Aggregation-enhanced theranostics: AIE sparkles in biomedical field

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Abstract
Theranostics referring to the ingenious integration of diagnostics and therapeutics has garnered tremendous attention in these years as it provides a promising opportunity for modern personalized and precision medicine. By virtue of the good biocompatibility, outstanding fluorescence property, easy processability and functionalization, promoted photosensitizing efficiency, as well as facile construction of multi-modality theranostics, fluorophores with aggregation-induced emission (AIE) characteristics exhibit inexhaustible and vigorous vitality in the field of theranostics. Numerous significant breakthroughs and state-of-the-art progression have been witnessed in the past few years. This review highlights the tremendous aggregation-enhanced superiorities of AIE luminogens (AIEgens) in disease theranostics mainly involving diagnostic imaging (fluorescence and room temperature phosphorescence), therapeutic intervention (photodynamic therapy), and feasibility in construction of multi-modality theranostics based on the experimental measurements and theoretical simulations. Additionally, the latest and advanced developments of AIEgens in disease theranostics in the aspect of corresponding strategies to design highly effective AIE-active theranostics through triggering aggregation formation are comprehensively summarized. Moreover, a brief conclusion with the discussion of current challenges and future perspectives in this area is further presented.

1 INTRODUCTION
Aggregate science that spotlights meso territory is captivating increasing research interests nowadays as it builds a significant bridge between the micro- and macrofields.11 Although the concept of aggregate science is newly put forward, the issues about aggregates based on various elements in terms of phenomenon, mechanism, and applications have actually long been studied.12 As a cutting-edge scientific research area, aggregation-induced emission (AIE) is the springboard of aggregate science and serves as a guiding light in the progression of aggregate science. The concept of AIE was first defined by Tang et al in 2001, when a unique phenomenon that the fluorophore exhibits much enhanced luminescence at aggregate level than its isolated molecular elements was observed.3 After 20 years of flourishing development, AIE research has shed new light on the fundamental understanding of chemistry and material science.4,5 Up to now, AIE-related research includes but not limited to the aggregation-induced fluorescence features, for instance, crystallization-induced emission (CIE), room temperature phosphorescence (RTP), clusterization-triggered emission (CTE), and many other extensive research area based on aggregate have been successfully explored and most of them have gained...
widespread research interests. Benefiting from their favorable superiorities, AIE luminogens (AIEgens) have been witnessed to be pervasively penetrating into various fields, such as chemo-/biosensing, optoelectronic devices, information encryption, biomedical applications, and other important areas (Figure 1).

Of all the explored applications based on AIEgens in the past two decades, the most intriguing advancements could be made in the biomedical field, particularly in the analysis of various bioactive species, monitoring of dynamic biological processes, giving much credit to the exploration of AIE-based turn-on probes and AIE-based nanoparticles (NPs). For instance, on the basis of its excellent fluorescence property, the smooth implementation of AIEgens in targeted cellular and subcellular imaging, vascular imaging, tumor imaging, and corresponding disease diagnosis has been witnessed.

Enthused by the outstanding performances of AIEgens in bioimaging, increasing efforts have also been channeled toward endowing AIEgens with additional therapeutically functional properties, such as photodynamic and photothermal behaviors, which largely accelerated the development of AIE theranostic systems, further expanding the application scope of AIEgens from sensing and imaging probes to theranostic agents. These systems allowing simultaneous diagnostic imaging and phototherapy intervention rightly satisfy the increasing demands of personalized and precision medicine in terms of therapeutic outcomes, representing an attractive approach to accelerate the progression of contemporary personalized and precision medicine. Compared with some of the traditional materials, such as quantum dots (QDs), carbon dots (CDs), and inorganic NPs, AIE-active theranostic agents possess a unique advantage, such as good biosafety, facile processability, easy functionalization, excellent fluorescence properties (e.g., high fluorescence quantum yield and quantum efficiency in aggregates, outstanding photostability, large Stokes shift, real-time and on-site activation ability) as well as promoted phototherapies. Moreover, along with the exploration of multifunctional AIE NPs, in which fluorescence and photoacoustic imaging together with photodynamic and photothermal therapies could be achieved simultaneously without the aid of any functional groups, multi-modality one-for-all theranostic systems have been successfully obtained by tailoring AIEgens for optimized multifunctionality.

In this review, we highlight the tremendous advantages of AIEgens in terms of diagnostic imaging, phototherapy, and feasibility in construction of multi-modality theranostics, and summarize the recent advances of AIEgens in disease theranostics. The systematic elucidation of aggregation-enhanced diagnostic imaging and therapy, involving fluorescence, reactive oxygen species (ROS) generation, multifunctionality, and RTP, from the perspective of experimental measurements and theoretical simulations, are first elaborated and put forward as a concept of aggregation-enhanced theranostics (AET) (Figure 2). Then, the latest advances observed in the field of AET based on AIEgens are introduced and classified into two parts primarily based on the aggregation approaches. In the first part, we summarize the turn-on theranostic systems based on in situ aggregation of AIEgens. The current developed theranostic systems involving various interaction-driven aggregation with biotargets and biological stimuli-responsive self-aggregation of AIEgens are showcased in detail. In the second part, enhanced theranostics based on preaggregation via supramolecular assembly is elaborated. Last, the current limitations, challenges, and perspectives for future opportunities in this research field are also discussed. It is expected that this review article cannot only provide an integrated picture of the up-to-date developments as well as a systemic elaboration of tremendous superiorities of AIEgens in theranostic applications, but also offer valuable insights for further research pursuit in promoting the translation of AIEgen-based theranostics for widespread clinical applications.

FIGURE 1 The overall development of AIE in terms of concept, wide application and splendid extension. Abbreviations: CIE, crystallization-induced emission; RTP, room temperature phosphorescence; AIDF, aggregation-induced delayed fluorescence; CTE, clusterization-triggered emission; ML, mechanoluminescence; CPL, circularly polarized luminescence.
2 | AGGREGATION-INDUCED SUPERIORITY OF AIEGENS IN THERANOSTICS

2.1 | Aggregation-enhanced fluorescence

Steaming from the increasing requirements of present-day theranostics, various modalities of diagnosis involving fluorescence imaging (FLI), photoacoustic imaging (PAI), magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), and ultrasound (US) as well as diverse therapeutic modalities including photodynamic therapy (PDT), photothermal therapy (PTT), chemotherapy (CHT), radiation therapy (RT), gene therapy (GT), and immunotherapy (IMT) have been employed to the construction of multifarious theranostic systems.[35–37] Among all these diagnostic modalities, FLI is an indispensable technique and plays an irreplaceable role in diagnostic imaging benefitting from its superb sensitivity, real-time and on-site responsiveness, easy operability, noninvasive attribute, and so forth. In addition, organic materials are considered as fantastic candidates for FLI by virtue of its high biosafety, tunable photophysical characteristics, good tailorability, and facile processability.[38] However, suffering from the aggregation-caused quenching (ACQ) effect, the intrinsic fluorescence signals of most traditional organic dyes are dramatically weakened or even vanish upon aggregation, ascribing to the intermolecular π-π stacking (Figure 3A).[7,39,40] Moreover, the structurally hydrophobic organic dyes with imperative aromatic rings and long conjugated chains are naturally inclined to forming aggregates in aqueous physiological conditions, leading to severe self-quenching phenomenon. Hence, organic dyes can only be used in very dilute concentrations for bioimaging (e.g., 25–500 nM for MitoTracker probes and 50–75 nM for LysoTracker dyes; Life Technologies), in which condition, however, the small amount of fluorophores is more susceptible to the light irradiation, resulting in poor photostability.[8,15] In addition, the limited number of fluorescent molecules preliminary penetrated into the cell often diffuse back to the extracellular media owing to the concentration gradient during the cell division process, which results in a decrease in the emission of the stained cells and a concurrent increase in the solution emission, leading to lowered imaging contrast and short cell tracking duration. These drawbacks largely compromise the performances of conventional organic dyes in diagnostic imaging.

