Aggregation-induced emission probes for cancer theranostics

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Conventional cancer therapy usually suffers from poor treatment efficiency and adverse effects. To improve the treatment efficiency, it is critical to precisely diagnose specific cancer types and monitor the therapy process in situ. Fluorescence imaging has the advantages of high sensitivity and easy operation, but conventional fluorophores suffer from aggregation-caused quenching (ACQ), and therefore, their applications for imaging or diagnosis are severely impeded. By contrast, aggregation-induced emission (AIE) probes have significant advantages in terms of excellent photostability and a lack of self-quenching, and can be conveniently incorporated into theranostic platforms by combining them with various therapeutic modalities. Here, we discuss and summarize the recent advances in the development of AIE probes for cancer theranostics.

Introduction
Cancer is a main cause of death worldwide and many efforts have been devoted to the development of methods for the early detection and treatment of cancer to increase the patient survival rates. However, conventional cancer therapies usually suffer from poor treatment efficiency and adverse effects, which are possibly the result of our poor understanding of therapy processes for different patients. Theranostics, which can simultaneously provide diagnostic and therapeutic information, have the potential to improve treatment efficiency and reduce adverse effects by image-guided therapy [1]. To date, various imaging methods have been adopted for cancer theranostics, including ultrasound, computed tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI). However, these imaging techniques suffer from one or more of the following limitations: poor resolution, potential radiation damage, and high equipment costs [2]. Compared with these imaging methods, fluorescence imaging has significant advantages of high spatial resolution, good biocompatibility, low cost, and easy accessibility [3]. However, because of their hydrophobic nature, conventional fluorophores usually suffer from aggregation-caused quenching (ACQ), high background noise in dilute solution, poor photostability, and difficulties associated with combining them with therapeutic functions [4].

In contrast to conventional fluorophores with ACQ drawbacks, AIE fluorogens (AIEgens) are almost non-emissive in dilute solution, but become highly emissive in the aggregated state because of restricted intramolecular motion [5,6]. AIEgens have several unique advantages, such as high emission efficiency in the aggregated state, low background noise in dilute solution, excellent photostability, and high sensitivity [7]. AIE nanoparticles (NPs) are especially suitable for in vivo imaging and have a deep penetration ability resulting from their large multiphoton absorption cross-sections, strong photobleaching resistance to high laser power, and high emission efficiency in the red/near-infrared (IR) bio-optical window (700–900 nm) [8,9]. Moreover, AIEgens with diverse skeletons can be easily incorporated with various therapy modalities, such as chemotherapy, photodynamic therapy, and gene therapy (Fig. 1). Here, we illustrate recent advances in the development of AIE probes for cancer theranostics and provide some primary examples.

Fluorescence image-guided chemotherapy
The integration of diagnostic imaging and therapeutic modalities in one molecule provides
a simple and efficient approach for monitoring drug distributions and evaluating therapeutic effects. For example, Hu et al. reported a mitochondria-specific probe (AIE-Mito-TPP, Fig. 2a) formed by conjugating an AIEgen of salicylaldehyde azide with lipophilic triphenylphosphonium (TPP) cation groups [10]. Given that cancer cells have a higher mitochondrial membrane potential (MMP) compared with normal cells [11], the AIE-Mito-TPP probe tends to accumulate in mitochondria of cancer cells and emits strong green fluorescence as a result of its restricted intramolecular motion. The accumulation of AIE-Mito-TPP in mitochondria can efficiently decrease the MMP, increase the intracellular level of reactive oxygen species (ROS), and inhibit ATP synthesis. As a result, AIE-Mito-TPP probe exhibits a higher cytotoxicity for cancer cells (HeLa, HepG2, U87-MG, MDA-MB-231, and MCF-7) than for normal cells (NHDF, HK2, MRC5, and NIH-3T3). Following on from this approach, Shin et al. developed a mitochondria-targeted and enzyme-activated AIE probe by decorating an AIE-active tetraphenylethene (TPE) skeleton with TPP cation groups and a NQO1-cleavable NAD(P)H masking unit [12].

Given that the NQO1 enzyme is overexpressed in cancerous tissues, this strategy further improved the targeted therapeutic ability via the preferential uptake of this probe into cancer cells and the subsequent induction of apoptosis. Similarly, Reedy et al. synthesized a series of TPE-based and mitochondria-targeted probes with lipophilic pyridinium cations as the targeting ligands, which show an efficient killing ability for metastatic melanoma cells through the disruption of mitochondrial metabolism [13].

