pairs to enhance the electron donating and accepting capability, and lengthen the HOMO-LUMO space by using the benzene ring as a linker. The principles could not only shift the fluorescence emission of AIEgen to the longer NIR wavelength region, but also provide singlet oxygen generation capability in aggregate state. Since the first
FIGURE 4 (A) Schematic representation of the multiple stimuli-responsive imaging guided chemotherapy based on the activable “light up” bioprobes. (B) Schematic representation of pH and caspase-3 triggered STD-NM transition from the “stealth” state to “activated” state and in vivo/ex vivo imaging of STD-NM. Reproduced with permission. Copyright 2018, Elsevier Ltd.[87]
two AIEgen-based PSs (TPE-red and TTD) were separately reported by Zhang’s group and Liu’s group in 2014, the developments of AIEgen-based PSs have attracted tremendous attention worldwide. Making the best use of dynamic changeable physicochemical property of AIEgen-based PSs offers a chance to develop the activatable PSs, which could achieve the aim of minimized side effects for therapy and increased signal-to-background ratio for imaging. Moreover, since almost stimuli-relative information we have introduced in the above, in the following section, we will focus more on the design strategy of each treatments, particular examples, and introduction of other new stimuli.

3.1 | Enzyme-responsive

In 2015, Liu et al. have modified common TPE structure and constructed AIEgens with different substituents conjugated to the dicyanovinyl group, which exhibited enhanced fluorescence and phototoxicity in the aggregate state (Figure 5). Further, they utilized newly synthesized AIEgen-based PSs (TPECM) and the CB-cleavable peptide substrate (GFLG) to design an enzyme-responsive theranostic bio-probe for targeted light-up imaging and activatable PDT. Due to the free intramolecular motions, the bioprobe cannot emit remarkable fluorescence and generate sufficient singlet oxygen in aqueous media. After being uptaken by the \( \alpha_5\beta_3 \) integrin overexpressed tumor cells (MDA-MB-231), GFLG peptide sequence would be cut by CB, leading to the aggregation of TPECM for cell-specific light-up imaging and photodynamic suppression of tumor cells. Moreover, to visualize the whole therapy process and evaluate the therapeutic effect, the same group has designed a light-up probe (TPETP-SS-DEVD-TPS-cRGD), which consisted of two AIEgens (tetraphenylethenethiophene [TPETP], a red-emissive AIEgen with ROS generation ability, and TPS, a green-emissive AIEgen). High GSH concentration triggered the TPETP aggregates, leading to the red fluorescence on and cytotoxic singlet oxygen generation. Caspases produced during the cell apoptosis cleaved DEVE sequence, resulting in the aggregation of TPS and green fluorescence on eventually.

Alkaline phosphatase (ALP) is known as a tumor biomarker that is overexpressed on the cell membrane of many cancers, including kidney tumors, osteosarcoma, as well as cervical cancer. The overexpressed ALP can induce
the dephosphorylation of tyrosine phosphate (pY). Utilizing the characteristics of ALP, Ding et al. have designed a theranostic probe (TPE-Py-FpYGpYGpYG) to achieve sensitive fluorescence diagnosis and on-demand PDT, which was composed of an AIE molecule TPE-Py and a peptide sequence containing three pY.[103] Owing to the modification of phosphotyrosine residues, TPE-Py-FpYGpYGpYG exhibited great aqueous solubility, thus leading to nonfluorescence and weak ROS generation ability in physiological environments. After ALP enzymatic dephosphorylation, the loss of hydrophilic phosphates in probe would result in the self-assembly of probe and impede the rotation of phenyl rings, which would demonstrate extensive emission of fluorescence and efficient production of ROS. The β-galactosidase (β-Gal) as a significant biomarker for cell senescence is overexpressed in the senescent HeLa (s-HeLa) cells. To selective identification and ablation of senescent cancer cells, Yang and co-workers have designed a bioprobe (TPE-ETh-R-GFFY(gal)ERGD) by using an AIEgen-based PS (TPE-ETh-R) with FR/NIR fluorescence.[104] After interaction with β-Gal, the probe converted to TPE-ETh-R-GFFYERGD, which was observed as worm-like nanofibers with strong fluorescence and ROS producing ability. Under the white light illumination, the nanofibers produced ROS efficiently, thus killing s-HeLa cells. Results demonstrated that this strategy could selectively eliminate senescent cells and elevate the efficacy of tumor therapy. Liu et al. used the activation of caspase-1 during the bacterial infection process of macrophages to synthesize an AIEgen-peptide conjugate (PyTPE-CRP) by simply linking the caspase-1-responsive peptide to PyTPE, which could be used as phototheranostic agents for the detection and extermination of intracellular bacteria.[105]

