Gathering brings strength: How organic aggregates boost disease phototheranostics

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Abstract
Phototheranostics that concurrently and complementarily integrate real-time diagnosis and in situ therapeutic capabilities in one platform has become the advancing edge of precision medicine. Organic agents possess the merits of facile preparation, high purity, tunable photophysical property, good biocompatibility, and potential biodegradability, which have shown great promise for disease theranostics. This review summarizes the recent achievements of organic phototheranostic agents and applications, especially which rationally utilize energy dissipation pathways of Jablonski diagram to modulate the fluorescence emission, photoacoustic/photothermal production, and photodynamic processes. Of particular interest are the systems exhibiting huge differences in aggregate state as compared with the solution or single molecule form, during which the intramolecular motions play an important role in regulating the photophysical properties. The recent advances from such an aspect for biomedical applications including high-resolution imaging, activatable imaging and therapy, adaptive theranostics, image-guided surgery, immunotherapy, and afterglow imaging are discussed. A brief summary and perspective in this field are also presented. We hope this review will be helpful to the researchers interested in bioprobe design and theranostic applications, and inspire new insights into the linkage between aggregate science and biomedical field.

KEYWORDS
activatable probe, aggregate, aggregation-induced emission, intramolecular motion, Jablonski diagram, phototheranostics

INTRODUCTION

Precision medicine requires noninvasive and accurate early diagnosis of diseases and appropriate treatments.1–3 Phototheranostics that concurrently and complementarily integrate real-time diagnosis and in situ therapeutic capabilities in one platform has become the advancing edge of precision medicine.4–7 Significant progress has been made in the development of new optical agents for diagnosis (e.g., fluorescence imaging, photoacoustic [PA] imaging and optical coherence tomography) and phototherapy (e.g., photodynamic therapy [PDT], photothermal therapy [PTT], and other photo-related therapies).8–12 Thus far, gold nanomaterials, semiconducting quantum dots, rare earth-doped upconversion nanoparticles (NPs), carbon-based nanomaterials, two-dimensional nanostructures, and organic/polymer materials have been developed for phototheranostic applications.13–22 Particularly, organic materials possess the merits of facile production, high purity, good biocompatibility, and potential biodegradability, which have attracted tremendous attention in recent years.23–26 And, the emergence of high-performance organic phototheranostic agents advances the rapid development and clinical translation of precision medicine.

Phototheranostics are based on the conversion of absorbed light into different forms of energy and/or signal for disease diagnostics and therapeutics. According to Jablonski diagram, there are several energy dissipation pathways after a chromophore is excited to the high-energy excited state (Scheme 1).27–29 When the absorbed light is reemitted as low-energy photons, it provides fluorescence/phosphorescence imaging with high sensitivity and spatiotemporal resolution.30–32 When the absorbed photons are converted into localized heat through vibrational deactivation to ground state (S0), this process can be used for PA/photothermal (PT) imaging and PTT of diseases.33–36 The electrons in the singlet excited state (S1) could also transfer to the triplet state (T1), which is used as a probe for photothermal therapy.

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to the triplet excited state (T₁) to produce highly toxic reactive oxygen species (ROS) for PDT.37–40 However, these photophysical energy relaxation processes usually compete with each other, and it will be preferable to maximize the energy dissipation as needed to boost the theranostic outcome. The energy transformation processes are closely related to the intramolecular motion, which would greatly impact the photophysical property. As nanomaterials display some unique properties at disease site such as the enhanced permeability and retention (EPR) effect.41–43 The manipulation of molecular motion of organic agents in the aggregate form represents an effective way to tune the theranostic efficacy, yet it is still undeveloped. From single molecule to solid/aggregate state, the material properties may undergo huge changes.45–46 For example, conventional organic dyes with planar conjugated structures usually show bright fluorescence in solution but the emission is strongly quenched in aggregate, which is called aggregation-caused quenching (ACQ) effect.47,48 An opposite photophysical phenomenon is aggregation-induced emission (AIE), of which the luminogens exhibit weak emission in dilute solution, but become strongly emissive in aggregate/solid states.49,50 These changes associate with different photophysical transition processes, which result in diverse imaging/therapeutic applications. Therefore, there is much room to explore from microscale to mesoscale, for example, the imaging/treatment performance of organic agents can also be readily tuned by changing the aggregate states.

In this review, we summarize the recent advances of organic phototheranostic agents and their applications, especially those exhibiting huge difference in aggregate state as compared with the solution or single molecule form. The excited-state energy dissipation process of optical agents is the main determining factor of theranostic modalities and performance, so we discuss the three energy dissipation pathways according to Jablonski diagram, namely, fluorescence emission via radiative decay, PA/PT through thermal deactivation, and ROS generation in T₁. In each part how the molecular motion and aggregate state tune the photophysical properties and phototheranostic efficacies will be explained. Stemming from the aggregate science, there are a lot of exciting biomedical applications such as high-resolution imaging, activatable imaging and therapy, adaptive theranostics, image-guided surgery, immunotherapy, and afterglow imaging. Due to the rapid development in this field and limited space, the progress in recent 3 years is highlighted. We hope the review will be helpful to the researchers working in bio-probe design and theranostic applications, and inspire more research interests to link the aggregate science and biomedi-cal area.

