Emerging Designs of Aggregation-Induced Emission Agents for Enhanced Phototherapy Applications

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Phototherapy, including photodynamic therapy (PDT) and photothermal therapy (PTT), that employs phototherapeutic agents to generate cytotoxic reactive oxygen species (ROS) or hyperthermia, is a promising approach for disease therapy. However, conventional organic phototherapeutic agents suffer poor photostability and aggregation-caused quenching (ACQ) in the aggregate state, restricting their therapeutic efficacy. Aggregation-induced emission (AIE) agents can solve these issues with strong emission in the aggregate state and reverse designation to generate heat. This review summarizes the recent advances in the development of AIE phototherapy agents for enhanced PDT and PTT performances in biomedical applications. First, design strategies of AIE agents that adjust the intersystem crossing process or intramolecular charge transfer to boost ROS generation or regulate ROS types are discussed. The AIE agents with ROS generation ability for biomedical applications including antitumor and antibacterial performances are then introduced. Next, designs and examples of AIE agents that enhance PTT performances through molecular motions are described. Finally, the current challenges and perspectives of AIE agents in the phototherapy field are discussed.

Keywords: aggregation-induced emission, structural designing, phototherapy, photodynamic therapy, photothermal therapy

Introduction

Phototherapy that utilizes photoirradiation to generate cytotoxic reactive oxygen species (ROS) for photodynamic therapy (PDT) or hyperthermia for photothermal therapy (PTT) is a promising approach for disease therapy because of its non-invasiveness, negligible drug resistance, high therapeutic selectivity and efficacy, and minimized off-target side effects. Organic contrast agents have attracted much attention in the phototherapy field due to their excellent optical properties and tunable molecular structures. However, the traditional small molecular organic contrast agents used for phototherapy such as rose bengal analogs, methylene...
blue, and cyanine derivatives, suffer from poor photostability and aggregation-caused quenching (ACQ) in the aggregate state after they are transformed into water-soluble nanoparticles, leading to a relatively low therapeutic efficacy.12−14

Organic contrast agents with aggregation-induced emission (AIE) phenomena are contrary to the traditional contrast agents with the ACQ effect.15−19 The AIE agents are almost non-emissive in the isolated molecular state but strongly emissive in the aggregate form.20−22 This phenomenon is due to the restriction of intramolecular motion (RIM) of AIE agents in the aggregated state, which avoids the nonradiative decay that dissipates the absorbed light, and thus leads to the enhanced fluorescence emissions.23,24 Meanwhile, the non-planar structure of AIE agents also contributes to circumventing π−π interactions and activates strong emission in aggregated state.25 Because of such a special molecular structure, AIE agents have been developed into optical probes for long-term cell tracking, tumor imaging, and image-guided surgery.26−28 Recently, AIE agents were reported to show strong photosensitization and high photothermal conversion efficiency through molecular engineering.29−31 Introducing an electron-rich anion−π* structure into AIE agents results in the highly efficient ROS generation for PDT.32,33 In addition, the disease microenvironment often suffers from hypoxia which limits the generation efficacy of singlet oxygen by AIE photosensitizers (PSs) through the energy transfer process from excited triplet AIE PSs to triplet oxygen.34,35 Another type of AIE PS that depends on the electron transfer from excited AIE PSs to oxygen to produce free radical ROS has obtained more and more attention.36−38 With the help of rotors and spacer structures, the molecular motions of AIE agents in the aggregate state can be tuned to enhance the nonradiative decay, providing guidelines for the development of AIE agents for PTT applications.39−42

In this review, we summarize the recent advances in the design strategies of AIE agents for enhanced PDT and PTT performances. The chemical structures of AIE agents used for enhanced PDT and PTT performances are summarized in Schemes 1 and 2. The design strategies of AIE agents through adjusting the intersystem crossing (ISC) process or intramolecular charge transfer (ICT) to boost ROS generation or regulate ROS types under laser irradiation are first discussed. Next, we review the AIE agents with ROS generation ability for biomedical applications including antitumor and antibacterial performances. Then, designs and examples of AIE agents to enhance PTT performances through molecular motions are described. Finally, a summary and current challenges and perspectives of AIE agents in the phototherapy field are given.

### AIE Agents with ROS Generating Ability

**Design strategies to enhance the photosensitizing performance of AIE agents**

PDT that relies on PSs and laser irradiation to produce ROS is an emerging therapeutic modality for diseases.43−45 According to the Jablonski diagram (Figure 1a), after the excitation light is absorbed by the PSs, the electrons of PSs are excited from the ground state (S0) to the excited state (S1), and the electrons at the Sn state dissipate the energy and return to the singlet excited state (S1) through the relaxation process. There are three major energy dissipation pathways from S1 to S0: radiative decay to produce fluorescence emission, nonradiative decay to generate heat for PTT, and ISC to the triplet excited state (T1) to produce ROS for PDT.39 The ROS produced by PSs mainly includes two types: Type I PSs produce radical species including superoxide anion (O2−) and hydroxyl radicals (OH−) through electron transfer from excited PSs to oxygen species, whereas type II PSs utilize energy transfer between triplet states of PSs and oxygen to produce singlet oxygen (O2).46,47 The key step for enhancing ROS generating efficiency is the ISC process.48,49 Promoting the ISC process and creating rich triplet-state energy levels show potential for improving the ROS generating efficiency.