For PDT, finding the appropriate light irradiation time and area is of great significance. To achieve imaging-guided precise PDT, Zhang’s group has designed a ratiometric fluorescence biosensor (TPPP), which was prepared by conjugating the PS protoporphyrin IX (PpIX) to the AIEgen TPE through an MMP-2-sensitive PEGylated PLGVR peptide.[106] TPPP could be cut by the overexpressed MMP-2 in tumor tissues, and then resulting in the detachment of TPE. The fluorescence ratio between TPE (blue fluorescence) and PpIX (red fluorescence) could estimate the MMP-2 concentration. The dual fluorescence imaging could accurately point out the tumor area, which guided the precise PDT with enhanced therapeutic efficacy.

3.2 | ROS-responsive

Apart from using the activation of caspase during apoptosis to estimate the therapeutic response of PDT, real-time monitoring the 1O2 generation is more direct but difficult owing to its short lifetime and limited radius. Liu et al. have developed a self-reporting probe by using a ROS-sensitive aminoacylinate (AA) linker to conjugate a red emissive AIEgen-based PS (TPETP) and a green emissive rhodol dye.[107] In the aqueous media, the probe could emit red fluorescence for self-tracking and generate ROS under light irradiation. Subsequently, the produced ROS cleaved the AA linkers, thus releasing the rhodol and turning on the green fluorescence, which could offer real-time and in situ signal of ROS production during photodynamic reaction.

The mentioned high content of endogenous H2O2 has been considered by Wang et al. to synthesize a H2O2 activatable fluorescent probe TPECNPB for imaging-guided PDT.[108] As shown in Figure 6, TPECNPB with a lipid droplet (LD) targeted pyrimidin pendant and a H2O2 activatable boronate ester, which attached to the pyrimidin pendant was designed for both monitoring in real time and photodynamic eradication of tumor cells. Due to the high expression of H2O2 in tumor cells and the high enrichment of LD, the amphiphilic TPECNPB can easily convert into hydrophobic TPECNP, and then aggregate to emit stark red fluorescence in LD. Moreover, the produced TPECNP can generate toxic 1O2 to trigger the cancer cell apoptosis under light conditions. Thus, the TPECNPB served as a powerful toolbox for H2O2-sensitive and LD-targetable imaging-guided PDT.

Different from traditional photoluminescence, bioluminescence or chemiluminescence imaging without external excitation source has deep tissue penetration ability and high signal-to-noise ratio in in vivo diagnosis. Based on this consideration, a good H2O2−triggered chemiluminescence imaging-guided PDT system was designed by Liu et al.[109] The AIE PSs TBD with FR/NIR emission and the bis[2,4,5-trichloro-6-(pentyloxycarbonyl)phenyl] oxalate (CPPO) were co-encapsulated in amphipathic pluronic F-127 and soybean oil to form C-TBD nanoparticles (C-TBD NPs). After CPPO-TBD was exposed to H2O2, 1,2-dioxetanediene intermediate was generated and directly excited TBD to produce cytotoxic 1O2 and emit FR/NIR fluorescence. Through the chemiluminescence imaging, C-TBD NPs could be used as a H2O2 probe to track tumor tissues precisely. Furthermore, subsequently chemiexcited 1O2 could induce apoptosis of tumor cells efficiently. The in vivo results indicated that C-TBD NPs could realize the specific chemiluminescence excited diagnosis and therapy.