**FLUORESCENCE EMISSION FOR PRECISE THERANOSTICS**

After first coined by Tang et al. in 2001, the AIE concept has received tremendous attention from a lot of scientists in the world.51–53 In solution state, the violent rotation and vibration of the propeller-like structures in AIE luminogens (AIEgens) could consume the excited-state energy, resulting in dominated nonradiative relaxation. Upon the formation of aggregate, these intramolecular motions are restricted and the nonradiative relaxation is blocked, in which the radiative decay is activated with strong emission. Therefore, restriction of intramolecular motions (RIM) is considered as the main working mechanism of AIE property, which has been employed for luminescent molecular design.54–57 AIEgens have been used in many areas including optoelectronic devices, process and environment monitoring, chemical sensing, and biological imaging.58–65 Thanks to the intrinsic merits of large Stokes shift, high brightness, good stability and biocompatibility, and turn-on property from solution to aggregate, AIEgens have also shown great promise in the field of biomedical applications.66–73

Recently, bioimaging in the second near-infrared (NIR-II, 1000–1700 nm) region has provided new opportunity for in vivo application of optical imaging techniques.74–76 As compared with the visible (400–700 nm) and first NIR (NIR-I, 700–900 nm) window, NIR-II light possesses much lower light–tissue interaction (e.g., scattering, absorption, and reflection), affording high penetration depth and spatial resolution.77–84 For example, the penetration depths of NIR-I and NIR-II fluorescence imaging are ~1 cm and 3 cm, respectively.85,86 Some materials such as carbon nanotubes, quantum dots, rare earth-doped NPs, and organic dyes have been developed as NIR-II emitters.87–90 However, they more or less face the problems of biosafety concern, bad reproducibility, and unsatisfied brightness. Accordingly, it is highly desirable to develop new emitters that could overcome
the aforementioned shortcoming, and AIEgens might be a good candidate. AIEgens are usually twisted structures, and the conjugation is reduced, so it is hard to realize low electronic bandgap or NIR emission.\textsuperscript{91,92} Recently, we and other groups have obtained NIR-II AIEgens by rational molecular design and the utilization of the fundamental rules of Jabloniski diagrams.\textsuperscript{93–98}

According to the “energy gap law,” the brightness of organic luminogens usually decreases with the bathochromic shift of emission wavelength, especially in the long-wavelength NIR region, because the nonradiative deactivation pathways become dominant when the electronic bandgap decreases.\textsuperscript{99,100} Thus, to obtain bright emitters with whole-peak NIR-II emission is really a hard task. Moreover, the low-bandgap chromophores usually possess large conjugation and high molecular weight, so it would be very difficult to process and synthesize them. The NIR-I emitters with partial emission in NIR-II region (off peak) might represent a good solution to realize NIR-II imaging in a fast way since NIR-I fluorophores are more easily available. The highly twisted structure endows AIEgens with lots of conformations, which result in broad emission band and show inherent advantage for off-peak imaging. As depicted in Figure 1(A), a donor–acceptor (D-A) type AIEgen with distorted phenyl and naphthyl substitutes has been reported by Tang and Qian.\textsuperscript{92} The NIR nanodots based on N,N’-(16,7-diphenyl-[1,2,5]thiadiazolo[3,4-g]quinoxaline-4,9-diy1)bis(4,1-phenylene)bis(N-phenylnapththalen-1-amine) (TQ-BPN) exhibited a high photoluminescence quantum yield (PLQY) of 14% with broad emission in the range 700–1200 nm. The in vivo off-peak NIR-II imaging was conducted based on TQ-BPN AIE dots. In vivo NIR-II imaging (Figure 1(B)) indicates that the vascular system of whole mouse body was unambiguously recorded, which showed much better resolution than that of conventional NIR-I window. The in vivo NIR-II microscopic imaging of mouse brain (Figure 1(C)) demonstrated that the blood vasculatures at various imaging depths were clearly visualized, and small capillary vessels could still be distinguished at the depth of 800 \( \mu \)m, which was among the best resolution of in vivo brain imaging. Interestingly, the NIR-II microscopic angiography helped to precisely monitor photothermobic ischemia (PTI) and blood–brain barrier (BBB) damage in mouse brain (Figure 1(D)), which was beneficial for fundamental research and early-stage treatment. Moreover, the AIE dots were capable of visualizing the EPR effect of tumors in different stages (Figure 1(E)), which represented the first example of EPR visualization in tumor blood vessels in situ and revealed that the EPR effect in early stage was more pronounced. This work brings up a new idea of off-peak emission for NIR-II imaging, and more researches with similar concept have been reported by others.\textsuperscript{101–103}

Many researches based on NIR-II imaging have been reported, yet most of which are focused on small animals such as mice (\( \sim 20 \) g), which is far from clinical case in terms of animal size.\textsuperscript{104,105} The exploration of NIR-II imaging in large-size animals would be more advantageous for clinical translation, but seldom reported due to the large tissue depth and increased light scattering. The application of NIR-II AIE dots in living rats (\( \sim 200 \) g) has been demonstrated recently, which showed very good imaging results.\textsuperscript{106} The in vivo long-wavelength NIR-II (\( \sim 1200 \) nm) fluorescence microscopic imaging of rat brain was able to resolve the capillary vessel as small as 9.1 \( \mu \)m in the depth of 700 \( \mu \)m, and helped to detect the blockage of single blood vessel during cerebral thrombosis as well (Figure 1(F)). More recently, the movement of AIEgen-based NIR-II imaging to nonhuman primates has been demonstrated.\textsuperscript{107} Tang, Zheng, and Li achieved clear imaging of the blood vessels of healthy cynomolgus monkeys (3–4 kg) and visualization of axillary artery in an unprecedented depth of 1.5 cm (Figure 1(G)), breaking the current limitation of millimeter-deep fluorescence technique. The blood chemistry and histological analysis in nonhuman primates during a study period of 36 days also suggested good biocompatibility of the NIR-II AIE probes. Although this is a primary study, it will inspire more investigations in large animals and promote clinical translation in human clinical trials.\textsuperscript{108,109}