AIEgens provide a straightforward protocol to this notorious problem. Unlike ACQ fluorophores, AIEgens exhibit fairly weak or nearly nonemission in dilute solutions but emit particularly enhanced fluorescence upon forming aggregates (Figure 3B).[7] When AIEgens remain dissolved state in solutions, the excitation energy is dramatically consumed by the active intramolecular motions through nonradiative thermal deactivation process, leading to fluorescence quenching. Upon aggregation, the intramolecular motions are largely restricted through the mechanism of restriction of intramolecular motion (RIM), which efficiently switch the consumption pathways from nonradiative relaxation to radiative decay, thus boosting fluorescence.[7,40] In addition, the nonplanar conformations of AIEgens also effectively hinder the detrimental π-π stacking that ubiquitously occurs in ACQ luminogens. Guided by the mechanism of RIM, a great deal of AIEgens with diverse molecular structures were successfully designed and developed, the emission color covers the entire visible spectrum and extends to the near-infrared (NIR) range.[41–47] Compared with the ACQ fluorophores, the feature of aggregation-enhanced fluorescence typically empowers AIEgens with the application feasibility at relative high concentration or in aggregate state with intense fluorescent brightness and extraordinary photostability, which are particularly favorable to high-quality FLI, long-term fluorescence tracking, and FLI-guided therapy.

2.2 | Aggregation-enhanced generation of ROS

Aside from the significantly enhanced release of fluorescence owing to the largely suppression of nonradiative thermal deactivation upon aggregation, the exciton may also undergo other deactivation pathways, for example, nonradiative intersystem crossing (ISC) from the lowest singlet state (S1) to the lowest excited triplet state (T1), to release energy accompanying with subsequent electron or energy transfer to triplet oxygen to generate ROS.[26] Photosensitizers (PSs) with efficient ROS generation efficiency play a decisive role in PDT application, in which cell apoptosis or necrosis, vascular damage, and probably the immunity response could be synchronously triggered by oxidative ROS.[48,49] AIEgens have been recently reported to be extremely efficient PSs for ROS generation as aggregates, because the effective suppression of nonradiative thermal dissipation could largely promote ISC process, consequently boosting the production of ROS.[52] Additionally, it has been demonstrated that the aggregation forming process of fluorophores from single molecules can give rise to energy splitting and generate more ISC channel between excited singlet and triplet states, thus resulting in reduced energy gap (ΔE S,T) and stimulated
ISC, which is known as aggregation-induced intersystem crossing (AI-ISC). According to this theory, the yield of the excited triplet state would be elevated at aggregate state owing to the promoted ISC rate, thereby boosting ROS generation. It is rational to conclude that the suppression of energy loss in excited state through nonradiative thermal deactivation process and reduction of $\Delta E_{S-T}$ could synergistically promote ISC and eventually improve the yield of triplet excited state, making AIEgens excellent PSs at aggregate state. This deduction was first verified by Liu in 2015, the ROS generation efficiency of AIEgen TPECM at both dissolved and aggregate state were evaluated by using 9,10-anthracenediy1bis-(methylene)dimalonic acid (ABDA) as an indicator (Figure 4A). There was almost no ROS production in both dilute dimethylsulfoxide (DMSO) and tetrahydrofuran (THF) solutions of TPECM, while extremely high ROS generation efficiency was measured in aqueous medium, in which condition TPECM existed as aggregates. Furthermore, the gradual enhancement of ROS generation along with the aggregates formation process was observed by Tang et al. As demonstrated in Figure 4B, the ROS generation efficiency of AIEgen TPANPF$_6$ was positively related to the aggregates formation along with the gradual increase of the water fraction ($f_w$) in DMSO solution. Moreover, the relationship between $\Delta E_{S-T}$ and ROS generation is also evidenced by experimental measurements. A series
of AIEgens exhibiting orderly reduced $\Delta E_{S-T}$ values were designed and synthesized by Wang and coworkers.\textsuperscript{53} Taking MTi, TPE-indo, and MeO-TPE-Bz, for example, the $\Delta E_{S-T}$ of these three AIEgens were calculated as 0.633, 0.664, and 0.882 eV, respectively (Figure 4C). ROS measurement results illustrated that MTi with the smallest $\Delta E_{S-T}$ value provided the greatest performance on ROS generation among these AIEgens, evidently substantiating that enhancing ISC efficiency through reducing $\Delta E_{S-T}$ can improve ROS generation efficiency.

This favorable phenomenon of much higher ROS generation efficiency at aggregates compared to isolated molecular species was coined as aggregation-induced generation of ROS (AIG-ROS).\textsuperscript{6} Besides the aggregation-enhanced emission feature, finely designed AIE PSs also exhibit AIG-ROS characteristics.\textsuperscript{26,54–56} Contrary to conventional PSs (e.g., porphyrin derivatives), which go through dramatically decreased fluorescence and ROS generation upon aggregation due to the strong $\pi-\pi^*$ stacking at aggregate state,\textsuperscript{57,60} AIE PSs make good use of aggregation that naturally occurs in biological system to exhibit enhanced fluorescence and ROS generation efficiency in aggregate state, showing great superiorities in FLI-guided PDT for disease theranostics.\textsuperscript{58,59} For instance, lots of AIE PSs have been reported to exhibit much higher ROS generation efficiency than the renowned PS Rose Bengal (RB).\textsuperscript{122,44,82} Additionally, as hydrophobic organic molecules naturally aggregate in aqueous medium and are usually utilized in theranostics in the form of NPs, AIEgens offer the unique opportunity to form nanoaggregates or NPs with high emission brightness and efficient ROS production. Most attractively, AIE NPs display almost linear loading-dependent increase in both brightness and ROS generation efficiency, which is essentially distinct from ACQ PSs-based NPs that exhibit compromised brightness and photosensitization at high loading density.\textsuperscript{60} This appealing feature promotes a rapid progression of AIEgens in FLI-guided PDT in these years.