To investigate the relationship between the molecular structure and the photosensitizing performance of AIE agents, four PSs, which were named TPAN, TPAPy, TPANPF6, and TPAPyPF6, composed of the electron-donating triphenylamine and electron-withdrawing azfluorenone and different substituents were designed and synthesized by Tang’s group (Figure 1b).39 The ionized compounds (TPAN and TPAPy) displayed higher molar absorption coefficients relative to their corresponding ionized counterparts (TPANPF6 and TPAPyPF6). Meanwhile, TPAN exhibited a typical ACQ effect, whereas the other three compounds were all AIE-active as verified by the enhanced fluorescence intensity in the high water fractions of organic solvent/water mixtures. The ionized compounds (TPANPF6 and TPAPyPF6) showed higher ROS generation capacity compared to their nonionized parent, when a general commercial ROS indicator 2,7′-dichlorodihydro-fluorescein diacetate (H2DCF-DA) that measures many types of ROS activity including type I and type II was employed. However, the singlet oxygen generation ability of TPAPy is higher than that of TPAPyPF6, indicating TPAPyPF6 produced both singlet oxygen and free-radical ROS. According to a previous report, enhanced ISC benefits the ROS generation through promoting spin-orbital coupling (SOC) and lowering the energy gaps.
between the $S_1$ state and $T_1$ state ($\Delta E_{ST}$). These four PSs showed similar SOC through theoretical calculation. Therefore, the $\Delta E_{ST}$ values should be the key factor to determine the ROS generation efficiency of these four PSs. However, such a theory cannot give a satisfactory explanation for the ROS generation efficiency order of these four PSs. Especially for TPAN, which has a small $\Delta E_{ST}$ but inefficiently generates ROS. Therefore, other factors such as the large difference in molar absorption coefficient or the aggregation state of PSs should also attribute to the ROS generation efficiency.

The singlet oxygen generation by type II PSs is the primary species in PDT. However, its oxygen dependence limits the generation efficiency in the hypoxic disease microenvironment. In fact, the oxygen consumption of the singlet oxygen generation process even aggravates the disease hypoxia and further reduces its treatment efficacy. Therefore, the design of type I PSs to generate free radicals in an oxygen-independent manner shows the potential to resolve this issue. The type I ROS, including superoxide radical ($O_2^-$), hydrogen peroxide ($H_2O_2$), and $OH^-$, are generated in a cascade.
reaction via electron transfer after laser irradiation. O₂⁻ is first converted to H₂O₂ and O₂ by intracellular superoxide dismutase (SOD)-mediated disproportionation, and the generated H₂O₂ is further catalyzed by ferrous ions to form highly cytotoxic OH⁺, resulting in the successful PDT (Figure 1c). To explore the molecular engineering strategy to fabricate AIE agents with type I ROS generation ability, Tang et al. included anion-π⁺ structures into the AIE agents to obtain strong ICT characteristics to promote electron transfer from excited AIE agents to adjacent substrates. The electron-rich group in the AIE agent structure could improve the electron density of the triplet excited state, which should facilitate the generation of free radical ROS. Four anion-π⁺ AIE agents, which were named TBZPy, MTBZPy, TNZPy, and MTNZPy, were prepared (Figure 1d). These AIE agents were composed of three parts: triphenylamine (TPA) or its methoxy-substituted triphenylamine (MTPA) units linking benzo[1,2,3]-thiadiazole (BZ) or naphtho[2,3-c][1,2,5] thiadiazole (NZ) moieties as the AIE-active electron donors, styrylpyridine cation as the electron acceptor, and iodide anion and collaborative donors to create electron-rich conditions for providing electrons to excited AIE agents. It was found that NZ-based AIE agents generated free radical ROS while TBZPy primarily generated ¹O₂ species. MTBZPy yielded both ¹O₂ and free radical ROS.
radical ROS due to the enhanced donor effect. Compared with BZ-based AIE agents, the NZ-based structure in AIE agents boosted the ICT intensity, leading to high free radical ROS generation efficacy. These results demonstrated the importance of the electron-rich anion-$\pi^+$ structure for the design of AIE agents with type I ROS generation ability.