Inflammation is an indicative omen of many significant illnesses such as diabetes, cancers, and cardiopathy.\textsuperscript{110,111} Thus, noninvasive specific detection and imaging of inflammation are critically beneficial to the early diagnosis of pathogenic processes. However, the brain inflammation can hardly be diagnosed by nanostructured materials, because the brain is protected by BBB, which gives tight control over the passage of substances moving from blood to the tissues of central nervous system.\textsuperscript{112} Currently, several strategies have been adopted to enhance the penetration of NPs across BBB, including incorporation of certain functional targeting ligands or special cell membrane on NPs.\textsuperscript{113,114} For example, neutrophils (NEs) have been reported to possess an innate ability to penetrate BBB, which has been utilized to deliver NPs into brain.\textsuperscript{15,116} Tang and Ding reported that NEs could be utilized as carriers to deliver NIR-II AIE dots for accurate diagnosis of brain inflammation.\textsuperscript{117} The hydrophobic molecule was encapsulated using polymer matrix to afford AIE dots, which were further conjugated with cell-penetrating peptides to enhance NE cell phagocytosis. To make a comparison, the AIE dots conjugated with HIV-1 transactivator of transcription (TAT) protein (AIE-dots-TAT) and indocyanine green (ICG) were, respectively, incubated with NEs (Figure 1(H)), most of which showed significant uptake of fluorescent probe due to the strong phagocytic capabilities. Afterwards, the obtained AIE@NE (AIE dots incubated with NEs) was used to pinpoint brain inflammation of mice. The brain inflammation model was constructed by injecting lipopolysaccharide (LPS) into the lateral cerebral ventricles of mice brains. The fluorescence signal at the inflamed site increased with the AIE@NE injection time and became the strongest at 12 h postinjection (Figure 1(I)). In comparison, the inflamed site was hardly differentiated from healthy tissue in the mice treated with ICG@NE, owing to its weak fluorescence. The quantitative study of the inflamed brain site revealed that the fluorescence intensity in the mice treated with AIE@NE was 6.5-fold higher than that of ICG@NE (Figure 1(J)). These results manifest that AIE dots possess deeper penetration depth and higher brightness, due to the restricted intramolecular motion and increased fluorescence decay.

Based on the RIM mechanism, the fluorescence of AIEgens lights up if changing from the free motion (solution) to restricted intramolecular motion (aggregate) state. This innate nature of AIEgens makes them excellent candidates for turn-on imaging, and a lot of activatable or stimuli-responsive systems have been reported for
FIGURE 1  (A) Chemical structure of \(N,N'-(6,7\text{-diphenyl-}[1,2,5]\text{thiadiazolo}[3,4-g]\text{quinoxaline-4,9-diyl})\text{bis}(4,1\text{-phenylene})\text{bis}(N\text{-phenylnaphthalen-1-amine})\) (TQ-BPN).  (B) NIR-II image of the mouse treated with TQ-BPN dots.  (C) In vivo NIR-II fluorescence microscopic imaging of mouse brain vasculature at various depths.  (D) NIR-II fluorescence microscopic images of brain blood vessels before (i, iii) and after (ii, iv) photothrombotic ischemia induction.  (E) NIR-II fluorescence microscopic imaging for visualizing enhanced permeability and retention (EPR) effect in the old and new tumor sites. The old and new tumors were the subcutaneously xenografted tumors for two and four weeks, respectively.  (F) In vivo NIR-II fluorescence images of rat brain blood vessels before and after the formation of cerebral thrombosis. Red arrows indicate the blocked blood vessels.  (G) In vivo NIR-II fluorescence imaging of the arm and deep axillary artery (∼1.5 cm) of a healthy adult cynomolgus monkey.  (H) Schematic illustration of the NE-mediated NIR-II AIE dots for brain inflammation imaging.  (I) Noninvasive time-dependent in vivo NIR-II fluorescence images of brain inflammation through intact scalp and skull. Red and yellow circles represent the inflamed and healthy brain tissue, respectively.  (J) The average fluorescence signals in the infected region at different time points. ICG: indocyanine green, PL: photoluminescence.  (A–E) Reproduced with permission.\(^\text{92}\) Copyright 2018, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.  (F) Reproduced with permission.\(^\text{106}\) Copyright 2020, Elsevier Ltd.  (G) Reproduced with permission.\(^\text{107}\) (H–J) Reproduced with permission.\(^\text{117}\) Copyright 2020, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim
theranostic applications. Wang and coworkers reported a smart nano delivery system that showed tumor microenvironment responsive targeting, efficient drug delivery, and precise evaluation of therapeutic effect in vivo (Figure 2(A)). The NPs kept “stealth” state in normal tissues and avoided the immune phagocytosis, and activated in the tumor acidic environment, which could actively target tumor tissues and treat them. Moreover, the nanoplatform contained AIEgens, so it exhibited weak emission in normal environment as TPE could rotate freely. The fluorescence “switched on” when apoptosis takes place because DEVD (Asp-Glu-Val-Asp) peptide was cleaved by the overexpressed caspase-3 and the hydrophobic TPE moiety formed bright aggregate. As a result, the system was capable of realizing turn-on fluorescence for precise in vivo imaging, tumor targeting therapy as well as apoptosis monitoring.

In many NPs form, the intraparticle AIEgens can only pack in a relatively loose manner, which significantly compromises their fluorescent brightness. To address this issue, Ding et al. reported a unique method of host–guest interaction to restrict the intramolecular motion of AIEgens and boosted the fluorescence brightness maximally for precise image-guided surgery. They synthesized a positively charged AIEgen, which was able to form host–guest complexation with calix[5]arene by electrostatic interaction (Figure 2(B)). In comparison with the normal DSPE-PEG–based AIE dots, this supramolecular approach exhibited a surprisingly high increase of 31.5 folds in the brightness with a very high PLQY of 72%, which was among the highest values of various red fluorophores. Further investigation indicated that the formation of host–guest complex could significantly turn off both the energy dissipation pathways of thermal deactivation and ROS production, enabling the absorbed excitation energy to focus on fluorescence emission mostly. Theoretical calculation demonstrated that the energy gap between the lowest singlet excited state and triplet excited state (ΔE<sub>ST</sub>) of the AIEgen-calix[5]arene complex was much larger than that of AIEgen itself, in which the intersystem crossing process was suppressed. The AIE dots were further used for fluorescence image-guided cancer surgery in peritoneal carcinomatosis-bearing mouse model. Intraoperative fluorescence imaging suggested that S-AIE dots helped to outline the intraperitoneal metastatic tumors in a more accurate way due to the prominent EPR effect (Figures 2(C) and 2(D)). Consequently, a very high signal-to-background ratio (SBR) of 48.5 was realized with S-AIE dots, which was much better than that of DSPE-PEG-AIE dots, and represented the best result of fluorescence image-guided surgery via intravenous injection. This work opens a new avenue on the utilization of host–guest interaction to boost the fluorescence brightness of AIEgens.