2.3 | Aggregation-enhanced multifunctionality

In addition to the tremendous advantages of AIEgens in FLI-guided PDT, AIE aggregates or NPs also offer huge opportunities in constructing one-for-all theranostic platforms based on single AIE component by deliberately modulating the balance among different energy deactivation routes. On the basis of the Jablonski diagram, after photoexcitation, the excited energy could undergo energy dissipation through three main pathways (Figure 5A).\textsuperscript{61} First, fluorescence is released through the direct radiative decay from $S_1$ to ground state ($S_0$), which is used for FLI and FLI-guided therapy. Second, the nonradiative thermal deactivation transition from $S_1$ to $S_0$ mediated by intramolecular motions as well as collisions with the surroundings results in the generation of heat energy, which can be directly used for PTT. Meanwhile, photothermally converted acoustic wave is desired for PAI. Third, efficient nonradiative ISC takes place when the $\Delta E_{S-T}$ is sufficiently small, which facilitates the formation of ROS, thereby enabling the PDT application. As the absorbed excitation energy is usually fixed for one molecule, meanwhile these three dissipation pathways are always fiercely competitive\textsuperscript{62} and very hard to balance in a single system, it seems impossible to obtain all-powerful theranostic agents to fulfill multi-modality imaging and therapy in terms of FLI, PAI, PDT, and PTT simultaneously based on single molecular species. Surprisingly, through elegant molecular design, AIEgens with well-tuned molecular structures make this feasible\textsuperscript{32,34} Together with the aggregation-facilitated radiative decay and ISC process, reserving part of molecular motions that guarantee considerable excited energy consumption through thermal deactivation in aggregate state is of crucial importance on the way to realize the all-round theranostics. Benefiting from the inherent twisted conformation in propeller-like shape and abundant freely motioned molecular rotators or vibrators in structures, AIEgens have proven to be extremely applicable in concurrently retaining all of these three energy dissipation pathways via forming aggregates with a feature of relatively loose-packed structures, where part of intramolecular motions are still active. By this means, the balance between radiative and nonradiative decays could be successfully achieved in aggregate state. These features make AIEgens good templates for balancing energy dissipations, as well as for constructing multi-modality theranostics (Figure 5B).\textsuperscript{33}

By taking fully advantages of the excited energy, several AIE-active multi-modality theranostic systems have been successfully constructed, some of which even displayed NIR-II emission.\textsuperscript{123,32–34,63} Compared with the theranostic systems with single imaging and therapy modality, the integration of multiple diagnostic and therapeutic modalities would definitely benefit to the fulfillment of wealthy and precise imaging information as well as synergistic therapeutic effect by coordinating the imaging quality and therapy efficiency. In addition, multifunctional AIEgens could endow the multimodal theranostics systems with precious one-for-all feature, which shows great advantages than all-in-one ones.
2.4 | Aggregation-enhanced RTP

According to the Jablonski diagram, besides transferring energy or electron to triplet oxygen to generate ROS, these triplet excitons can also undergo radiative transition from $T_1$ to $S_0$ accompanying with phosphorescence release.\[61\] Distinct from fluorescence, the lifetime of phosphorescence is far longer, ranging from micro-seconds to seconds. Organic luminogens with phosphorescence, particularly persistent RTP with long lifetime (>100 ms) have attracted great attention as they hold great potential in ultrasensitive in vivo afterglow imaging that can minimize the influence of biological auto-fluorescence/background interference, hence providing excellent signal-to-background ratio (SBR) and penetration depth.\[64\] Remarkable as it is, the realization of pure organic RTP luminogens is still a challenging task. Based on the comprehensive understanding of the phosphorescence mechanism, it is rational to anticipate that aggregation can effectively contribute to the RTP owing to the mechanism of AI-ISC\[65–68\]. For example, some crystals with extremely compact molecular packing and substantial intermolecular electronic interactions were reported to exhibit RTP.\[69\] Besides, efficient RTP can only be achieved in crystals rather than in amorphous aggregates, further providing solid evidence to verify the contributions of aggregation in promoting RTP ascribing to the maximum molecular packing extent and furthest facilitation of ISC process in crystals. Under this circumstance, for the purpose of biomedical application, nanocrystallization strategy is introduced to fabricate NPs for bioimaging. Such NPs bearing an intense afterglow in aqueous milieu are successfully used for background-independent and high-contrast in vivo imaging with minimized background interference.\[70–73\]

Additionally, crystallization is also regarded as an effective strategy to further elevate the fluorescence brightness and ROS generation efficiency of many AIEgens, as the packing degree of molecules increases to the maximum through crystallization, which remarkably minimizes the intramolecular motions.\[74,75\] Liu’s group reported that the singlet oxygen ($^1O_2$) generation efficiency and fluorescence quantum yield (QY) of a PS could be elevated by 50% and over twofold at the same time after being fabricated into NPs through nanocrystallization method.\[76\] In addition, as a general and simple method to realize closer and ordered molecular packing, crystallization could also contribute to enhance the molar extinction coefficients with red-shifted absorption maxima for some AIEgens.\[75\]

2.5 | Other advantages

In addition to the above-mentioned four outstanding superiors involved in theranostics profited from aggregation, AIEgens have displayed several other merits including good biocompatibility (the essential feature of materials for bioplications), facile processability, readily tuneable photophysical properties, ease of functionalization, as well as perfect nanoaggregation size for enhanced permeability and retention (EPR) effect. Particularly, many other distinct superiorities of AIEgens have also been witnessed and deserved to be highlighted here: First, as a kind of phototheranostics, AIE-active theranostics represents high controllability, as the theranostic effects take place only in the presence of both AIEgens and light irradiation, which greatly improves the theranostic specificity with minimal side effects and damage to normal tissues or organs.\[32\] Second, theranostics based on AIEgens is feasible to be endowed with turn-on feature on the basis of RIM mechanism, the fluorescence and ROS could be momentarily turned on upon aggregation responsive to specific biotargets such as subcellular structures, disease biomarkers, external stimuli, or microenvironment changes, largely improving the theranostic specificity and minimizing systematic toxicity.\[13\] Moreover, the turn-on feature of AIEgens also plays a role in signaling or monitoring of the delivery and activation of drugs, as well as therapeutic outcomes. Third, AIE nanoaggregates or NPs possess excellent aggregation-enhanced retention capability, which is preferred for therapeutic drugs in cancer treatment because the long retention time can largely prolong the action time in lesion area.\[15\] Fourth, besides one-photon theranostics, some aggregation-induced enhancement of nonlinear optical effects, such as aggregation-enhanced multiphoton (two-/three-photon) absorption, has also been discovered. Multiphoton excitation using NIR light can achieve higher penetration depth due to the lower scattering and absorption by biological molecules in that region. Last, some natural products have been reported to inherently exhibit AIE characteristics.\[77,78\] Taking berberine (BBR), an isoquinoline alkaloid isolated from herbal plants, for example, recent study has shown that BBR with natural AIE features was successfully applied in cancer and bacteria theranostics in terms of FLI-involved diagnosis and PDT, giving a wonderful demonstration of the promising applications in disease theranostics of the explored natural AIEgens.\[79\]

3 | TURN-ON THERANOSTICS BASED ON IN SITU AGGREGATION

The AET feature endows AIEgens with a favorable theranostic turn-on nature in terms of fluorescence and ROS generation, showing significantly enhanced theranostic efficacy with potentially improved specificity and minimized systematic toxicity. By taking advantage of the RIM mechanism, a class of turn-on theranostic systems based on AIEgens have been developed via specifically aggregate with biotargets or undergo self-aggregation process responding to biological stimuli in a hydrophilic environment, resulting in largely improved therapeutic outcomes (Figure 6).\[8,10,80\] In addition to the disease diagnosis and therapy, the real-time monitoring of delivery and activation of theranostic agents, as well as therapeutic responses can also be successfully achieved by virtue of the turn-on feature of these activatable theranostic systems.