The electron transfer mechanism of type I PSs exists not only in ICT but also in charge transfer between substrates.62–64 In most luminophore systems, photon excitation triggers energy level transition and is dissipated through radiative or nonradiative decay processes. Besides laser irradiation, chemical reaction provides another pathway for subsequently controlling the excitation of the surrounding species.55–57 Photochemical reactions such as photochromism and photocyclization in tetraphenyl ethylene (TPE) and stilbene have been reported.68–70 Under UV light irradiation, stilbene undergoes trans-cis isomerization, which induces subsequent photocyclization of cis-stilbene to form trans-4a,4b-dihydrophenanthrene. However, this unstable structure reverts to cis-stilbene if not trapped. Utilizing oxidative trapping, dihydrophenanthrene is oxidized to irreversibly yield phenanthrene, which is the pivotal step in photocyclization. Therefore, improving the photochemical efficiency of TPE could be used for the design of type I PS. Based on the electron-rich anion-$\pi^+$ structure with a photochemical reaction feature, Tang et al.71 designed...
and synthesized an isoquinolinium organic salt derivative with a TPE structure feature, named TIdBO, to investigate the type I ROS generation efficacy (Figure 1e). This AIE agent displayed strong ICT and potential photocyclization reactivity, providing opportunities for electron transfer and improved free radical ROS in prospect. Compared to its similar AIE agent, TiOdBP, which possesses photoreduced crystallization with an emission enhancement (PCE) property, exhibited high photocyclization reactivity in the dispersive state and sufficiently produced type I ROS upon aggregation due to the translocation of the electron-donating methoxy group. To reveal the structure-function relationship in this photochemistry-based type I PS, single crystals of TIdBO were obtained to confirm its molecular structure and conformation. The tetraphenyl ethylene group showed a twisted propeller conformation, leading to less π–π stacking interaction, which could suppress the ACQ effect. Tetrafluoroborate anions were interspersed on different sides of the twisted plane and exhibited a similar effect to avoid emission quenching. TIdBO in phosphate-buffered saline (PBS) solution showed a robust ROS generating capacity under white light irradiation, and only OH radical free radicals were detected when using 2,7-dichlorodihydrofluorescein (DCFH), 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA), with oxygen sensor green (SOSG), dihydro-rhodamine 123 (DHR123), and hydroxyphenyl fluorescein (HPF) as ROS general or specific indicators, confirming the efficient free radical ROS generation of TIdBO. However, the ROS generating efficiency of TIdBO in Dulbecco’s modified eagle medium (DMEM) + 10% fetal bovine serum (FBS) mixtures showed the opposite results. The photocyclization process was completely inhibited in PBS solution, but obvious in DMEM + FBS mixtures. The photocyclization process was impacted by the excited-state molecular conformational adjustment, which occurred easily in solution or loose aggregates but hardly in tight aggregates because the twisted dihedral angles impeded planarization in photocyclization. Therefore, in the aggregated state such as in PBS solution, TIdBO could not acquire the molecular conformation, which is necessary for the photocyclization process, and thus impels the electron in the excited state to interact with oxygen to dissipate energy, leading to the generation of type I ROS.

Various design strategies have been explored to create AIE agents with high ROS generating efficiency. For example, a strong electron donor-acceptor interaction in the molecular skeleton facilitates ICT, thus leading to the enhanced singlet oxygen generation efficiency. Based on these design strategies, Tang et al. synthesized several zwitterionic compounds, BITT, BTB, ITT, and ITB, which consist of dimethylaniline or triphenylamine moiety as the donor, thiophene fragment as a donor and π-bridge, double bond for the π-bridge, and a quaternary ammonium salt unit as acceptor. Here, the strong D-A effect contributed to the separation of highest occupied molecular orbital (HOMO)–lowest unoccupied molecular orbital (LUMO) distribution and a reduced energy gap for ISC, which critically enhanced the ROS generation. In a similar work, Tang et al. designed two AIE agents TTP and MeTTP containing a pyridium unit (as A), double bond (π-bridge), thiophene fragment (D and π-bridge), and triphenylamine segment (D). Twisted conformation of the triphenylamine moiety can enlarge the intermolecular distance, which generally prevents π-π stacking interaction. This aggregation-related character promoted the ISC process, while the enhancement of the D-A strength also contributed to the ROS generating efficacy of AIE agents. Moreover, the D-π-A molecular structure is widely adopted in the design of AIE agents for strong charge transfer and certainly benefits the adjustment of the HOMO and LUMO. A new AIE agent, MeO-TPE-indo (MTI), designed according to this strategy, contains an electron donor methoxy group and an acceptor indo moiety modified on the TPE conjugated skeleton and exhibits the effective generation of ROS for PDT. The spin-orbit coupling constant (ξST) is an ISC efficiency factor that essentially influences ROS generation. However, ξST in traditional organic fluorophores could be adjusted in narrow range. Introducing heavy atoms, such as bromine, into the structure of AIE agents could potentially solve this issue. Tang et al. synthesized two red-emissive AIE agents, named TBP-1 and TBP-2, with addition of bromine atoms in the anion-π structure. Results demonstrated that charge transfer occurring from the bromine atoms to the π-conjugated core in TBP-1 and TBP-2 could reduce ΔE barrier and increase energy levels of the excited triplet states, thus achieving efficient ROS generation.