Tamoxifen (TMX) is a breast cancer drug that acts by forming a TMX–estrogen receptor (ER) complex. Inspired by the structural similarity between TMX and TPE–TMX, Zhao et al. (FIGURE 2)

Fluorescence image-guided and organelle-targeted chemotherapy. (a) Schematic illustration of intracellular tracking and therapeutic effect of aggregation-induced emission (AIE)-Mito-triphenylphosphonium (TPP) in cancer cells. Adapted, with permission, from Ref. [10]. (b) Molecular structures of tamoxifen (TMX), and tetraphenylethene (TPE)–TMX. Bright-field and fluorescent images of MCF-7 [estrogen receptor (ER)+] and MDA-MB-231 (ER−) breast cancer cells treated with TPE–TMX. Adapted, with permission, from Ref. [14].
explored TPE–TMX as a theranostic agent for the simultaneous imaging and treatment of ER+ breast cancer (Fig. 2b) [14]. As a result of the basic amino group of TPE–TMX, this complex can selectively accumulate in acidic lysosomes, where it emits fluorescence as a result of restricted intramolecular movement. Although TMX and TPE–TMX are structurally similar, they exhibit different working mechanisms. The therapeutic effect of TMX results from the formation of a TMX–receptor complex and further entry into the nucleus of cancer cells; by contrast, TPE–TMX selectively kills ER+ breast cancer cells by inducing autophagy, which is verified by the increased number of autolysosomes, formation of vacuoles, and swelling of lysosomes in these cells.

Another approach to combine chemotherapy and fluorescent diagnosis is the covalent or noncovalent linking of AIEgens to chemical prodrugs. Yuan et al. reported a series of AIE theranostic systems based on a Pt(IV) prodrug linked with AIEgens via enzyme-cleavable hydrophilic peptides. For example, the AIE-Pt(IV) prodrug system can comprise a cancer cell targeting cRGD ligand (cyclic arginine–glycine–aspartic acid), an AIE-active tetraphenylsilole (TPS) derivative, an apoptosis-responsive DEVD peptide linker, and a chemotherapeutic prodrug Pt(IV) [15,16]. The AIE-Pt(IV) prodrug is able to selectively enter cancer cells overexpressing the \( \alpha_p\beta_3 \) integrin receptor; Pt(IV) is then reduced in the cytoplasm to generate the anticancer drug Pt(II), resulting in cancer cell death via apoptosis. This therapeutic process can be visualized in situ by the fluorescence of TPS aggregates induced by cleavage of the hydrophilic DEVD peptide linker through activated expression of caspase-3/7 enzymes during apoptosis.

Xue, Chen, and Zhang et al. developed several pH-sensitive self-indicating drug delivery systems (SIDDs) based on the combination of TPE derivatives with the anticancer drug doxorubicin (DOX) through several different methods, including the self-assemble of TPE-COOH and DOX into NPs via electrostatic interactions, conjugation of TPE with DOX via a pH-sensitive hydrazone bond, and encapsulation of DOX within TPE-mPEG micelles [17–19]. As a result of Förster resonance energy transfer (FRET) between TPE and DOX and the ACQ effect of DOX, a ‘double quenching’ phenomenon was observed. After entry into cancer cell lysosomes, DOX is released in response to the lower pH environment (pH 5.0) and induces cell apoptosis by entering the cell nucleus accompanied with red fluorescence emission. Meanwhile, the TPE carriers remain in the cytoplasm and emit strong blue fluorescence. This dual light-up fluorescence clearly indicates the drug activation process. Wang et al. also constructed AIE polymeric carriers for DOX delivery based on TPE-decorated dextran and a PEG-PSS polymer; both carriers showed in situ monitoring ability and efficient anticancer activity [20,21].