3.3 | Other stimuli-responsive

Other small molecules can also play a role in the selective release of PSs, which can reduce the side effects of PSs on normal tissues by reducing the intrinsic photo-toxicity of PSs and increasing selectivity. In the initial state, the systems are without ROS generation ability and fluorescence imaging ability. After the stimuli triggering, the AIEgen-based PSs generated. For instance, Liu et al. designed a probe based on the GSH activatable PSs, which could selectively recognize, imaging, and kill the tumor cells (Figure 6).[110] TPETF-NQ-cRGD consisted of a GSH-active quencher moiety and ααββ integrin targeted cRGD. To begin with, TPETF-NQ-cRGD remained nonfluorescent and no ROS generation under light treatment, while after ααββ integrin receptor mediated endocytosis into the cell, the fluorescence and ROS generation ability could be switched on by the activation of GSH. Then, released TPETF with efficient ROS generation ability can be applied to imaging-guided PDT with high selectivity. In view of the fact that the disulfide bond is also a GSH-cleavable moiety, Kim et al. designed a GSH-activated probe (TPEPY-S-Fc) for imaging-guided PDT.[111] TPEPY-S-Fc consisted of a ferrocene as a quencher moiety and vinyl pyrimidin substituted tetraphenyl-ethylene (TPEPY) as AIE PS, which were linked by a disulfide bond. TPEPY-S-Fc initially showed nonfluorescence and...
PDT blocked through a photo-induced electron transfer mechanism. After adding GSH, the disulfide bond in TPEPY-S-Fc was cut to generate TPEPY-SH that induced red fluorescence enhancement and $^{1}\text{O}_2$ release under light irradiation, thereby leading to selective imaging-guided cell apoptosis.

Besides the monomolecular structure, nanoparticles could also realize the stimuli-responsive imaging-guided PDT. Tang and Liu et al. reported a pH-sensitive probe for multiple purpose imaging and therapy.\(^\text{[112]}\) The probe PLL-g-peg/DPA/TPS/PheA was prepared by AIEgen TPS, pheophorbide A (PheA), pH-sensitive DPA, and PEG block, which can be coupled to RGD targeting ligand. Under physiological conditions (pH 7.4), PheA in nanoparticles exhibited weak fluorescence and low phototoxicity, while TPS emitted remarkable green fluorescence for self-tracking.

After being taken up by tumor cells and trapped by lysosomes, PLL-g-peg/DPA/TPS/PheA was disassembled under acid activation to produce weak fluorescent TPS and release strong red fluorescent PheA. The phototoxicity was activated and restored, thereby destroying lysosomes and causing apoptosis under light. After that, the probe was leaked into the cytoplasm (pH 7.2), and the fluorescence of TPS was restored for in situ prediction and visualization of the therapeutic response. Recently, Liu et al. constructed a carrier-free hybrid nanosphere for image-guided enhanced PDT through Fe\(^{3+}\)-activated Fenton reaction and inhibition of Bcl-2 simultaneously overcoming ACQ, tumor hypoxia, as well as the intrinsic oxidative resistance.\(^\text{[113]}\) The nanosphere consisted of Fe\(^{3+}\) as Fenton reaction initiator, TPEDCC as an AIE PS, and sabutoclax as a Bcl-2 inhibitor. After the nanosphere was internalized by tumor cells, $\text{O}_2$ concentration...
in cells would increase through the Fenton reaction driving by Fe³⁺. Meanwhile, sabutoclax was released to inhibit the activity of Bcl-2 and decrease the inherent oxidative resistance through reducing the GSH concentration, eventually leading to the cytochrome c release.

Currently, the light source in most of above systems is still restricted to the white light, whose penetration depth in tissue is about a few hundreds of micrometers and out of “optical transparency window” of biological tissues. [94] Exploiting more AIEgens PSs that can be excited by NIR light would have a wider application. Besides, the ROS generation efficiency is another feature needed being optimized. There is still far to go to apply the PDT-based theranostic systems as biomaterials practically.