AIE agents for PDT applications

PDT has been widely applied in the biomedical field due to its noninvasive and controllable characteristic. Although design strategies for enhancing ROS generation by AIE agents have been well developed, the absence of their disease targeting ability could cause phototoxicity toward normal tissue. Recently, in situ synthesis of antitumor drugs inspired a PS design that largely decreases toxicity and specifically targets the PSs to the tumor region. Liu et al. designed a nanoscale metal–organic framework (nMOF) that carries AIE precursors and utilizes an F127 coating to form precursor-loaded MOF nanoparticles (PMOF NPs) (Figure 2a). A Cu-embedded nMOF, MOF-199, was chosen as the carrier due to the high loading efficiency of PS precursors and catalytic activity of the Cu-based skeleton. TPA-alkyne-2+ and MePy-N3, having negligible photoactivity before coupling, were chosen as the precursors. This nanosystem could specifically generate Cu(I) from glutathione (GSH) reduction of the Cu(II) skeleton in...
Figure 2 | (a) Synthetic mechanism of TPATrzPy-3+ in situ and components of PMOF NPs. (b) GSH-induced in situ PS synthesis for PDT. (c) Viability of HeLa cells after 24 h incubation with a range of concentrations of different agents under light irradiation. (d) Confocal laser scanning microscopy (CLSM) images of HeLa cells incubated with precursors or TPATrzPy-3+ for 24 h before staining of commercialized mitotracker for 30 min. (e) The relative tumor volume changes of zebrafish under different treatment. (f) Chemical structure of organelle-targeting AIE agents and schematic illustration of multi-organelle killing PDT. *, **, *** represent different person correlation coefficients (p); *p < 0.05, **p < 0.01, ***p < 0.001. (g) Confocal microscopy images of HeLa cells for co-localization test of different AIE agents with commercialized organelle trackers: (A) TFPy; (B) TFVP; (C) TPE-TFPy; (D) All three AIE agents. (h) Viability of HeLa cells incubated with different concentrations of AIE agents under light irradiation. Figures a–e are reprinted with permission from ref 82. Copyright 2021 Wiley-VCH. Figures f–h are reprinted with permission from ref 83. Copyright 2020 Wiley-VCH.
cancerous cells; a subsequently induced in situ click reaction of TPA-alkyne-2+ and MePy-N₂ provides photosensitive TPATrzPy-3+, which performed highly efficient ROS generation (Figures 2b and 2c). Moreover, the co-localization of TPATrzPy-3+ and MitoTracker Green under confocal laser scanning microscopy revealed the mitochondria-targeting property of TPATrzPy-3+, which assists the killing of cancer cells (Figure 2d). Thus, an in vivo antitumor experiment was conducted on zebrafish, showing an excellent phototherapy effect via this in situ catalysis strategy (Figure 2e).

Tang et al. put forward an innovative strategy for PDT that combines multiple AIE agents with different targeting abilities to achieve a 1 + 1 + 1 > 3 photosensitizing effect (Figure 2f). Three novel AIE Ps, TFPy, TFVP, and TPE-TFPy, were tailored based on the D–A feature with slight adjustments, and could specifically endow mitochondria, cell membrane, and lysosome target ability, respectively, to perform full-scale photodynamic killing of cancer cells. The high efficiency of electrophoretic transmembrane migration and binding affinity of the positively charged pyridinium moiety toward the negatively charged interior of the transmembrane potential of mitochondria contribute to the mitochondria-staining ability of TFPy. The high free-energy barrier caused low membrane permeability coefficients of TFVP and led to specific accumulation of TFVP in the cell membrane. TPE-TFPy generally formed nano-sized aggregates due to high hydrophobicity; whereas in situ aggregates targeted lysosomes through endocytosis. The colocalization of these three AIE agents with the commercial organelle fluorescent probes including MitoTracker Green, CellMask Green, and LysoTracker Green under confocal laser scanning microscopy revealed the mitochondria-targeting property of TPATrzPy-3+, which assists the killing of cancer cells (Figure 2d). Thus, an in vivo antitumor experiment was conducted on zebrafish, showing an excellent phototherapy effect via this in situ catalysis strategy (Figure 2e).