3.1 | Aggregation with biotargets

3.1.1 | Aggregation via physical interaction with biotargets

Physical interactions, especially electrostatic interaction and hydrophobic effect, have been widely used as natural driving
FIGURE 6  Schematic illustration of turn-on theranostics based on in situ aggregation of AIEgens. (A) Various interactions-driven aggregation with biotargets. (B) Self-aggregation responding to biological stimuli

forces to facilitate the aggregation between AIEgens and biotargets. Charged AIEgens are prone to accumulate on the oppositely charged biotargets via electrostatic interactions, where the AIEgens are compelled to be restricted in poky space, leading to the restriction of intramolecular motion and thus remarkably enhanced fluorescence and ROS production. Majority of organelle-targeted cancer theranostics as well as bacterial discrimination are demonstrated to rely on this mechanism.[81–83] It is worth noting that hydrophobic interactions also contribute to these specific imaging processes, particularly for those molecules with aromatic or hydrophobic structures. In addition, the hydrophobic interaction also plays an important role in real-time monitoring the folding or fibrillation processes or conformational transitions of disease-associated proteins, such as Alzheimer’s or Parkinson disease.[84,85]

Cell membrane serving as a protecting boundary between a living cell and its surroundings is essentially important in maintaining the integrity of the cell. The locally generated ROS in the cell membrane could efficiently result in the changes of membrane permeability as well as the losses of membrane fluidity and integrity through oxidating the cholesterol and other unsaturated phospholipids, thus leading to necrosis-mediated cell death.[86] Consequently, cell membrane is recognized as an ideal target for PDT. Aiming to achieve effective cancer cell ablation effect via cell membrane-targeted PDT, Tang’s group reported a water-soluble NIR-emissive AIEgen, namely TTVP (Figure 7A).[87] The positively charged TTVP initially maintains nonemissive in aqueous condition thanks to its good water solubility, and its NIR fluorescence quickly turns on upon embedding into the phospholipid bilayer of cell membrane driven by electrostatic and hydrophobic interaction. Unlike the commonly used plasma membrane-specific probes (e.g., DiO and CellMask) that require tedious washing procedures,[86,88] this fluorescence turn-on feature endows TTVP with good cell membrane imaging ability bearing high SBR in a wash-free manner, which do not need postwashing process after cell staining. Besides, the extremely ultrafast staining ability was attributed to the quick diffusion of TTVP. Additionally, TTVP was also proven to be a powerful ROS generator and can effectively kill cancer cells at a very low concentration (1.0 μM) with the aid of white light irradiation via cell membrane-mediated PDT.
Mitochondria are cell’s energy stations and play a vital role in cell’s growth and division. The dysfunction of mitochondria can effectively induce cell apoptosis, making it a promising target for cancer therapy. Since the mitochondrial membrane potential (MMP) of cancer cells (−180 mV) is more negative than those of normal cells, a myriad of mitochondria-targeted theranostic AIEgens have been developed for selective cancer cells imaging and ablation based on the stronger electrostatic interaction between the probes with the mitochondria of cancer cells.[89] Zhao and coworkers developed a mitochondria-targeted cationic AIE probe TPE-DQN with photodynamic activity (Figure 7B).[90] Due to its suitable cationic nature and unique AIE feature, TPE-DQN could successfully discriminate cancer cells over normal cells through specifically turning on its NIR fluorescence upon effective accumulation on the mitochondria of cancer cells driven by electrostatic interaction. Of the two AIEgens they developed in this work, TPE-DQN exhibited more efficient 1O2 generation (0.83) owing to the smaller singlet-triplet energy gap. In vivo evaluation of TPE-DQN was subsequently performed in CT26-colon tumor-bearing mice, showing considerable tumor inhibition effect through FLI-guided PDT.

In order to monitor the treatment progress and therapeutic effect of PDT in situ, a self-reporting AIE-active theranostic probe TPE-4EP+ was developed by Tang’s group (Figure 7C).[91] Similarly, the positively charged TPE-4EP+ can specifically stain cancer cells against normal cells through assembly with the more negatively charged mitochondrial membrane driven by electrostatic interaction. Upon white irradiation, cell apoptosis can be induced by the intrinsic 1O2 generation of TPE-4EP+, accompanying with the mitochondria-to-nucleus translocation of the red fluorescence, thereby enabling the real-time monitoring of apoptosis process. It is demonstrated that the strong interaction with DNA driven by its multivalent positive charge accounted for the in situ translocation of TPE-4EP+. This work empowered the theranostic probes new opportunities for monitoring the process of photodynamic therapy so that the end point of PDT could be exactly judged expediently. In addition, some natural AIEgens are also reported to exhibit similar self-reporting abilities in the therapeutic process.[179]

Recently, a molecular AIE platform with synergetic mitochondria-targeted combinatory therapy of PDT and CHT was developed by Liu and coworkers.[92] The single-molecular AIE theranostic agent TNPT was designed based on AIE core of TPE, positively charged pyridine, and chemotherapeutic drug of cisplatin. With balanced hydrophobic-hydrophilic property, electro-donating (D)-accepting (A) strength, and intramolecular charge transfer effect, TNPT exhibited predominant mitochondria location of cancer cells, high chemotherapeutic efficacy similar to that of cisplatin, and strong ROS generation capability better than that of chlorin e6 (Ce6). Importantly, obvious C6 glioma cells apoptosis resulted by synergetic photodynamic-chemotherapy was observed, which was further explained by the inhibition of DNA replication.

### 3.1.2 Aggregation via specific recognition of biotargets

High specificity of imaging and therapy toward lesion region is of crucial importance in disease theranostics as it can maximize therapeutic efficacy and minimize side effects. Considering the complexity in living systems, diagnostic imaging
based on physical interactions is often perturbed by interfering analytes, leading to false positive/negative diagnosis, greatly compromised the theranostic effect. To address the specificity issue, novel design principles based on the specific recognition between biotargets and targeting ligands conjugated with the AIE cores have been proposed. In order to prepare targeted theranostic agents with turn-on nature, hydrophilic targeting ligands such as proteins, peptide, aptamer, and targeted drugs are selected to conjugate to the AIE core. The high affinity between these targeting ligands and receptors on the biotargets activates the RIM process of AIEgens, thus resulting in the turn-on response.