## 4 | GENE THERAPY-BASED THERANOSTIC SYSTEMS

Gene therapy by utilizing an effective vector to convey nucleic acid in targeted cells could modulate specific gene expression, or restore deficient production of a protein.[114] The gene delivery system should be developed to realize high transfection efficiency, simultaneously have low cytotoxicity. One of big challenge for gene delivery is that the common endocytosis process would cause the nucleic acids to be destructed owing to the abundance of enzymes in lysosomes. Thus, the equipment of endo-/lysosomal escape ability for gene delivery system is necessary. Beside, real-time and long-term tracking of targeted nucleic acid is beneficial to understand the detailed delivery and release processes, thus optimizing the treatment.

### 4.1 | Photo/ROS-responsive

To realize the endo-/lysosomal escape and controlled unpacking of gene, in 2015, Liu’s group reported a ROS-responsive polymer (P(TPECM-AA-OEI)-g-mPEG) based on AIE PSs for light-controlled gene delivery. As shown in Figure 7, under light irradiation, the production of ROS from PS could destroy the endo-/lysosomal membrane and break the polymers containing ROS-labile linkages, leading to light-triggered escape and gene release.[115] Similarly, Gao’s group has reported an oligoethyleneimine (OEI)-cross-linked polycation (OEI-SeSex-AIE) consisting of a functional AIE component and an ROS-sensitive diselenide linkers.[116] In addition, a core-shell nanostructure (AIE PS as core/Bcl-2 oligonucleotide [OSA] as shell) for light controlled gene delivery with endo-/lysosome escape capacity was developed by Liu’s group.[117] Under light irradiation, the core-shell nanostructure could generate sufficient amount of ²¹ O₂ to rupture the lysosome structure and let the nanostructure to escape, thus resulting in the released OSA to induce apoptosis of tumor cells.

### 4.2 | GSH-responsive

In 2017, a multifunctional system consisting of two TPE molecules as the hydrophobic parts and linear PEI-galactose as the hydrophilic part was synthesized.[118] The amphiphilic TPE-ss-IPEI-galactose could self-assemble to form micelles and be used for cell imaging. The disulfide bonds facilitated a higher transfection efficiency. Gene transfection results demonstrated that the disulfide bonds may promote DNA release with lower cytotoxicity and higher gene transfection efficiency in the presence of serum.

### 4.3 | Enzyme-responsive

Very recently, our group has reported a multifunctional peptide-conjugated AIEgens (T₁₂-NCP) as a targeted gene vector for real-time and long-term tracking, as well as efficient gene delivery.[119] The system contained four main segments: an integrin-targeted peptide, a cell-penetrating peptide, a nuclear localization signal peptide, and the hydrophobic PyTPE. The cellular uptake of the probe was observed by CLSM imaging of MDA-MB-231 cells. The yellow fluorescence of T₁₂-NCP was observed quickly at the cell membrane within 5 min and was sustained for 150 min, which could prove the superior cell imaging ability for real-time and long-term tracking. Moreover, the probe possessed a superior encapsulation capability of therapeutic genes.

Nowadays, two-photon fluorescence microscope imaging systems possessing lower autofluorescence and longer wavelength excitation are widely used in biomedical areas. He et al. modified benzylideneimidazolone (BI), which is a chromophore of green fluorescent protein analogue, with the popular unit for two-photon absorption triphenylamine (TPA) to synthesize TPA-BI as the hydrophobic moiety, and further connected hydrophilic tomacrocyclic polyamine[12]eneN₃ unit to construct the nonviral gene vectors with AIE features, large Stokes shifts, as well as large two-photon absorption cross sections.[120] Especially, the compound TPA-BIC could realize the pH- and lipase-triggered DNA release, two-photon fluorescent tracing DNA delivery, and effective transfection genes. The EGFP-encoding plasmid delivery process in zebrafish by TPA-BI-C/DOPE was conducted. The successful protein expression results in zebrafish indicated that TPA-BI-C/DOPE nanoparticles could successfully overcome the hindrances in the physiological environment and realize the gene transfection in vivo. Furthermore, a similar TDM-B/DOPE system was also successfully implemented in zebra fish embryos[121] and another gemini-type amphiphiles DEDPP-8/DOPE obtained excellent transfection efficiency.[122]