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PDT used in antibacterial treatment has also been explored to discriminate Gram-positive (G(+) ) and Gram-negative (G(−) ) bacteria or to conquer drug tolerance caused by antibiotic drug abuse. To investigate the relationship between antibacterial efficiency and chemical structure of AIE agents, Tang et al. designed two red-emissive AIE agents (TBP-1 and TBP-2) with the same luminogenic core but a different number of positive charges. The importance of the number of positive charges of AIE agents on the antibacterial performance was found by revealing the different behavior of TBP-1 and TBP-2 under incubation with G(+) and G(−) bacteria. Both TBP-1 and TBP-2 exhibited dark toxicity to G(+) bacteria and obvious ROS activation under light irradiation (Figure 3a). However, TBP-1 barely killed G(−) bacteria either in dark or under light irradiation, whereas TBP-2 showed much higher phototoxicity toward G(−) bacteria (Figure 3b). Further investigation into the underlying mechanism of antibacterial activity by TBP-2 under laser irradiation found that both TBP-1 and TBP-2 accumulated in the bacteria wall and cytoplasm of G(+) bacteria, demonstrating the strong interactions between AIE agents and the G(+) bacteria lead to effective dark toxicity (Figure 3c). However, TBP-2 showed stronger accumulation in the bacteria wall of G(−) bacteria, indicating that TBP-2 interacts better with bacteria G(−) compared to TBP-1. The difference in accumulation is probably due to the difference in the number of positive charges of the AIE agents (Figure 3d). When the TBP-2-incubated G(−) bacteria was treated with Mg²⁺, obvious fluorescence quenching of TBP-2 was found, indicating the interactions between lipopolysaccharide (LPS) and TBP-2 were blocked by the divalent cations (Figure 3e). This phenomenon revealed that sufficiently positive-charged TBP-2 could interact more strongly with the negatively charged LPS on the outer membrane of G(−) bacteria and compete with the divalent cations (Ca²⁺ or Mg²⁺) that are bonded to stabilize the LPS structure. This process may cause cracks in the permeability barrier, benefiting the penetration of TBP-2 into the periplasmic space of G(−) bacteria for effectively destroying the biomolecules of G(−) bacteria. Moreover, TBP-1 and TBP-2 showed selective photoactivation of G(+) and G(−) bacteria, respectively, over mammalian cells.

Recently, antibiotic drug abuse has become an intractable problem that directly induces tolerance of antibiotics in the bacterial population. A typical antibiotic, vancomycin (Van), possesses a pesticide effect on G(+) bacteria by specific binding affinity to the peptidoglycan sequence N-acyl-γ-Ala-γ-Ala presented on G(+) bacterial cell walls. However, the occurrence of Van-resistant Enterococcus (VRE), having a largely decreased binding affinity for Van, impedes the therapeutic effect on infection problems. Synthesis of dimer or oligomer types of Van is an alternative method to maintain the binding ability. Liu et al. reported a novel AIE agent conjugated with two Van groups for antibacterial treatment, named AIE-2Van. AIE-2Van showed a high binding affinity toward G(+) bacteria and retained the binding affinity toward VRE bacteria. Under restricted concentration, AIE-2Van performed satisfactory ROS generation when incubated with VRE bacteria. Therefore, although AIE-2Van showed low killing efficiencies on VRE bacteria in the dark due to the resistance of VRE bacteria to Van, the maintained binding ability of AIE-2Van to VRE bacteria boosted its antibacterial effect through ROS generation under light irradiation, indicating the great potential for PS-assisted antibacterial therapy against antibiotic-tolerant bacteria.

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AIE Agents with Photothermal Conversion Performances

Design strategies to enhance the photothermal conversion efficiency of AIE agents

Under laser irradiation, the nonradiative decay of excited-state energies is inevitable due to natural vibration and rotation.92 Utilizing dissipated energy as the thermal effect for PTT has been well investigated in the biomedical field. Various structure-dependent design strategies have been explored for boosting the nonradiative relaxation of photothermal agents.93,94 For instance, Pu et al.95 quenched the fluorescence of semiconducting polymer nanoparticles by intraparticle photoinduced electron transfer to achieve heat generation. Lee et al.96 modified the semiconducting polymer chains with light-harvesting groups to increase the photothermal properties. Cheng et al.97 constructed π–π stacking structures for the ACQ effect that consumes the excited energy as heat. However, structural design of AIE agents for nonradiative decay is conducted in a quite different way. Molecular motions are conceptually proposed as an energy-consuming pathway and closely correlate to light-controllable molecular machines with a rotatable moiety.98 Similar rotor structures in AIE agents transform optical energy into mechanical energy, thus generating a thermal effect.99 However, AIE agents are sterically encumbered in an aggregated state, which largely impedes the molecular motions and suppresses the nonradiative decay.24 To solve the inconsistent mechanism, Tang et al.100 reported the spacer-supported molecular motion in an aggregate state as a strategy to design photothermal AIE agents (Figure 4a). Here, the introduction of long alkyl chains into the highly twisted AIE agents as spacer decreases the restrictions on molecular motion and stabilizes the twisted ICT (TICT) state, which prefers the nonradiative decay.101 The synthesis of three AIE agents, naphthalene diimide-fused 2-(1,3-dithiol-2-ylidene) acetonitrile (NDTA), 2TPE-NDTA, and 2TPE-2NDTA, confirmed this hypothesis. Appended were a TPE group as the intramolecular rotor and a NDTA moiety as the acceptor. The twisting characteristic of the TPE group acts as a strong donor while the NDTA possesses bulky π-conjugation and electron-withdrawing ability, which forms a typical structure favoring TICT. The long alkyl chain is coupled to enable the intermolecular spatial isolation in aggregates to produce the necessary room for intramolecular rotation of the phenyl ring in TPE groups. To prove the preference of the structure toward nonradiative decay, excited-state dynamics revealed that the lifetime for the S1 state of E. coli G(−) bacteria in PBS upon increasing Mg2+ concentration.
Moreover, obvious fluorescence at low temperature confirmed that molecular motion is a critical factor for fluorescence suppression. The change in orbital contribution to the HOMO and LUMO, which demonstrates a great ICT effect, also verified the highly twisted structure underwent the TICT process. The solid-state nuclear magnetic resonance (SSNMR) experiment showed a rapid relaxation time of the TPE moiety in 2TPE-NDTA than in a single TPE molecule, which indicates free motions in 2TPE-NDTA. Therefore, due to the spacer-promoted intramolecular motions, nanoparticles encapsulating AIE agents 2TPE-2NDTA and 2TPE-NDTA possessed robust photothermal conversion efficiencies of 54.9 and 43.0%, respectively (Figure 4b). In a similar work, Tang et al.\textsuperscript{102} applied the design to another AIE structure and confirmed the universality of this strategy among a broad fluorophore species.