Wu et al. developed a prodrug, DCM-S-CPT, with an excellent tumor-inhibiting ability based on dicyanomethylene-4H-pyran derivatives as the NIR-emissive AIEgens and camptothecin (CPT) as the cancer drug [22]. Both in vitro and in vivo experiments showed concomitant drug release and light-up fluorescence, which clearly exhibited the advantages of in situ monitoring of the therapeutic process. Zhang et al. recently synthesized TPE-based rhomboidal Pt(II) polymers via metal–ligand coordination interactions [23]. The AIE-active Pt(II) polymers showed selective enrichment in the tumor and lung over other organs after being intratumorally injected into a mouse bearing an MDA-MB-231 xenograft tumor, indicating the potential of such polymers for in vivo cancer therapy. Ding et al. developed an AIE-active nanoprobe Net-TPS-PEI-DMA, whose surface charges can become positive in the acidic extracellular environment of tumors (pH ~6.5). Thus, this nanoprobe can selectively enter cancer cells and activate apoptosis by inhibiting the Akt pathway [24]. Huang et al. reported a series of NPs derived from pyridinium-substituted tetraphenylethylene with different counter anions; these NPs were able to specifically accumulate in tumor sites and exhibited efficient tumor suppression during in vivo experiments [25]. Wang et al. recently fabricated a theranostic nanoplate, HSA-PhENH2-Ppy-PTX-cRGD, with the following features: bright fluorescence from AIE-active PhENH2, photothermal therapy from polypyrrole (Ppy), chemotherapy from paclitaxel (PTX), and cancer cell targeting ability from cRGD. This multifunctional nanoplate shows great potential as a fluorescence image-guided multimodal cancer therapy [26].

**Fluorescence image-guided photodynamic therapy**

Photodynamic therapy (PDT) is an attractive therapeutic technique for eliminating cancer cells by generating ROS under light irradiation. However, conventional photosensitzers (PSSs), such as porphyrin and methylene blue, suffer from both fluorescence quenching and a serious decrease in their photodynamic efficiency in the aggregated state [27,28]. In contrast to conventional PSSs, the nonradiative pathway of AIEgens is efficiently blocked in the aggregated state, which can activate both the radiative and intersystem crossing (ISC) pathways to emit strong fluorescence and simultaneously react with oxygen to efficiently generate ROS.

Recently, Gui et al. developed a positively charged AIEgen TPE-IQ-20, which can not only distinguish cancer cells from normal cells via their different mitochondrial membrane potentials, but also act as an efficient PS to kill cancer cells through ROS generation under white light irradiation (Fig. 3a) [29]. Moreover, TPE-IQ-20 is able to differentiate cancer cells (HeLa cells or MDA-MB-231 cells) from normal cells (COS-7 cells or MDCK-II cells) even when they are co-cultured in the same dish, which indicates its potential application for the in vivo fluorescence imaging and photodynamic ablation of cancer tissues. Li et al. reported AIE-active T-TTD dots for image-guided PDT using a human cholangiocarcinoma (CC) xenograft mouse model [30]. The T-TTD dots could selectively accumulate in tumors via the enhanced permeability and retention (EPR) effect. The specific interaction between integrin \( \alpha_v\beta_3 \) overexpressed on CC cells and cRGD on T-TTD dots promoted the endocytosis of T-TTD dots into the targeted tumor cells. Upon light irradiation, the T-TTD dots not only emitted red fluorescence to illustrate the tumor sites, but also exhibited excellent photodynamic activity for the efficient induction of apoptosis or necrosis of the tumor cells. As a result, the AIE-active T-TTD dots show excellent in vivo antitumor effects (Fig. 3b) [30]. Yuan et al. also reported a pH-responsive polymeric probe containing an AIE-active TPS fluororogen and a phophorhobe (PheA) PS with ACQ properties. In a pH-neutral environment, the probe self-assembles into NPs with green emission from TPS; after entrapment in acidic lysosomes, the NPs disassemble and yield red emission from PheA. Upon light irradiation, the generated ROS from PheA efficiently disrupts lysosomes to induce cell apoptosis. The probe then leaks into the neutral cytoplasm to reform NPs and restore the green emission from TPS. This ‘AIE + ACQ’ probe design represents an efficient strategy for the in situ visualization of the therapeutic process and response [31]. Han et al. designed and synthesized a matrix metalloproteinase-2 (MMP-2)-responsive probe containing an AIE-active TPE unit and a photodynamic active protoporphyrin IX (PpIX) moiety. Not only could the probe efficiently differentiate cancer cells from normal cells through MMP-2 induced ratiometric fluorescence changes, but also could be used to monitor the photodynamic therapy process in situ [32].
Fluorescence image-guided photodynamic cancer therapy. (a) Schematic illustration of tetraphenylethene (TPE)-IQ-2O for selective mitochondrial imaging and photodynamic ablation of cancer cells (i). Fluorescence images of different cancer cells (MCF-7, PC-9, MDA-MB-231, and A549) and normal cells (HEK-293 and MDCK-II) stained with TPE-IQ-2O (ii). Adapted, with permission, from Ref. [29]. (b) Aggregation-induced emission (AIE)-active T-TTD dots for in vivo photodynamic therapy (PDT) in a human cholangiocarcinoma xenograft mouse model to induce cancer cell apoptosis or necrosis via reactive oxygen species (ROS) generation under light irradiation. Adapted, with permission, from Ref. [30]. Abbreviation: EPR, enhanced permeability and retention.
Hu et al. developed an AIE probe, TPPDC-2AP2H, comprising two 2AP2H (IHGHHIIIWG) peptides for the specific targeting of lysosomal transmembrane 4 beta (LAPTM4B) proteins overexpressed on cancer cell membranes. Under light irradiation, the generation of $^{1}\text{O}_2$ efficiently kills cancer cells, but causes no harm to normal cells [33]. Zhang et al. developed a light-up probe, TPETH-2T7, by conjugating TPETH with a TfR-targeting peptide, T7. The probe emits light-up fluorescence after binding to TfR-overexpressed cancer cells and showed good therapeutic potential by the disintegration of cancer cell membranes via ROS generation [34]. Yuan et al. further introduced a dual-targeted probe by decorating an AIE PS with a lysosomal cathepsin B enzyme-responsive peptide (GFLG) and a cancer cell targeting peptide (cRGD). This design guarantees the probe-selective entry and activation in cancer cell lysosomes, dramatically decreasing the viability of cancer cells following light irradiation [35].