PTD has been used in clinic to treat some superficial or localized tumors and other diseases. However, the relatively low efficiency caused by tumor microenvironment (e.g., hypoxia) and shallow penetration depth of excitation light limit its large-scale real applications. Some strategies have been proposed to overcome the shortcoming of PTD. Tang and Li reported the tumor-exocytosed exosome/AIEgen hybrid nanovesicles (DES) to facilitate efficient tumor penetration during PTD (Figure 2(D)). The tumor-derived exosomes were employed to couple with an AIEgen that could produce ROS efficiently. The obtained DES displayed good tumor-targeting ability both in vitro and in vivo. They further used dexamethasone to normalize the vascular function and reduce local hypoxia, thereby significantly enhancing the PDT efficacy of DES. The in vivo applications revealed that this combined approach greatly enhanced the PDT performance. Recently, Zhang’s group demonstrated the first example of using AIEgen to realize sonodynamic therapy (SDT). SDT was first proposed by Umemura et al. in 1989, and regarded as a good candidate to increase the penetration depth of PDT as ultrasound (US) has good tissue penetration ability. The AIE NPs were able to generate ROS upon US irradiation (Figure 2(E)). More interestingly, the comparison of ROS generation triggered by light and US with the obstruction of different thicknesses of pork lean (Figure 2(F)) clearly manifested the advantage of SDT in terms of tissue penetration depth. The good penetration of SDT may prove a new avenue for the clinical translation of AIEgens, for example, to treat the deeply located tumors. Since PDT and SDT possess similar working mechanism, some efficient AIEgen-based PDT agents are expected to be applicable for SDT.

**PA IMAGING AND PTT**

PA imaging is an emerging noninvasive and nonionized biomedical imaging modality that has attracted considerable interest as it could provide high penetration depth, spatial resolution, and 3D images. And, the penetration depths of PA imaging in NIR-I and NIR-II region are as high as 5 cm and 11 cm. The PA signal is generated by the pulsed laser excitation to convert photons into localized heat to induce transient thermo-elastic expansion and wideband acoustic waves for imaging. Therefore, two processes are involved in PA transition: photo-to-thermo and thermo-to-acoustic transitions, in which the first pathway is closely related to the nonradiative deactivation and the second process is influenced by the thermodynamic property. To realize high PT/PA transition, it is preferable to make as much absorbed energy as possible concentrate on the nonradiative deactivation pathway through chemical or physical methods. At present, the fluorescence quenching and intramolecular motion facilitated PT conversion have appeared as powerful strategies for boosting in vivo theranostics.

The planar organic molecules are easy to form a large aggregate through intermolecular interaction like π–π stacking, which usually quenches the fluorescence significantly. This process can be utilized to realize amplified PA/PT property according to Jablonski diagram and enhance agent accumulation at disease site as well. For example, Yan’s group reported a supramolecular strategy to fabricate PT nanodots through peptide-modulated self-assembly of photoactive porphyrins. As shown in Figure 3(A), the PT nanodots of around 25 nm were fabricated by tuning the molecular interactions of the porphyrin–peptide conjugates. The absorption spectra of TPP-G-FF and PPP-NDs (Figure 3(B)) revealed a bathochromic shift and broadening for Soret and Q bands, indicating that the π–π stacking of tetr phenyl porphyrin (TPP) contributed to the formation of PPP-NDs. They further investigated the photophysical properties of the nanodots for PT application. The PLQY of TPP-G-FF and PPP-NDs were calculated to be...
FIGURE 2  (A) The peptide decoration format and their transition process on the surface of STD-NM. (B) S-AIE dots and DSPE-PEG AIE dots and chemical structure of CC5A-12C. (C) Representative fluorescence imaging of the intraperitoneal tumor nodules after intravenous injection of S-AIE dots and DSPE-PEG-AIE dots. (D) Illustration of tumor exocytosed exosome/AIEgen hybrid nanovesicles (DES) facilitating efficient tumor penetration and PDT. (E) Illustration of AIE mediated sonodynamic therapy (SDT). (F) The ROS generation of AIE NPs triggered by light and ultrasound (US) with the obstruction of pork lean with different thicknesses. The absorbance of 1,3-diphenylisobenzofuran (DPBF) at 416 nm was used as the indicator. (A) Reproduced with permission.125 Copyright 2018 Elsevier Ltd. (B, C) Reproduced with permission.128 (D) Reproduced with permission.132 (E, F) Reproduced with permission.133 Copyright 2020, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim
FIGURE 3  (A) Self-assembly of the peptide-porphyrin conjugate (TPP-G-FF) into PT peptide-porphyrin nanodots (PPP-NDs). (B) Absorption spectra of TPPG-FF and PPP-NDs. (C) PA images of mice after intravenous injection of PPP-NDs. (D) Tumor volume of the mice with different treatments. (E) Chemical structure of the probe and the macrophage chemotaxis-instructed S. aureus infection detection in vivo. (F) Absorption spectra of PRC in H2O/DMSO mixture with different volume ratios (from 0% to 100%). (G) PA intensity curves of PRC dimers and assemblies. (H) PA image of intracellular infection in vivo. (I) Chemical structure and the CAF-instructed self-assembly of nanofibers in situ for enhanced tumor imaging. (A–D) Reproduced with permission.146 Copyright 2017 American Chemical Society. (E–H) Reproduced with permission.149 Copyright 2018 American Chemical Society. (I) Reproduced with permission.150 Copyright 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim
0.062 and 0, respectively, suggesting that the fluorescence process was greatly blocked for the strong intermolecular interaction. Another competitive pathway of thermal deactivation is the generation of ROS. Interestingly, the singlet oxygen (\(1O_2\)) quantum yield of PPP-NDs was 0 by monitoring the characteristic phosphorescence emission band of \(1O_2\) at 1270 nm, indicating a quenching yield of higher than 99%. The suppression of both fluorescence and \(1O_2\) generation in the porphyrin-peptide aggregate could be attributed to the strong \(\pi-\pi\) stacking, which significantly boosted the vibrational deactivation channel, and led to a relatively high light-to-heat conversion efficiency of 54.2%. The nanodots were successfully used for PA imaging and PT antitumor therapy in vivo (Figures 3(C) and 3(D)).