Although the chemical decoration of AIE agents with long and flexible alkyl chains can create sufficient free volume for intramolecular motions in an aggregate state to promote efficient photothermal conversion, the improved motion remains sensitive to the external...
However, the synthesis of new photothermal AIE agents inevitably requires a huge investment of time. Intramolecular bond stretching vibration is a molecular motion with high frequency that generally exists within a short distance along the bond axis. Such molecular motion keeps stable towards the environmental restraint and spontaneously produces dark-state energy for boosting the photothermal effect. To investigate the impact of bond stretching vibration compared with structural rotation, Tang et al. synthesized two D–A conjugated AIE agents, DCP-TPA and DCP-PTPA (Figure 4c), with a pyrazine unit and aromatic amine as the electron acceptor and donor, respectively. The pyrazine units also served as bond stretching vibrators to promote intramolecular motions in the excited state. Basic photophysical properties and optimized S0 geometry showed more vigorous molecular motions in DCP-PTPA due to the additional phenyl group, which indicates enhanced vibration modes. The single-molecule reorganization energy ($\lambda$) is one of the key factors to evaluate the influence of molecular motion on the nonradiative decay process. The high-frequency component associated with the bond-stretching vibration showed a primary contribution to the total $\lambda$ (Figure 4d). Moreover, the pyrazino(2,3-b)pyrazine moiety boosted the high-frequency molecular motion and accounts for most of $\lambda$. More than 86% of $\lambda$ in DCP-TPA was contributed by the bond length variation, which also implies the importance of bond stretching vibration in the excited-state relaxation process. Although the proportion of bond stretching vibration in $\lambda$ of DCP-PTPA decreased to 76%, a contribution to stretching vibration from phenyl ring rotation increased from 9.5% to 21% by enhanced vibration modes in the low-frequency region, leading to a higher total $\lambda$ in DCP-PTPA than DCP-TPA. Therefore, DCP-PTPA showed stronger photothermal conversion efficiency due to the presence of more benzene groups. Nevertheless, the intramolecular bond stretching vibration maintained dominance in the motion model.

Strategies utilizing spacer or rotor modification and bond stretching vibration have essentially overcome the restriction on the AIE-based photothermal effect. However, spacer and rotor structures are sensitive to the biological surroundings, and the uncertain design principles limit the application of bond stretching vibration. To achieve sufficient photothermal conversion efficiency in nanoparticles, Tang et al. created disordered amorphous aggregates with loose packing, where molecular motions were still active. The AIE agent TFM, which obtained a twisted D–A–D structure and packing manner of amorphous form in the aggregate state, was synthesized. TFM aggregates displayed a disordered packing in molecular dynamics (MD) simulations (Figure 4e), which indicate deficient resistance for molecular motions. Dihedral angle was also investigated to show a partially restricted rotation in the $\pi$-conjugated system favored by the loose packing (Figure 4f). After encapsulated in nanoparticles, TFM-based nanoparticles showed a great photothermal conversion efficiency of 51.2%, demonstrating that the structure of AIE agents with amorphous packing formed in the aggregate state facilitates the nonradiative decay for PTT.