Transferrin receptor (TfR), a type of transmembrane glycoprotein, plays a crucial role in iron transport and cellular respiration. Generally, it presents in both normal cells and cancer cells, while cancer cells express much higher TfR than normal cells due to the much faster proliferation ubiquitously occurs in abnormal cells. Thus, TfR could serve as a potentially biomarker for early diagnosis of cancer and excellent delivery target for therapeutic agents. Liu’s group prepared a cancer cell-targeted AIE-active theranostic probe TPETH-2T7 by conjugating two TfR-targeting peptide T7 to AIEgen TPETH (Figure 8A). The introduction of the hydrophilic T7 linker makes this probe water-soluble and able to keep in a dissolved and nearly nonemissive state in aqueous condition. Through the specific recognition between T7 and TfR, TPETH-2T7 can specifically turn on its red fluorescence on the cell membrane of TfR-overexpressed cancer cells against normal cells. The unique on-site fluorescence turn-on response of TPETH-2T7 also enables the study of interaction between the probe with TfR and real-time tracking of TfR. A better therapeutic performance was observed when TPETH-2T7 localized on the plasma membrane than in cytoplasm, indicating the improved PDT treatment efficiency due to the membrane-targeted location.

In addition to TfR, many types of tumors also over express transmembrane receptor tyrosine kinase EphA2, which has been considered as one of the most attractive targets for the design of antitumor drugs. Tang et al. designed an
EphA2-specific targeting AIEgen (TPE-Py-FFGYSA) by introducing a targeting peptide sequence YSAYPDSPVMMS (YSA, in short) to a typical AIE moiety TPE-Py (Figure 8B). In addition, tripeptide FFG as a self-assembly-aided unit was incorporated between TPE-Py and YSA moieties, since dipeptide FF (F: phenylalanine) has been widely demonstrated to be able to favor supramolecular self-assembly by intermolecular aromatic–aromatic interactions. The integration of targeting group and self-assembly-aided unit resulted in the formation of TPE-Py-containing assemblies on EphA2 protein cluster. The tight intermolecular stacking between FFG sequences strongly blocked the intramolecular rotations of TPE unit, leading to a much higher fluorescent signal output for tracking EphA2 cluster, as compared to TPE-Py-YSA that lacks FFG sequence. What is more, they also demonstrated that the intracellular oxidative environment evoked by TPE-Py-FFGYSA-bound EphA2 clusters upon light irradiation could significantly amplify the antitumor activity of paclitaxel with a synergistic effect of “0 + 1 > 1.” Western blot studies further revealed that such synergistic antitumor efficacy was attributed to the enhanced inhibition of p-Akt, which led to a mitochondria-originated apoptosis. Similarly, Kim et al. synthesized a heteroatom-containing spiropolymer P1a2b, which could intrinsically target MDM2 protein that is overexpressed in various cancerous cells. P1a2b exhibited the binding between P1a2b and MDM2 inhibited the anti-apoptotic p53/MDM2 interaction, causing the release of p53, a cancer suppressor protein, ultimately leading to apoptosis in cancerous cell lines, without appreciable cytotoxicity toward normal cells. Apart from the enhanced theranostics via assembly with protein overexpressed in cancerous cells, bacteria can also be specially diagnosed and treated by modifying a specific bacteria targeting group to an AIE-active moiety. Liu et al. designed a sensitive probe for diagnosis and treatment of Gram-positive bacterial infection. The probe is consisted of a short peptide sequence DFDFDYDEnGDK (n = 1, 2, or 3) as the self-assembly unit, a TPE derivative connected to the N-terminal as the AIE-active moiety and vancomycin connected to the C-terminal as the targeting group (Figure 8C). D-confuguration amino acids were used in order to enhance the stability of these probes in vivo. To adjust the hydrophilicity and study the self-assembly performance, three probes (E-probe, EE-probe, and EEE-probe) were synthesized with different numbers of glutamic acids (n = 1, 2, and 3). Among them, E-probes have a lowest critical assembly concentration in aqueous medium, indicating the best self-performance of E-probe. The combination of targeting group and self-assembly unit enabled the selective binding and in situ self-assembly of the probes on the surface of Gram-positive bacteria, resulting in a strong fluorescence turn-on, which favored the sensitive detection of Gram-positive bacteria both in vitro and in vivo. Furthermore, the authors also demonstrated that E-probe could generate more ROS in the aggregation state as compared to the free E-probe, thus E-probe exhibited excellent therapeutic effect to Gram-positive bacterial infection in vivo while the bare AIEgen without targeting group and self-assembly unit modification did not. They speculated that this assembly-induced ROS enhancement was attributed to the adjustments of HOMO-LUMO distributions or enhanced ISC efficiency.

### 3.1.3 Aggregation via chemical/biological reaction with biotargets

Apart from the noncovalently recognition, the aggregation of AIEgens with biotargets can also be mediated by chemical or biological reactions, such as bio-orthogonal click reaction and metabolic engineering, which are garnering increasing interests recently. This appealing approach relies on the conjugation of functional reactive groups such as bio-orthogonal clickable or metabolizable groups to the AIE cores. The molecular motion of AIEgens will be largely restricted when a unique and specific chemical or biological reaction takes place, and the theranostics thus are subsequently turned on. Taking advantages of its high specificity, various examples based on AIEgens have been developed for cancer theranostics as well as bacterial labeling, identification, and killing.

Liu’s group developed an AIE-active theranostic probe (TPEBAI) for real-time bio-orthogonal turn-on labeling and image-guided photodynamic cancer cell ablation (Figure 9A). A dual-responsive metabolic precursor cRGD-S-Ac3-ManNAz was preliminary developed and utilized to selectively label the cell membrane of tripeptide glutathione (GSH) and αvβ3 integrin overexpressed cancer cells with azide groups through generating unnatural glycans. Driven by its AIE characteristic, TPEBAI exhibited weak fluorescence in aqueous medium or in the absence of specific biomolecules, the bright red fluorescence of which specifically turned on due to the restriction of the intramolecular motion upon a click reaction with azide groups on the surface of MDA-MB-231 cells with GSH and αvβ3 integrin overexpressed, enabling specific cancer cell imaging with high SBR. Due to the low expression level of αvβ3 integrin, the fluorescent signal is much weaker in MCF-7 cancer cells. Similarly, the fluorescence intensity of normal 293T cells is the lowest, owing to the low expression of both αvβ3 integrin and intracellular GSH concentration. Moreover, the strong ROS generation efficacy of TPEBAI resulted in an efficient cancer cell ablation upon white light irradiation than that of normal 293T cells. The integration of cancer cell-targeted metabolic precursor and AIE-active PS with turn-on features is promising for selective imaging and ablation of specific cancer cells through coupled metabolic labeling and bio-orthogonal click reaction.