### 4.4 | Surgical therapy-based theranostic systems

Through systemic investigation and rational utilization of energy dissipation pathways of AIEgens after light irradiation, Tang and Ding et al. subtly designed a photo-switched AIEgen (DTE-TPECM), which consisted of a DTE core and two TPECM units for significant enhancement of tumor surgery outcomes.[123] On account of the closed-ring and open-ring isomers of TPECM units in DTE-TPECM, it could be reversibly switched via UV/visible light illumination between the ring-closing form for PA imaging with deep imaging depth and ring-opening form for fluorescence imaging with excellent sensitivity and ROS generation. After using
amphiphilic lipid-$\text{PEG}_{2000}$ as the doping matrix, the prepared nanoparticles could improve the efficacy of tumor surgery through the preoperative photoacoustic imaging and treat the residual tumors through the intraoperative fluorescent visualization imaging-guided PDT, thus ensuring total removal of tumor.

5 | COMBINATION THERAPY-BASED THERANOSTIC SYSTEMS

Combination therapy could enhance monotherapy outcomes at low therapeutic dosage, hence reducing side effects to normal tissues. Moreover, the combined tactics would solve some difficulties for monotherapy. Taking the common combination of chemotherapy and PDT as an example, chemotherapy could enhance the sensitivity of tumor cells to cytotoxic ROS generated during PDT. In turn, the ROS have the ability to reduce the activity of drug-efflux relative proteins, thus overcoming the multidrug resistance. Besides, the drug delivery efficiency could also be improved by PDT-induced “super-enhanced permeability and retention.” Herein, the theranostic systems with different combinations were introduced.

5.1 | GSH-responsive

Liu et al. designed a GSH-responsive dual-prodrug, TPEPY-S-MMC, with GSH activation for combinatorial PDT
and chemotherapy through a disulfide bond to conjugate an AIEgen (TPEPY) and chemo-prodrug (mitomycin C [MMC]) (Figure 8A). Initially, TPEPY-S-MMC exhibited no fluorescence and ROS generation ability because of the quenching effect of MMC. However, when GSH was present, the TPEPY-S-MMC system was activated, which could simultaneously release active TPEPY and MMC for AIEgens imaging-guided combined treatment.[124] Liu and co-workers developed a fluorescence turned-on platinum pro-drug TPECB-Pt-D5-cRGD via Pt(IV) prodrug conjugated with an AIE TPECB and a hydrophilic peptide D5-cRGD for combined PDT and chemotherapy against cisplatin-resistant cancer cells (Figure 8B). Thus, prepared prodrug system was fluorescence off, which could be activated by intracellular GSH, and the released AIE TPECB was simultaneously light-up for drug activation monitoring and cancer cell imaging.[125] Our group designed a GSH-responsive micelles TB@PMP via the polymeric prodrug PMP encapsulating an AIEgen TB with red emission for imaging-guided PDT and chemotherapy (Figure 8C). Due to the high content of GSH in tumor cells, after the TB@PMP was uptake, the disulfide bonds of PMP were cleaved to release the PTX for chemotherapy, while TB acted as imaging agents and produced cytotoxic ROS to induce cell death upon white light irradiation. Thus, fabricated co-delivery system exhibited synergistic enhancement effect for inhibiting the tumor growth when being compared to PDT or chemotherapy only.[126] Following this, Tang and his colleagues further used the same polymeric prodrug (PMP) to encapsulate an AIEgen (DEB) and a drug resistance inhibitor tariquidar (TQR) to eradicate multidrug resistance cancer. Due to the presence of disulfide bonds in PMP, when DEB/TQR@PMP micelles were taken up by cells and irradiated with white light, GSH triggered PTX, DBE and TQR release, and then DBE produced ROS for PDT, PTX served as chemotherapy.[127] Moreover, Li and co-workers encapsulated AIEgens (TTD) into cRGD-siVEGF-TTD NPs using biocompatible PEG-lipid as the encapsulation matrix, then modified with cRGD peptide and siVEGF. Thanks to the presence of disulfide bond, once cRGD-siVEGF-TTD NPs were internalized and irradiated with white light; it could produce ROS, while the elevated GSH in cytoplasm released siVEGF at the same time, achieving a synergistic effect with combination of imaging-guided PDT and siRNA therapy to enhance the total treatment outcomes.[128] Our group reported the MnO2-DNAzyme-TB nanocomposite (MDT) for imaging and PDT and gene combination therapy through GSH-responsive MnO2 nanosheets co-loading AIEgen TB and DNAzymes. TB and DNAzyme would release when MDT was internalized into the tumor cells. Meanwhile, GSH decomposed MnO2 into Mn^{2+} that acted as a cofactor of DNAzyme, which made gene knockdown possible. Furthermore, TB could produce ROS to kill tumor cells with white light illumination. Thus, imaging-guided photodynamic
combined with gene therapy would significantly improve the anti-tumor efficiency.\textsuperscript{[129]}