Wang and coworkers proposed a kind of aggregation-induced retention (AIR) effect, in which the formation of nanostructured materials by supramolecular self-assembly at the disease site could extend the retention time, amplify the imaging signal, and improve the treatment outcome (e.g., hyperthermia therapy, antitumor hydrogel, and autophagy-mediated chemotherapy).\(^{147-151}\) Several self-assembled nanosystems have been developed based on this design concept. They reported a chlorophyll-peptide-based PA agent (MPC) for in vivo bioimaging of bacterial infections in phagocytic cells (Figure 3(E)).\(^{149}\) The probe contained three motifs: (i) mannose as an active-targeting ligand for specifically recognizing macrophages; (ii) peptide YVHDC as a responsive unit for cleavage by the activated caspase-1; (iii) P18-AL derivative as a PA signal pertaining for assembly-induced PA signal enhancement. The macrophage could uptake MPC, which would be delivered to the infectious site in a highly efficient manner. The enzyme (i.e., caspase-1) could be immediately activated upon the infection of macrophages by bacteria cells, which efficiently cleaved the peptide-based probe. Afterwards, the chlorophyll derivative self-assembled into J-type aggregates and accumulated inside the macrophage. As a result, the AIR effect significantly enhanced the probe accumulation at the infectious site and the J-type aggregate greatly amplified PS signal. The formation of J-type aggregates was confirmed by the UV–vis absorption spectra (Figure 3(F)), as the original Qy absorption band at a 676 nm decreased, broadened, and redshifted to 697 nm upon self-assembly. The PA measurement (Figure 3(G)) suggested that the PA signal of the self-assembled J-aggregate increased for more than twofolds, which could be assigned to the intensified absorption intensity in the NIR spectral region. Based on the in vivo self-assembly approach, the average PA signal in the infected site was enhanced by about 2.6-fold due to the ATR effect and PA enhancement (Figure 3(H)). J-aggregation usually leads to predominant photoexcited decays via nonradiative channel, which can be utilized to develop highly efficient PA imaging and PT therapy.\(^{152-155}\) For instance, the absorption spectrum undergoes bathochromic shift when forming J-type aggregation, which would be advantageous for sensitive turn-on/ratiometric imaging.

Recently, the same group further developed a peptide-based NIR probe that was responsive to fibroblast activation protein-\(\alpha\) (FAP-\(\alpha\)), and specifically formed nanofibers on the surface of cancer-associated fibroblasts (CAFs) in situ.\(^{150}\) The peptide probe consisted of a hydrophilic part, a tailoring motif, a self-assembly unit, and a cyanine dye. As depicted in Figure 3(I), the peptide probe was specifically tailored by FAP-\(\alpha\) on CAFs, which resulted in a highly efficient in situ construction of \(\beta\)-sheet fibers that induced enhanced accumulation and retention on the membrane of CAFs. Owing to the formation of large-size nanofiber in situ, the probe showed a long blood half-life of up to 77 min, which was much longer than that of free cyanine dye such as ICG (2.5 min). The pronounced AIR effect of the probe around tumor displayed a 5.5-fold signal enhancement in the tumor 48 h after administration as compared to that of control peptide-probe molecule. ICG dye underwent quick clearance from the whole body in 4 h, but was hard to accumulate in the tumor. In contrast, the probe exhibited a long detectable time of 48 h for tumor diagnosis. More importantly, the targeted assembly of the probe resulted in higher intensity in tumor, which was over four- and fivefold higher than that of liver and kidney, respectively.

The RIM mechanism of AIEgens reveals that the restricted intramolecular motion could intensify fluorescence, whereas the molecular motion, on the other side, consumes the excited state energy through thermal deactivation, benefitting for PA and PT applications.\(^{156-158}\) Unfortunately, the currently available molecular rotors only show intense intramolecular motion in solution, which is significantly suppressed in aggregate or solid state.\(^{159,160}\) Tang and Ding introduced the NIR-absorbing molecule with intramolecular motion-induced phototherapy (iMIPT) in aggregate state with amplified PT/PA signal (Figure 4(A)).\(^{161}\) The comparison of the molecules possessing different molecular rotors/motors indicated that strong intramolecular motion in the solid state was observed in the TPE-containing structures. It is noted that the long alkyl chain plays an important role in promoting the excited-state intramolecular motion in aggregate since it enables the intermolecular spatial isolation to produce some necessary room. By virtue of the active excited-state intramolecular motion and strong light absorptivity, the NPs displayed a high photothermal conversion efficiency (PCE) of 55% (Figures 4(B) and 4(C)). The NPs were used as a high-performing agent for PA imaging of tumor in vivo (Figure 4(D)). The concept of iMIPT will inspire more interest in the exploration of superior PA imaging probes with active excited-state intramolecular motion in aggregate state.