This two-step bio-orthogonal labeling method was also applied in the field of bacterial identification and killing by Liu and coworkers. A theranostic probe TPEPA was developed for the discrimination and precise ablation of certain type of bacteria. As illustrated in Figure 9B, D-alanine (D-Ala) derivative D-Ala-N3 and 3-deoxy-D-mannooctulosonic acid (Kdo) derivative Kdo-N3 were chosen as the metabolic precursors to be incorporated into peptidoglycan (PG) layer of Gram-positive bacteria and lipopolysaccharide (LPS) inserted outer membrane of Gram-negative bacteria, respectively. Then, the subsequent introduction of TPEPA with two terminal alkyne groups could effectively label bacteria through a click reaction. Thanks to the AIE property of
TPEPA, the water-soluble TPEPA was nonemissive in aqueous medium, whereas a strong fluorescence appeared due to the restriction of the intramolecular motion after click reaction. Owing to the ROS generation of TPEPA under light irradiation, the probe also exhibited selective bacterial ablation with high specificity. To simplify the labeling steps, Liu et al. reported another metabolic probe TPACN-D-Ala consisting both AIE PS and metabolic motif in one molecule to achieve precise in vivo bacterial detection and photodynamic killing (Figure 9C) [109]. Due to the high selectivity of D-Ala toward Gram-positive bacteria, TPACN-D-Ala could precisely label the bacteria inside living host cells or hidden in biofilms, while showing good biocompatibility to normal tissues during the photodynamic treatment. More attractively, after intravenous injection of TPACB-D-Ala in vivo, the invasive bacteria in the infected site could be clearly labeled and effectively eliminated upon light irradiation.

### 3.2 Self-aggregation responding to biological stimuli

Apart from aggregation with biotargets, in situ self-aggregation of AIEgens triggered by the stimulation of biological stimuli represents another appealing approach to design turn-on probes for enhanced theranostics. The development of stimuli-responsive theranostic system based on AIEgens has attracted considerable attention for specific disease diagnosis and treatment in these years. Since AIEgens are intrinsically prone to aggregate in aqueous medium due to their structural hydrophobicity, the turn-on feature could be tactfully obtained by designing water-soluble AIEgens and duly triggering the aggregation through decreasing the solubility responding to biological stimuli. These water-soluble AIEgens are generally designed to consist hydrophobic AIE cores as emitting centers, hydrophilic moieties guaranteeing the overall hydrophility of the AIE conjugates and/or reactive stimuli-cleavable groups as the linker. Due to the AET feature, the fluorescence and ROS generation of these AIEgens maintain “OFF” state in aqueous medium and could be significantly boosted upon the stimuli-responsive formation of aggregates due to the decreased solubility, resulting from the leaving of hydrophilic moieties or self-polymerization (Figure 6B). Recently, some endogenous stimuli, such as enzymes, GSH, and ROS, have been exploited to trigger the self-aggregation of AIEgens to achieve precise disease theranostics with minimal side effects.
### 3.2.1 | Self-aggregation responding to enzyme

The overexpressed specific enzyme in disease sites can act as a profitable stimulus to activate self-aggregation of AIEgens for turn-on theranostics. Generally, these enzyme-responsive systems are designed by incorporating enzyme cleavable peptide sequences. For instance, Liu’s group reported a caspase-1 (casp-1)-responsive AIE bioconjugate-based fluorescent bioprobe (PyTPE-CRP) by conjugating an AIE fluorophore (PyTPE) with an enzyme cleavable peptide linker (NEAYVHDAP) for detecting bacterial infection and eliminating survival bacteria inside macrophages.\(^{[110]}\) As described in Figure 10A, the peptide substrate of PyTPE-CRP could be specifically cleaved by the activated caspase-1 in bacterial infection macrophages, then the resultant residues self-aggregated into stable spherical aggregates and accumulated on the bacterial phagosomes, which resulted in the inhibition of nonradiative energy dissipation, leading to fluorescence turn-on inside macrophages. Meanwhile, the ROS generating ability could effectively kill the intracellular bacteria under white light irradiation with minimal cytotoxicity toward macrophages. This caspase-1-responsive self-aggregation system showed high feasibility for in vivo selective and sensitive detection of bacterial infection via fluorescence turn-on feature and efficient intracellular bacteria eradication through photodynamic killing.

Alkaline phosphatase (ALP) is a well-known tumor biomarker overexpressed on numerous cancer cell membranes. Recently, Ding et al. constructed a fluorescence and photoactivity dual activatable probe (TPE-Py-FpYGpYGpY) for selective imaging and photodynamic ablation of ALP-overexpressed cancer cells.\(^{[111]}\) The tyrosine phosphates (pY) in the tripeptide of the probe could be dephosphorylated by ALP and the hydrophobic catalytic products could self-assemble into nanostructures, leading to remarkable activation of both fluorescence and ROS generation ability. TPE-Py-FpYGpYGpY could selectively target ALP-overexpressed cancer cells (Saos-2) and exert efficient cancer cell ablation under white light irradiation via a diagnosis and therapy dual turn-on manner.

### 3.2.2 | Self-aggregation responding to tripeptide GSH

GSH, taking part in controlling a variety of cellular processes, is recognized as a cellular protection agent, the deficiency or overexpression of which will cause dysfunction of related cellular processes. Compared with normal cells, the expression level of GSH in many cancer cells is typically higher (about 10 mM).\(^{[112]}\) Therefore, lots of AIE-active theranostics based on the reduction reaction of GSH for cancer cell targeted activation and/or release of diagnostic and therapeutic agents have been developed these years.\(^{[80]}\)

Liu’s group synthesized a fluorescent turn-on targeted prodrug (TPECB-Pt-D5-cRGD) comprising a targeted peptide, a platinum (IV) prodrug, and a functionalized AIE-active PS for real-time monitoring of drug activation and PDT-CHT synergistic therapy against cisplatin-resistant cancer cells (Figure 10B).\(^{[113]}\) TPECB-Pt-D5-cRGD could be selectively uptaken by \(\alpha_v\beta_3\) integrin overexpressed cancer cells and activated by intracellular GSH to release cisplatin and the AIE residues, which were prone to aggregate in aqueous environment, thus turning on the fluorescence and ROS generation. Upon light irradiation, TPECB-Pt-D5-cRGD exhibited remarkably enhanced cytotoxicity (IC\(_{50}\) = 4.2 \(\mu\)M) in the cisplatin-resistant MDA-MB-231 cells, while its dark cytotoxicity was similar to cisplatin (37.1 \(\mu\)M).

### 3.2.3 | Self-aggregation responding to ROS

It has been reported that the expression levels of ROS, such as hydrogen peroxide (H\(_2\)O\(_2\)), hydroxyl radical (\(^{\cdot}\)OH), superoxide radical (\(^{\cdot}\)O\(_2^-\)), hypochlorous acid (HOCI), and 1\(_2\)
Figure 11 Turn-on theranostics based on in situ aggregation via self-aggregation responding to biological stimuli. (A) Molecular structure and \( \text{H}_2\text{O}_2 \)-responsive oligomerization of TT catalyzed by peroxidase. (B) Schematic illustration of selective turn-on imaging and inhibition of inflammatory cells after incubation with TT. Reproduced with permission: Copyright 2017, John Wiley and Sons[114].