5.2 | Photo-responsive

Singh et al. developed the light-responsive TPE(Cbl)\textsubscript{4} NPs with AIE features for imaging-guided chemotherapy and PDT. The authors first used the covalent combination of AIEgens (TPE derivatives) and chlorambucil (Cbl) to generate TPE(Cbl)\textsubscript{4}, then obtained the TPE(Cbl)\textsubscript{4} nanoparticles via precipitation methods. Under visible light irradiation, TPE(Cbl)\textsubscript{4} nanoparticles simultaneously generated \(1\text{O}_2\) for PDT and sequentially released Cbl for chemotherapy.\textsuperscript{[130]}

5.3 | Enzyme-responsive

To achieving the aim of each agent of multiple-agent-therapy systems entering the cells in an optimal dominant way, our group constructed an MMP-2- and CB-responsive multiple agent therapy system (FC-PyTPA).\textsuperscript{[131]} FC-PyTPA contained three components: an amphiphilic structure with 16-carbon alkyl chain and GGGH peptide segment (F), which could self-assemble to form fibers, a positively charged cell penetrating peptide GRKKRRQRRR (C) loaded with siRNA for gene interference, and an AIE-based PS (PyTPA) for image-guided PDT (Figure 8D). These three parts were linked together by the peptide sequence specifically recognized by MMP-2 and CB. Once approaching the location of targeting tumor, FC\textsubscript{siRNA}-PyTPA was cleaved into two parts owing to the overexpressed MMP-2 in the tumor microenvironment. FC\textsubscript{siRNA} was internalized mainly by means of micropinocytosis and other part PyTPA entered cells simultaneously mostly through caveolae-mediated endocytosis. Subsequently, FC\textsubscript{siRNA} was hydrolyzed by CB, which facilitated F self-assembly to form fibers and siRNA escape to play the role in gene interference. Finally, through the regulation of dual enzymes, we achieved precise release, high-efficiency cell internalization, and combined therapy of three therapeutic agents.

6 | CONCLUSION

In this review, we summarized the developments in the construction of stimuli-triggered theranostic systems, which can achieve simultaneous imaging and therapy. We focused on the features of different therapy modes (chemotherapy, PDT, gene therapy, surgical therapy, combination therapy), types of responses (external and internal stimuli), design strategy of systems (molecules, probes, nanomaterials), as well as the application fields (in vitro and in vivo).

As summarized, the characters of AIEgens in theranostic systems are as follows: (1) as the hydrophobic part to participate in the construction of nanocarriers, (2) as the therapeutic agents (PSs) for therapy, and (3) as the fluorescent agents for imaging. In fact, owing to the excellent optical performance and unique emission pattern of AIEgens, the application of AIEgens in stimuli-responsive theranostic systems is diverse, the “always on” fluorescence can be used for long-term tracking the systems. The dynamically changeable fluorescence can be utilized for detecting the stimulus concentration, monitoring the drug release, evaluating therapeutic response. To be more specific, by virtue of ingenious design, the fluorescence of systems could be changed along with the appearance of stimuli, for example, ratiometric fluorescence through FRET, “light up” fluorescence through aggregation, and “light off” fluorescence through disassemble (Scheme 1). This fluorescent change could indicate some important signals. Making full use of AIEgens could maximize the advantages of imaging-guided therapy and reduce its limitations as well.