### AIE agents for PTT applications

Structural design strategies have theoretically boosted the photothermal effects of AIE agents. Further applications in the biomedical field require biocompatible packing of AIE-based photothermal agents. Inspired by the different behaviors of the D–A–D group in polar and non-polar solvents, Lu et al. synthesized BPBBT and utilized its specific affinity toward human serum albumin (HSA) to create HSA-bound BPBBT nanoparticles for near infrared (NIR)–II fluorescence imaging-guided PTT. Van der Waals interactions and forming hydrogen bonds through Ser454 and Lys195 assist the HSA pocket to catch BPBBT. The photophysical properties of BPBBT/HSA complexes were well investigated to reveal the effect of HSA after the specific binding. In non-polar solvents, BPBBT prefers a planar structure and emits intense fluorescence by local excitation (LE), whereas in polar solvents, the TICT state is formed and enhances the nonradiative transition. After binding to HSA, intramolecular rotation in BPBBT was restricted in the protein pocket as an aggregate-like state that suppresses nonradiative decay and activates the NIR-II fluorescence. However, the dihedral angles of BPBBT were changed to reduce planarity after binding to HSA, which formed a largely twisted geometry to drive equilibrium toward the TICT state. The charge transfer in the twisted structure improved the photothermal conversion efficiency of BPBBT. The specific intermediate state provided NIR-II emission and photothermal effect and showed great potential in imaging-guided PTT (Figure 5a). BPBBT/HSA nanoparticles (BPBBT/HSA NPs) maintained the binding activity of HSA toward secreted protein acidic and rich in cysteine (SPARC) following the nonformulation. Meanwhile, the photothermal conversion efficiency of BPBBT/HSA NPs (27.5%) was higher than that of BPBBT in 5% tetrahydrofuran (THF) (22.0%). BPBBT/HSA NPs were then applied for NIR-II fluorescence image-guided PTT in the orthotopic model and metastatic lesions. BPBBT/HSA NPs displayed colocalization with luminophore-labeled cancer cells and SPARC in the tumor, demonstrating the excellent tumor targeting effect of BPBBT/HSA NPs through the albumin-SPARC binding (Figures 5b and 5c). Moreover, the NIR-II imaging specifically identified the two metastatic lesions of the same mouse, which evidenced the sensitivity of NIR-II imaging guidance. Intraoperative NIR-II image-guided PTT utilizing BPBBT/HSA NPs precisely identified both primary and metastatic tumors and achieved a great photothermal therapeutic effect (Figure 5d).

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For traditional PTT, inexhaustive quenching of fluorescence limits the nonradiative decay of photothermal agents, leading to relatively low photothermal conversion efficiency. Since PTT requires irradiating targets with real-time guidance and precision, Tang et al. designed pH-responsive AIE photothermal nanoparticles for photoacoustic (PA) imaging-guided PTT. In this work, AIE agents, NIRb6, NIRb10, and NIRb14, possessing alkyl chains with different lengths as a spacer and the typical structure for TICT state, were synthesized. NIRb14 displayed an excellent photothermal effect, which promised great quality for PA imaging and PTT. To improve tumor uptake and retention in vivo, poly(β-amino ester)-block-polycaprolactone (PAE-b-PCL), a pH-responsive polymer, was employed to encapsulate NIRb14 (Figure 5e). The rapid pH-responsive ability of the PAE moiety in the positively charged shell of NIRb14-PAE/PEG nanoparticles (NIRb14-PAE/PEG NPs) enhanced the binding toward the cytomembrane for retention in the acidic tumor microenvironment. In vitro PA imaging experiments comparing NIRb14-PAE/PEG NPs with other AIE agents with nanoparticles displayed outstanding PA intensity of the designed structure, while in vivo PA imaging on tumor-bearing mice showed that PAE-included nanoparticles possessed better tumor retention and performed the more accurate presentation of tumor zones (Figure 5f). PA-imaging guided PTT were further investigated on the xenograft 4T1 tumor mouse model. A much higher temperature rise in the NIRb14-PAE/PEG NPs treatment group proved the heat generation superiority was retained in the biological environment and achieved convincing phototherapy results (Figure 5g).

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Although PTT through AIE photothermal agents has been commonly effective against general tumor models, glioblastoma (GBM) remains extremely difficult to treat because of the existence of blood–tumor barriers (BTBs) and low penetration of laser irradiation.105 Modulating the permeability of the BTB and receptor-mediated
penetration is a feasible way to enhance the delivery of therapeutic probes for GBM therapy. Tang et al. focused on the kinin B1 receptor (B1R) as a particular target to achieve the transportation of NIR-II AIE photothermal agents into GBM. B1R is specifically inducible in inflammatory diseases, and it assists kinin peptides to work as modulators of the tone and permeability of vessels. Since GBM occurs in an inflammatory environment with tumor proliferation and angiogenesis, B1R is preferred as the pharmaceutical target to minimize side effects. Therefore, [des–Arg]−bradykinin (BK, a type of B1R agonist) was applied as a modified fragment conjugated to the bared carboxylic acid group on the shell of AIE NIR-II nanoparticles to form BK@AIE NPs (Figure 6a). Upon transport to brain cancer lesions, the kinin ligand BK effectively bound on B1R and thus amplified the permeability of BTB, which improves the delivery of BK@AIE NPs. PTT under irradiation of 980 nm laser showed better therapeutic effects because of its deep-tissue penetrating capacity. In vitro experiments were conducted to verify the targeting efficiency of BK@AIE NPs, and PTT-induced immunogenic cell death (ICD) was observed through the exposure of calreticulin proteins. A U87-MG/human vascular endothelial cell (HUVEC) co-culture model was designed to show not only the increased passing rate of BK@AIE NPs but also effective PTT targeting of GBM with minimal damage to the adjacent cells (Figure 6b). In vivo application of BK@AIE NPs displayed excellent brain accumulation (Figure 6c), while PTT performed on orthotopic brain tumor models exhibited a great killing effect toward tumor

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**Figure 6** | (a) Schematic diagram of PTT treatment on glioma-bearing mice and induced local immune-response activation. (b) Scheme of PTT on U87-MG cells and normal human astrocytes in the basal chamber and confocal fluorescence imaging for the viability of cell types in different groups after treatment. (c) In vivo fluorescence imaging on mice at different times after AIE NP and BK@AIE NP injections. (d) Congo red and terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) staining of the brains of glioma-bearing mice following PBS + laser, AIE NP + laser, and BK@AIE NP + laser treatments (scale bar: 100 μm). Reprinted with permission from ref 106. Copyright 2021 WILEY-VCH.