ROS, such as hydrogen peroxide \( (\text{H}_2\text{O}_2) \), and peroxynitrite \( (\text{ONOO}^-) \), are usually much higher in pathological cells or tissues. On basis of this fact, several ROS-responsive turn-on systems based on AIEgens have been developed for lesion-specific theranostics.[20]

Abnormal level of \( \text{H}_2\text{O}_2 \) production has proven to be closely associated with inflammation, neurodegenerative disease, diabetes, and cancer. Xia et al. developed a \( \text{H}_2\text{O}_2 \)-responsive theranostic turn-on probe (TT) to implement detection and treatment methods for inflammatory cells (Figure 11).[114] TT consisted of one TPE moiety as an emissive core, two tyrosine (Tyr) moieties at both ends as \( \text{H}_2\text{O}_2 \)-activatable units, and emission mediators. Owing to its good water solubility, TT is nearly nonemissive in aqueous solution. Through \( \text{H}_2\text{O}_2 \)-responsive and myeloperoxidase (MPO)-catalyzed dityrosine formation inside inflammatory cell, TT molecules could cross-link with each other via dityrosine linkages to form TT oligomers and exhibit intense blue emission, exhibiting fluorescence turn-on feature (Figure 11A). The TT molecules with high selectivity of \( \text{H}_2\text{O}_2 \) and MPO could distinguish inflammatory cells, cancer cells, and normal cells based on the distinction in the expression levels of intracellular \( \text{H}_2\text{O}_2 \) and MPO. The \( \text{H}_2\text{O}_2 \)-dependent and MPO-catalyzed self-polymerization of TT in inflammatory cells also led to selective inhibition of cell growth through mitochondrial damage, which might provide a promising tool for precise inflammation theranostics in biomedical systems (Figure 11B).

Figure 12 Schematic illustration of enhanced theranostics based on pre-aggregation via supramolecular assembly by means of (A) loading in nanocarriers formed by synthetic polymers, natural/synthetic lipid or natural protein, (B) self-assembly and (C) macrocycle-guided assembly via host–guest interaction.

4.1 Loading of AIEgens in nanocarriers

The AIE characteristics empower AIEgens with superior functionalities in aggregate state. Except for taking advantage of aggregation to achieve in situ turn-on theranostics by driving aggregation with biotargets or self-aggregation responding to various biological stimuli, enhanced AIE-active theranostic systems can also be achieved through actuating preaggregation of AIEgens into nanomaterials via supramolecular assembly, potentially facilitating the further in vivo applications.[14,115] As illustrated in Figure 12, various supramolecular assembly strategies have been exploited to construct the AIEgen-based nanotheranostics nowadays. These tactics can be summarized as three general approaches, including loading of AIEgens in nanocarriers, self-assembly of AIEgens into NPs, and macrocycle-guided assembly via host–guest interaction.

4 | ENHANCED THERANOSTICS BASED ON PREAGGREGATION VIA SUPRAMOLECULAR ASSEMBLY

The AIE characteristics empower AIEgens with superior functionalities in aggregate state. Except for taking advantage of aggregation to achieve in situ turn-on theranostics by driving aggregation with biotargets or self-aggregation responding to various biological stimuli, enhanced AIE-active theranostic systems can also be achieved through actuating preaggregation of AIEgens into nanomaterials via supramolecular assembly, potentially facilitating the further in vivo applications.[14,115] As illustrated in Figure 12, various supramolecular assembly strategies have been exploited to construct the AIEgen-based nanotheranostics nowadays. These tactics can be summarized as three general approaches, including loading of AIEgens in nanocarriers, self-assembly of AIEgens into NPs, and macrocycle-guided assembly via host–guest interaction.
include amphiphilic polymers, such as 1,2-distearoyl-snglycerol-3-phosphoethanolamine-N-poly(ethylene glycol) (DSPE-PEG), poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide) (Pluronic F127), and poly(lactide-coglycolide)-b-poly(ethylene glycol) (PLGA-PEG), as well as natural/synthetic lipids and natural proteins. For the purpose of the bathochromic absorption/emission wavelengths, most of the AIE fluorophores and other organic fluorophores are designed to possess large $\pi$-conjugated molecular structures that cause their highly hydrophobic nature. Loading of those AIEgens into the nanocarriers is also a very effective way to endow them with good water solubility (Figure 12A). More importantly, the aggregation of AIEgens within the limited space of nanocarriers is also able to efficaciously restrain their active molecular motion, which dominates the energy dissipation when molecularly dissolved in solution; as a consequence, the excited energy can thus be harvested for bright fluorescence as well as high ROS generation.

4.1.1 Loading of AIEgens in nanomicells formed by amphiphilic polymers

In a general preparation process, the AIEgens and amphiphilic polymers are primarily fully dissolved in an organic solvent, which should be miscible with water, followed by quickly transferring them into a certain volume of water under ultrasound sonication. The change of solvent environment gives rise to the aggregation of AIEgens and concurrently drives the assembly of the amphiphilic matrices to form AIEgen-loaded NPs with hydrophilic chains corona and hydrophobic AIEgens core.\[115] Moreover, the selection of encapsulation matrices is capable of regulating the performance of wrapped AIEgens. Prefunctionalization of encapsulation matrices or postmodification of yielded NPs can also endow AIE NPs with specific biological functions, such as additional targeting capability, stimulus responsiveness, and so forth. Furthermore, this facile nanofabrication method allows co-loading of other functional components such as active drugs, upconversion materials, and chemical fuels (e.g., peroxalates), assisting the construction of highly efficient theranostic systems with synergistic therapeutic outcomes or largely enhanced penetration depth. More importantly, it has been proven that the balance of radiative and nonradiative pathways of AIEgens could be subtly regulated by loading elaborately designed AIEgens in the nanomicelles, serving as an attractive strategy to construct one-for-all multimodal theranostics.

DSPE-PEG, a PEGylated derivative with amphiphilic structure, is a most widely used amphiphilic polymer for the construction of nanocarrier systems by virtue of its superior biocompatibility and biodegradability, easy surface functional modification, minimized nonspecific interactions in the bloodstream, and desired blood retention capacity. By using DSPE-PEG as matrix, an AIE-active fluorescent molecule AP4 was chosen for the preparation of AP4 NPs, which exhibited more than 10-fold higher fluorescence QY and over three fold higher $^{1}\text{O}_2$ generation efficiency as compared with the C66 NPs.\[116] Coupled with FR/NIR emission and negligible dark in vivo toxicity, AP4 NPs were successfully used for FLI-guided PDT for cancer treatment. To further regulate and optimize the fluorescence and ROS production of AIEgens, Tang et al. changed the flexible DSPE to corannulene, which is a superhydrophobic and rigid bowl-shape structure for amplifying the intraparticle confined microenvironment.\[117] As shown in Figure 13A, a rotor-rich AIEgen (TPP-TPA) was encapsulated with DSPE-PEG and corannulene-decorated PEG (Cor-PEG) separately, yielding DSPE-AIE dots and Cor-AIE dots. Cor-AIE dots showed four fold amplified fluorescence QY and 5.4-fold enhanced ROS production as compared to DSPE-AIE dots (Figure 13B and C), because the intraparticle rigidity and strong interactions between AIEgens and corannulene largely restricted the intramolecular motion of AIEgens, thus strongly suppressing the thermal consumption and markedly facilitating the radiative pathway and ISC process (Figure 13D). Moreover, the density functional theory (DFT) calculation results suggested the reduced $\Delta E_{5-7}$ of TPP-TPA in the presence of corannulene as well. As a result, these distinct advantages greatly promoted the theranostic efficiencies of Cor-AIE dots involving NIR FLI-guided cancer surgery and photodynamic treatment on a peritoneal carcinomatosis bearing mouse model. This study brought a new perspective for the design of advanced AIE nanotheranostics.