In spite of many designs and promising results, the development of theranostic systems is still in the proof-of-concept stage. There are several urgent problems of AIEgen-based theranostic systems for the future clinical translation. Herein, we outline the following challenges that should be overcome and also propose some promising directions in future progresses. First, the pool of AIEgens needs to be expanded urgently. The most AIEgens mentioned in this review are blue to red emitting and excited by white light. Actually, it is hard to obtain a satisfying in vivo imaging result, not to mention clinical application. Obviously, AIEgens with longer
wavelength absorption and emission is attractive and promising, considering the light penetration ability and the location of deep tumor tissues. By adjusting the structure of AIEgens through the design principles mentioned, researchers have reported some AIEgens with NIR absorption and longer wavelength emission.\textsuperscript{[98,132]} But modifying or conjugating these AIEgens to realize dynamic change of fluorescence and stimuli-triggered imaging-guided therapy is still a hard problem owing to the nonexistent or limited functional site in AIEgens. Systemically investigating Jablonski Diagram of AIEgens and exploiting more AIE molecules with outstanding fluorescent performance, high fluorescence quantum yield, longer emitting wavelength, as well as modifiable ability is the foundation of AIEgens in biomedical areas. Besides, to the AIEgens-based PSSs, utilizing the internal light source methods might be a promising way, like afterglow luminescence.\textsuperscript{[133]}

Then, despite possessing the delicate design and utilizing the excellent AIE fluorescent performance, these theranostic systems are more of an ideal situation. Considering the complexity of in vivo environments, current evaluation of theranostic systems remained in initial phase. For instance, the experiment periods of therapy and biosafety are relatively short (about 15 days to 2 months). Assessing the efficiency of theranostic systems in different tumors modes, such as subcutaneous tumor models, orthotopic tumor model, as well as metastatic tumor model, contributed to understanding therapeutic mechanisms comprehensively.\textsuperscript{[134–136]} Moreover, a great deal of work is necessary to estimate the biocompatibility, long-term toxicity, and pharmacokinetico-pharmacodynamic analysis of AIEgens before more in-depth study.

Last but not least, at this stage, the treatment modes are more focused on chemotherapy and PDT on this topic instead of surgery and immunotherapy, which are also promising to achieve the clinical translation.\textsuperscript{[137,138]} Further, encouraged by the feasibility of using AIEgens to evaluate the therapeutic response of chemotherapy, is it possible to assess the therapeutic response of immunotherapy by stimuli-responsive theranostic systems based on AIEgens, since monitoring the therapeutic feedback of immunotherapy is still a big challenge currently? We hope that this manuscript has summarized the up-to-date status on this theme and offered several promising directions in the war against cancer.

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AUTHOR BIOGRAPHIES

Jing-Jing Hu received her Ph.D. (2019) in polymer chemistry and physics from Wuhan University, China under the supervision of Prof. Xian-Zheng Zhang. She is currently an associate professor in Faculty of Materials Science and Chemistry at China University of Geosciences (Wuhan). Her scientific interest is focused on the biomedical applications of multifunctional drug delivery systems.

Xiaoding Lou received her Ph.D. in organic chemistry from Wuhan University in 2012 under the supervision of Prof. Zhen Li. Then she worked as a research associate in Prof. Ben Zhong Tang’s group at the Hong Kong University of Science and Technology. In 2013, she joined Huazhong University of Science and Technology (Wuhan). Her scientific interest is focused on the chemical and biosensor field.

Fan Xia studied physical chemistry at the Institute of Chemistry, Chinese Academy of Sciences and received his Ph.D. in 2008 under the supervision of Prof. Lei Jiang. He then worked as a postdoctoral scholar with Profs. Alan J. Heeger, Kevin Plaxco, and Herb Waite at the University of California, Santa Barbara. He joined Huazhong University of Science and Technology as a professor in 2012. Currently, he is a professor and Dean of Faculty of Materials Science and Chemistry, China University of Geosciences (Wuhan). His scientific interest is focused on bioanalytical chemistry.

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