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tissue (Figure 6d), demonstrating the advantage of receptor-mediated penetration of BTB combined with deep-tissue NIR-II PTT.

Summary and Outlook

This review summarizes recent advances in design strategies of AIE agents as PSs or PTT agents. Since energy transfer or electron transfer between the AIE agents and surroundings are largely dependent on the formation of the triplet state, adjustments of $\Delta E_{ST}$ in the chemical structure is a feasible way to improve the ISC efficiency of AIE agents for enhanced PDT. Meanwhile, controllable synthesis of type I or type II AIE-based PSs attracted much interest and various electron-transfer-prefering structures have been developed. Amplified $\pi$-conjugation with the electron-donating group enhances ICT, favoring the type I route. Moreover, the photoreactive structure TPE also contributes to the type I ROS generation by restricted photocyclization for electron transfer toward oxygen species in an aggregate state. Biomedical applications of AIE-based PSs mainly focused on antitumor treatment and antibacterial therapy. Specific PDT with low normal-tissue toxicity led to the development of therapeutic strategies, such as in situ catalyzed AIE-based PSs and a combination of multi-organelle targeting AIE agents. In addition, the charge effect in killing bacteria and the binding affinity of antibiotic-oligomers-modified AIE agents were also discussed. In a reverse thought to boost the nonradiative decay for PTT, photothermal AIE agents were designed with a typical D-A-D structure, which generally preferred the TICT state and enhanced the heat generation of AIE agents. With the introduction of spacers or rotors, the photothermal effect could also be improved by the enhanced intramolecular rotations. Meanwhile, bond stretching vibration was critical in the excited-state relaxation process and dominated the high-frequency component of molecular motions that prefer the nonradiative decay. When the AIE agents were encapsulated in nanoparticles, disordered amorphous aggregates with loose packing were necessary to maintain the rotations of the photothermal AIE agents for PTT. The design strategies to enhance the PDT or PTT performances of AIE agents are summarized and compared in Table 1.

Chemical structures of AIE agents are subtly designed, and various biomedical applications also show great potential in practical treatment. However, there remain several issues to be solved. First, structural design principles are hardly universal in different molecular skeletons, and illustrations of the photophysical mechanism are often provided by theoretical calculations instead of convincing experimental results. For instance, it was supposed that restriction on planarization in TPE prompts the electron transfer from PSs to oxygen species. However, no further verification was conducted to confirm it. Second, either D-A type PSs or D-A-D type photothermal agents highly rely on TPE or triphenylamine moiety as rotors, which largely restricts the tuning of the emission spectrum in all-scale wavelength. Innovative structures with AIE character need to be explored to extend the AIE agents for phototherapy. Moreover, regardless of loose or tight aggregate formation, nanoparticles remain sensitive to biological environments. New methods to construct

### Table 1 | Designing Strategies and Phototherapy Applications of AIE Agents

<table>
<thead>
<tr>
<th>AIE Agents</th>
<th>Designing Strategy</th>
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<tr>
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<tr>
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<td>ICT</td>
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<td>NIRb14</td>
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<td>BBT-C6T-DPA</td>
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biocompatible AIE agents should be developed to guarantee the stability and specificity for in vivo applications. Third, the inherent mechanism of de-excitation in the photothermal conversion process largely depends on inter or intramolecular interaction of AIE agents. Apart from the design strategies of chemical structure to regulate the photothermal effect, the supramolecular self-assembly strategy could also facilitate the nonradiative decay for heat generation.\textsuperscript{107–109} AIE agents with supramolecular interaction should have great potential to achieve high photothermal conversion efficiency and perform well in PTT. Finally, the in vivo fate of AIE agents needs to be carefully examined to advance to clinical applications. Tang and co-workers tested liver function markers including alanine transaminase (ALT) and aspartate transaminase (AST) and renal function markers including creatinine, uric acid, and blood urea nitrogen of mice after intravenous injection of AIE agents for 1 week. The blood routine indexes including white blood cells, lymphocyte, monocyte, granulocyte, hemoglobin, hematocrit test, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, and mean platelet volume of AIE agent-treated mice were also examined. All the measured parameters were in the normal levels, indicating the biosafety of AIE agents in mice.\textsuperscript{10} However, metabolism and degradation of AIE agents in vivo have not been reported in recent studies, which is also important for clinical applications of AIE agents. The biodegradable amphiphilic polymers such as poly(lactide-co-glycolic acid) (PLGA) could be used to encapsulate AIE agents, whereas endowing AIE agents with biodegradability without compromising their optical properties remains a chemical challenge.

Conflict of Interest
The authors declare no conflict of interest.

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