Yu et al. recently reported a mitochondria-targeted AIEgen of DPA-SCP by using pyridinium salt as the targeting moiety, resulting in an elevated level of $^{1}\text{O}_2$ in mitochondria and enhancing the radiosensitivity of cancer cells with a supra-additive synergistic effect of ‘$0 + 1 > 1$’ [36]. Based on a similar strategy, the authors further developed an AIEgen-based PS (TPE-Py-FFGYSA) that can serve as a nontoxic adjuvant to amplify the anticancer efficacy of PTX, which also exhibits a synergistic effect of ‘$0 + 1 > 1$’. Cytotoxicity and western blot studies revealed that the ROS generated by AIEgens of DPA-SCP and TPE-Py-FFGYSA under light irradiation do not kill cancer cells, but instead enhance the radiotherapy and chemotherapy effect of the drugs by increasing the

![FIGURE 4](image-url)
oxidative level in the intracellular environment [37].

**Fluorescence image-guided gene therapy**

Gene therapy is a potential cancer therapy method that uses nucleic acid transfer. Hu et al. developed two polymeric nanovectors to deliver small interfering (si)RNA for pancreatic cancer treatment via silencing of mutant K-ras [38]. They used Pluronics F127 and DSPE-PEG to encapsulate the AIEgen of PE-TPA-DCM to form NPs, which then formed a further complex with siRNA to monitor the transfection process. Quantitative PCR experiments showed that the expression of the mutant K-ras oncogene in pancreatic cancer cells was successfully suppressed. Jin et al. reported a theranostic platform comprising cRGD, small interfering vascular endothelial growth factor (siVEGF), and an AIEgen of TTDc that efficiently improved the therapeutic efficiency via synergistic PDT and gene silencing therapy by efficient ROS generation and simultaneous inhibition of the expression of VEGF [39].

Yuan et al. developed an AIE polymeric carrier P(TEP-MAA-OE)-g-mPEG for light-controlled gene delivery (Fig. 4) [40]. The polymer can self-assemble into NPs with DNA via electrostatic interactions. After entry into cancer cells lysosomes via endocytosis, the ROS generated by TPECM under light irradiation efficiently decomposed the polymer by cleavage of the ROS-responsive amino-acrylate linker and disrupted the lysosomal membranes to facilitate the escape of DNA for gene expression. This design was able to reveal the dynamic polymer decomposing and DNA transfection process.

**Concluding remarks**

The combination of fluorescence imaging, chemotherapy, PDT, and gene therapy into anticancer AIE theranostic platforms provides an efficient strategy for precise cancer diagnosis and personalized therapies. Future AIE theranostic systems could be developed based on: (i) the preparation of new AIEgens with large multiphoton absorption cross-sections and high NIR emission efficiency to facilitate in vivo tumor imaging; (ii) conjugation of antibody drugs with AIE-active PSs to realize antibody-targeted photodynamic therapies against specific cancer cells; and (iii) integration of fluorescence imaging and different therapeutic modalities in one platform to further improve the cancer treatment efficiency and overcome drug resistance. We anticipate that recent progress in the development of AIE-based cancer theranostics should inspire more scientists to join this promising area of research.

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