Complete removal of tumor tissues is critically important for tumor surgery and even curing cancers.\(^{162}\) However, it is really difficult for surgeons to differentiate tiny tumor nodules and the margin between tumor and normal tissues by naked eyes. The image-guided surgery has emerged as a promising method to address this issue, which calls for diverse imaging methods to meet the requirements of cancer operation in different stages.\(^{163,164}\) Thus, the multimodality imaging agents with tunable functions would benefit to improve the surgery outcome. To realize precise image-guided surgery, we have developed a kind of one-for-all organic agent with three optimal optical imaging properties by making full use of the intramolecular motions for precise cancer surgery (Figure 4(E)).\(^{165}\) The intramolecular motion was changed in different aggregate states, in which the resulting fluorescence, PA, and Raman properties could be tuned precisely. As a result, the nanoagent with maximal fluorescence-PA-Raman signals was obtained thanks to both the large conjugated substitutes and intense intramolecular motions. The NPs were employed to decipher tumor information at different surgical stages (Figure 4(F)). By making full use of
FIGURE 4  (A) The working mechanism of iMIPT. (B) IR thermal images and (C) temperature changes of various NPs. (D) PA intensities of tumor and muscle tissues as a function of time after intravenous injection. (E) Chemical structure of the FL-PA-Raman agent (OTPA-TQ3). (F) Schematic illustration of the one-for-all organic agent for image-guided cancer surgery in different stages. (G) Representative fluorescence and PA images of tumor-bearing mice after intravenous injection of OTPA-TQ3 NPs. (H) Representative fluorescence images, Raman imaging and H&E-stained tissues of the operative incision site after S1. FLI: fluorescence imaging, PAI: photoacoustic imaging, RI: Raman imaging. (I) Survival curves for the tumor-bearing mice with various treatments. (J) Representative H&E-stained images of the lung slices from different groups. Control: the tumor-bearing without any surgery, S1: the tumor-bearing mice underwent surgery with the experience of a surgeon, S2: the tumor-bearing mice without any intraoperative FLI and RI signals. (A–D) Reproduced with permission.161 (E–J) Reproduced with permission.165 Copyright 2019, Elsevier Inc.
the high sensitivity of fluorescence and good spatial resolution and penetration depth of PA technique, the preoperative fluorescence and PA imaging (Figure 4(G)) were capable of providing comprehensive information about tumors. During surgery, the fast, real-time, and sensitive fluorescence imaging helped to determine the tumor sites but there were still some suspicious areas with faint fluorescence. The high-contrast zero-background Raman imaging in the cell-silent region (1800–2800 cm⁻¹) delineated the residual tiny tumors and tumor-normal tissue margins in a sensitive high-contrast manner (Figure 4(H)). The smart multimodality nanogent could help the surgeon accurately remove all of the tiny residual tumors, which greatly prolonged the lifetimes of mice and reduced metastases (Figures 4(I) and (J)). This kind of one-for-all agents favors comprehensive cancer surgery, rendering great promise for clinical translation.

More researches about the intramolecular motion within NPs for promoting nonradiative deactivation have been reported and applied in biomedical field. Lee and coworkers developed conjugated oligomer NPs with ultra-high PCE for cancer theranostics (Figure 5(A)). The π-conjugated oligomer was designed with strong “A−D−A” interaction, which not only led to a low energy bandgap with NIR light-harvesting ability, but also triggered strong intramolecular charge transfer with almost completely quenched fluorescence. Moreover, the ethylene link between D and A moieties enabled more flexible intramolecular rotation, together with the twisted molecular structure and alkyl substituents, the PCE reached as high as 82% in NPs. The NPs could be used for PA imaging and PT therapy of tumor in vivo (Figure 5(B)). Moreover, the nanogent could also be biodegraded by endogenous enzymes to avoid long-term toxicity concerns. Peng and Sun reported a kind of barrier-free group rotation promoting PCE for PT therapy. They synthesized a BODIPY derivative with −CF₃ moiety, which could rotate freely without an energy barrier to result in ultra-efficient nonradiative decay and maximize the conversion of light into heat (Figure 5(C)). The microscopic dynamics study demonstrated that the dehedral angle of the molecule was widely distributed between −180° and 180° in both solution and NPs aggregate states, suggesting free rotational motion without restriction by steric hindrance (Figures 5(D) and 5(E)). Importantly, the agent exhibited an ultrahigh PCE of 88% thanks to the introduction of −CF₃ moiety in the meso-position of BODIPY scaffold. The “barrier-free” rotation of −CF₃ provided a pathway for efficient nonradiative decay, thus enabling excellent PCE. In vitro and in vivo experiments (Figures 5(F) and 5(G)) indicated that the new agent served as an efficient probe for PA imaging and PT ablation of tumor under safe NIR laser irradiation (0.3 W cm⁻², 808 nm). Recently, Li’s group adopted a double bond-based molecular motor concept to develop highly efficient PT agents. They synthesized an imino-based molecular motor that induced easier intramolecular motions through the double-bond torsion-causing photoinduced nonadiabatic decay effect. Upon light irradiation, the double bond was twisted because of the strong twisted intramolecular charge transfer (TICT) effect, thus the excited agents underwent nonradiative deactivation through the conical intersection (CI) of internal conversion (Figure 5(H)). Surprisingly, such an agent possessed a high PCE of 90% in aggregate and NPs (Figure 5(I)), which was the highest reported PCE for organic/polymer chromophores. The NPs were used for low-temperature PT therapy of tumor in the presence of a heat shock protein 70 (HSP70) inhibitor, apoptosis (Apo). Collectively, the new concept of intramolecular motion boosting PT/PA conversion will inspire the development of advanced PT agents and expend the biomedical applications.

**ACTIVATABLE ROS GENERATION**

According to Jablonski diagram, the electron in S₁ could transfer to the more reactive T₁, in which it may produce ROS or ¹O₂, being able to cause cell damage and apoptosis. There are mainly two types of mechanisms for PDT. In the first pathway (Type I), the photosensitizer (PS) in T₁ interacts with activated substrate (e.g., proteins and nucleic acids) to transfer the energy to oxygen directly, and free radical such as hydroxyl radical (OH⁻), superoxide anion (O₂⁻), or hydrogen peroxide (H₂O₂) is generated.

In Type II PDT, direct energy transfer from PS to oxygen leads to the generation of ¹O₂. The Type II PDT is more dependent on the concentration of oxygen than Type I, which might reduce the efficiency in hypoxia environment such as tumor site. An interesting phenomenon is observed in AIEgens, that is, the ROS or ¹O₂ generation ability increases greatly in aggregate state, accompanying with the enhanced fluorescence efficiency. The aggregation-induced ROS generation has emerged as a promising new type of PDT that can boost the theranostic outcome in a great deal. The unique ROS production capacity of AIEgens in different aggregate states can be used for exploiting phototheranostic agents with turn-on property and reduced undesired side effect. Liu’s group reported an activatable AIE theranostic probe for detecting bacterial infection and killing survival bacteria inside macrophages. The AIE probe contained two moieties: One part was the responsive peptide that could be cleaved by the activated casp-1 during bacterial infection, and the other part was the AIE-active PyTPE unit. The probe had good water solubility at first, so it was in the silent state with weak fluorescence and negligible phototoxicity with low ROS generation. After cleaved by casp-1, the residual hydrophobic AIEgen would self-assemble into aggregates and accumulate on the bacterial phagosomes and produce ROS to kill the intracellular bacteria under light irradiation (Figure 6(A)). The cellular experiments suggested that the probe assembled on the phagosome containing S. aureus because the active casp-1 accumulated on the phagosomes. The AIEgen could further eradicate the bacteria surviving in macrophages through PDT under white light irradiation (Figure 6(B)). This kind of molecular design represents an effective strategy to develop activatable phototheranostic probes.

Immunogenic cell death (ICD) plays a pivotal role in cancer immunotherapy as the released damage-associated molecular patterns could improve immunogenicity. The development of ICD inducer has attracted considerable interest, for example, some PSs such as chlorin e6 (Ce6), hypericin, and temoporfin have been reported to be able to induce ICD of cancer cells through causing oxidative stress under light irradiation. However, the immunotherapy efficacy of these PSs against cancer is unsatisfied. Ding et al. reported a new way of using AIE-based PSs to induce mitochondrial...
oxidative stress to evoke abundant and large-scale ICD. The positively charged molecule TPE-DPA-TCyP was mainly distributed in mitochondria after incubation with 4T1 cancer cells, which lit up the fluorescence and ROS generation ability thanks to the AIE signature (Figure 6(C)). Since cancer cells possess high negative mitochondrial membrane potential, the electrostatic interaction enables the aggregation of TPE-DPA-TCyP in mitochondrial membrane. Interestingly, the mitochondrial-anchoring TPE-DPA-TcyP caused focused mitochondrial oxidative stress under light excitation that was able to greatly amplify ICD induction (Figure 6(D)), which was even better than the widely reported...
FIGURE 6  (A) Schematic illustration of the macrophage-mediated intracellular bacterial infection diagnosis and elimination and chemical structure of PyTPE-CRP. (B) Confocal images showing the localization of PyTPE-CRP and ROS detection inside macrophages. (C) Chemical structure and dihedral angle of TPE-DPA-TCyP. (D) Confocal images of 4T1 cancer cells treated with TPE-DPA-TCyP or TPE-DPA-TCyP NPs, which were co-stained with commercial MitoTracker Deep Red FM. Scale bar: 20 μm. (E) The proposed mechanism of TPE-DPA-TCyP as an effective ICD inducer for antitumor immunity. (F) Schematic illustration of using a prophylactic tumor vaccination model to evaluate the in vivo ICD immunogenicity of different ICD inducers. (G) Plot of tumor volume in different groups versus the time post 4T1 cancer cell inoculation. (A and B) Reproduced with permission. Copyright 2020 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (C–G) Reproduced with permission. Copyright 2020 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim
ICD inducers including Ce6, and oxaliplatin. The working mechanism of TPE-DPA-TCyP in inducing antitumor immunity and immune-memory effect was simultaneously triggering both innate and adaptive immune systems (Figure 6(E)), which had been verified by immune cell analyses. The in vivo tumor treatment results indicated that TPEPEDA-TCyP-treated cell vaccine could significantly inhibit tumor growth, more effective than other PS-based ICD inducers (Figures 6(F) and 6(G)). This work demonstrates the first example that AIEgens serve as an effective ICD inducer, which would inspire more insights in this area.

Although efficient, PDT also faces some drawbacks, for example, the dark toxicity for the “always-on” model and low tumor specificity. After injection, the PS also exists in healthy organs and tissues, which will generate toxic ROS and affect body health adversely upon exposure to light. So patients have to stay in the dark for a long period (usually a few weeks) after administration of the “always-on” PDT agent. As such, the development of adaptive or switchable PSs is an ideal solution. Huang and Liu constructed a supramolecular switch by changing the host–guest interaction of an AIEgen PS (G) and a water-soluble pillar[5]arene (WP5) in different pH environments (Figure 7(A)). The alkyl chain of G was threaded into the cavity of WP5 in pH of 7.4, in which both the fluorescence and ROS production turned off. After adjusting pH to 5.0, the carboxylate group in WP5 was protonated, resulting in more hydrophobic product P5. The neutral P5 moved to the endmost pyridinium unit and complexed with it through cation-π interactions, which switched on the fluorescence and ROS generation (Figures 7(B) and 7(C)). Since the tumor sites were acidic, this kind of pH-switchable PSs could be used for turn-on tumor imaging and therapy. To avoid the undesired dark toxicity, Tang, Ding, and Feng proposed the substitution activated phototheranostics using host–guest strategy for activatable PDT. The host–guest complexation between positively charged TPE-PHO and negatively charged p-sulfonatocalix[4]arene (SC4A) with negative charge could greatly decrease the dark toxicity of TPE-PHO itself and enhanced the biocompatibility (Figure 7(D)). The addition of 4,4′-benzidine dihydrochloride (BZD) competed and displaced TPE-PHO from the cavity of SC4A, which allowed the dissociative hydrophobic TPE-PHO to form aggregate and restored the dark cytotoxicity and ROS production ability under light irradiation (Figure 7(E)). In vivo applications verified that the activatable agent showed improved tumor inhibition property as compared with the silent host–guest complex (Figure 7(F)). This work represents a feasible approach to realize activatable PDT, for example, disease biomarker or microenvironment responsive platforms could be expected.

Afterglow luminescence, also called persistent luminescence, which is generally caused by the slow release of photons from energy traps in the materials after stopping light excitation, holds great potential for advanced biomedical applications due to the enormous advantages such as independence to external light source and elimination of tissue autofluorescence. Nevertheless, high-performance organic persistent luminescence materials are still undeveloped. Recently, Ding et al. reported a kind of NIR AIE dots with long luminescence time and high tumor-to-liver ratio for image-guided surgery. The afterglow luminescent (AGL) AIE dots could emit persistent luminescence for more than 10 days after single light excitation through a series of chemical and photochemical processes, including \( ^1 \text{O}_2 \) production by 2-(2-(2-(4′-(2,2-bis(4-methoxyphenyl)-1-phenylvinyl)-[1,1′-biphenyl]-4-yl)vinyl)-4H-chromen-4-ylidine)malononitrile (TPE-Ph-DMC), Schaap’s dioxetane formation, chemi-excitation by dioxetane decomposition, and energy transfer back to TPE-Ph-DMC (Figure 8(A)). Thanks to the AIE signature, both fluorescence brightness and \( ^1 \text{O}_2 \) generation ability increased in the aggregate state, which were beneficial for boosting the afterglow luminescence. Interestingly, the AGL AIE dots exhibited persistent luminescence lasting for more than 10 days after the cessation of excitation (Figures 8(B) and 8(c)), which was the longest time of all the available organic and polymeric afterglow luminescent probes. The AGL AIE dots exhibited great advantages in the image-guided tumor surgery (Figure 8(D)). An interesting phenomenon was that the afterglow signal quenching rate of AGL AIE dots in the homogenates of liver and spleen and kidney was very fast and nearly disappeared in 2 h, while the afterglow signals in tumor decreased in a much slower manner, leading to high tumor-to-normal tissues ratios. In-depth study revealed that the afterglow signal of AGL AIE dots could be greatly quenched by aspartate aminotransferase (AST), which was known to distribute in liver and kidney largely. This unique property affords high-performance imaging as most nanomaterials tend to accumulate in liver.

Fluorescence and PA imaging techniques possess their own strengths and weaknesses, and more importantly, they have complementary advantages. Fluorescence technique holds the advantage of excellent sensitivity but lacks spatial resolution. On the hand, PA imaging shows centimeter-depth penetration ability but suffers from low sensitivity. Therefore, the integration of fluorescence and PA imaging modes is decidedly beneficial for precise imaging with good penetration and high sensitivity. However, fluorescence and PA imaging correspond to radiative and nonradiative decays, respectively, which are competitive processes according to Jablonski diagram. Therefore, it is highly desirable but really difficult to develop one organic agent with high fluorescence and PA signal on demand. To overcome this difficulty, we introduced a smart organic agent whose absorbed excitation energy could mostly concentrate on either the pathway of thermal deactivation for PA imaging, or the opposed routes for fluorescence imaging and PDT, which was simply triggered by external light stimuli (Figure 8(E)). Interestingly, the closed-form isomer was a relatively planar core structure, which possessed strong intermolecular interaction to suppress radiative decay and the maximal PA signal could be obtained. On the other hand, the open form structure was highly twisted, which afforded AIE feature with excellent fluorescence emission and ROS production. Thus, the photophysical energy transition processes could be facilely controlled by simple external light trigger (Figure 8(F)). The smart nanoagents exhibited excellent performance in promoting in vivo cancer surgery outcomes by preoperative PA imaging and intraoperative fluorescent visualization/PDT of residual tumors (Figures 8(g)–8(I)).
FIGURE 7  (A) Chemical structures and adaptive mechanism of the host-guest system. (B) The ROS generation property and (C) fluorescence spectra of different systems. (D) Schematic illustration of the substitution-activated phototheranostics. (E) Confocal images of HeLa cells stained with TPEPHO⊂SC4A and MTDR, with and without BZD treatment. (F) Tumor growth curve of the mice with different treatments. (A–C) Reproduced with permission. Copyright 2020 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (D–F) Reproduced with permission. Copyright 2020 American Chemical Society.
FIGURE 8  (A) Schematic illustration of the mechanism for amplified NIR afterglow luminescence for promoting image-guided cancer surgery. (B) NIR afterglow decay images and (C) intensity of AIE dots at different time post white light (0.2 W cm\(^{-2}\)) preirradiation for 2 min. (D) The number of tumor nodules versus nodule diameter excised via unguided and AIE dots afterglow imaging-guided surgery. (E) Photo-controlled reversibility and optimized geometric structures of DTE-TPECM molecules, and photographs of RClosed-DTE-TPECM and ROpen-DTE-TPECM powders. (F) Schematic illustration of the controllable photophysical processes of RClosed-DTE-TPECM and ROpen-DTE-TPECM. (G) Plot of PA intensity in tumor versus postinjection time. Inset shows the PA image of tumor site. (H) Bright-field and fluorescence images, and H&E stained tissues during tumor surgery. (I) Quantitative analysis of bioluminescence intensities of residual tumors from the mice with various treatments. (A–D) Reproduced with permission\(^{194}\) Copyright 2018, American Chemical Society. (E–I) Reproduced with permission\(^{198}\)
SUMMARY AND OUTLOOK

Organic agents have shown excellent performance for phototheranostic applications, especially by tuning the aggregate states. The significant property changes from single molecule to aggregate provide promising opportunities for the revolution of precision medicine. In this review, we summarize the recent advances of organic aggregates for boosting disease phototheranostic outcome from the viewpoint of various energy dissipation pathways driven by Jablonski diagram. The intense brightness enhancement of AIEgens from solution/free motion to aggregate/restricted-intramolecular-motion state greatly improves the disease diagnosis and treatment results. For instance, NIR-II AIEgens have shown very excellent penetration depth and spatial resolution for in vivo imaging, not only in small animals, but also large animals like nonhuman primates. The considerable increase of fluorescence brightness and ROS generation ability when AIEgens form aggregate brings superb diagnostics and therapy outcomes. Further researches can be focused on several aspects of in vivo phototheranostic systems based on aggregate science. The first-in-human NIR-II imaging-guided surgery with ICG has been reported recently, which will motivate more relevant researches. The NIR-II AIEgens with superior imaging performance and long-term biosafety can be used for clinical trials and even translation. The intramolecular motion elevating PT/PA signal is interesting but still undeveloped. The next step should concentrate on how to make full use of this exciting result, for example, the activatable bioprobes with tunable PA imaging and PT therapy. Phosphorescence imaging represents a bright imaging modality because the interference from background autofluorescence can be eliminated from time scale. Although there are some reports about in vivo phosphorescence imaging, still in the infancy. The room temperature phosphorescence (RTP) agents with red/NIR light excitation, improved stability, intravenous injection, and integration with therapeutic effect are favorable for in vivo applications. Most papers about phototheranostic agents are focused on tumor imaging and therapy. More attention should be paid to the precise diagnosis and treatment of other intractable diseases such as Alzheimer’s disease (AD), Parkinson’s disease (PD), and cardiovascular and cerebrovascular diseases. In conclusion, the manipulation of aggregate represents a powerful strategy for boosting the in vivo phototheranostic performance and we hope that this review will inspire more thoughts into the linkage between aggregate science and biomedical area.

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