Non-specific location of AIE nanotheranostic agents would reduce imaging accuracy and cause undesired phototoxicity to normal tissues during PDT. To increase the specificity of AIE NPs to lesion sites, a variety of bioactive targeting moieties including small molecules, peptides, proteins, and so on, can be modified on the surface of NPs for specific delivery.\[26] Folic acid, a small molecule vitamin, possesses high affinity to folate receptor that is overexpressed on the surface of a wide range of cancer cells. Folate functionalization is therefore a low cost but effective strategy for targeted transport of NPs to tumor sites through folate receptor-mediated endocytosis. Liu and co-workers reported for the first time a cancer cell and mitochondria dual-targeted AIE NPs by loading a red-emissive AIE PS of DPBA-TPE in DSPE-PEG-assembled nanomicelles with folate and triphenylphosphine moieties decorated on the periphery.\[118] As expected, the as-prepared AIE NPs could specifically recognize the folate receptor-positive MCF-7 breast cancer cells, accumulate in mitochondria and direct the site-specific generation of ROS, resulting in markedly enhanced PDT outcomes. Certain types of integrin receptors are also closely associated with cancers. For instance, the overexpression of $\alpha_v\beta_3$ receptor has been discovered in many cancer types but undetectable in most of the normal organs, making it useful as tumor-specific marker. Tripeptide with the sequence of arginine-glycine-aspartic acid (RGD) or cyclic RGD (cRGD) is found to selectively target $\alpha_v\beta_3$ receptor on the surfaces of tumor cells and tumor vascular endothelial cells, and therefore has been widely applied to modify drugs, nanomaterials, etc., to implement tumor specificity. Li et al. prepared the TPETS dots with TPETS as AIE PS and DSPE-PEG as the matrix through physical encapsulation method and further modified the nanodots with cRGD through a click reaction to yield the integrin $\alpha_v\beta_3$-targeted T-TPETS dots (Figure 14A).\[119] Upon receptor-mediated endocytosis, the nanodots accumulated in the acidic organelles (endosome and lysosome) of $\alpha_v\beta_3$-overexpressed cancer cells. With light irradiation, the ROS produced by TPETS led to the lysosomal disruption and mitochondrial damage accompanying with a rapid leakage of lysosomal protease and
mitochondrial cytochrome-c throughout the cytosol, which activated the downstream caspases (caspase-9 and caspase-3) and eventually induced cell apoptosis. In vivo experiments demonstrated that the nanodots with bright fluorescence emission and effective $^1$O$_2$ generation exhibited excellent tumor-targeted imaging and imaging-guided PDT for tumor ablation in a hepatocellular carcinoma model. Additionally, the systemic toxicity evaluation demonstrated the appreciable biocompatibility of the nanodots.

Similarly, Liu’s group fabricated another kind of AIE NPs based on PTPEAQ with anti-HER2 affibody as the targeting moiety. Owing to the extremely high affinity and specificity of antigen–antibody interactions, the obtained AIE NPs showed selective internalization into HER2-overexpressed SKBR-3 cells over NIH-3T3 normal cells. Coupled with extraordinary ROS generation capability, the PTPEAQ NPs could be successfully used to selectively kill HER2-overexpressed cancer cells upon white light illumination.

Recently, the vectorization of diagnostic agents or therapeutic drugs using endogenous cells or bacteria has been proposed as a strong potential strategy for targeted drug delivery. Neutrophils (NEs), the most abundant type of immune cells, have been reported to possess a native ability to traverse the blood–brain barrier (BBB) and penetrate into brain tissues. In view of this fact, Tang et al. synthesized a bright NIR-II AIEgen, encapsulated the AIEgen in the hydrophobic cores of DSPE-PEG-formed nanomicelles, and subsequently loaded the yielded AIE NPs into NEs for accurate diagnosis of the inflammation areas in mouse brain tissue (Figure 14B). They proved that AIE NPs-loaded NEs (AIE@NE) could easily migrate into the inflamed brain and noninvasively identify the inflammation sites at a depth of about 3 mm within a mouse brain through the intact scalp and skull. Notably, NIR-II imaging of AIE@NE gave a SBR of 30.6 for in vivo diagnosis, which was 22.5-fold higher than that of ICG, allowing highly sensitive brain inflammation diagnosis.

On the other hand, natural killer (NK) cells, lymphocytes of the innate immune system, could identify abnormal cells (such as cancer cells) with the aid of receptors expressed on their plasma membranes. More recently, inspired by the design and concept of nanorobots, Tang et al. developed a type of NK-cell-mimic AIE NPs (NK@AIEdots) by wrapping the NK cell membranes on AIE-active polymer-based NPs. Owing to the AIE feature and soft-matter characteristics of the conjugated polymer PBPTV, the afforded NK@AIEdots showed superb NIR-II fluorescence with the QY about 7.9% and good biocompatibility. Attractively, these NK@AIEdots could trigger an intracellular signaling cascade, causing the disruption of tight junctions (TJs) and reorganization of actin cytoskeleton to form an intercellular “green channel,” thus assisting them to cross BBB smoothly. As a consequence, they were capable of actively accumulating in glioblastoma in the complex brain matrix for in situ through-skull-scalp FLI with high contrast as well as FLI-guided phototherapeutics under an 808 nm NIR laser irradiation.

As a typical light-activated noninvasive treatment paradigm, PDT has been approved in the clinic and achieved success in improving life quality and median survival of patients with high controllability. Nevertheless, the single PDT performance is yet unsatisfactory sometimes. Combination therapy has been proven to be one of the promising approaches to improve treatment outcomes. For example, the cooperation of CHT and PDT could compensate for
each other and achieve enhanced treatment effects.\textsuperscript{125,126} Tang’ group constructed a reduction-sensitive co-delivery system named DEB/TQR@PMP NPs by encapsulating a NIR-emissive AIE-active PS (DEB) and a drug resistance inhibitor (TQR) inside the polymeric prodrug PMP-formed nanomicelles.\textsuperscript{127} Experimental measurements proved that the DEB/TQR@PMP NPs exhibited a outstanding PDT-CHT synergistic lethal effect against multidrug-resistant SKOV-3 cells and could apparently enhance the antitumor effect as compared to sole PDT or CHT on the SKOV-3 tumor-bearing mice. In addition, the low systemic toxicity induced by DEB/TQR@PMP NPs was also confirmed by mouse body weight measurement and histological examination.

Aside from combination with chemotherapy, Li et al. reported a photodynamic-gene therapy combinatory platform for synergetic therapy.\textsuperscript{128} Small interfering RNA-vascular endothelial growth factor (siVEGF) has been widely used to suppress the expression of vascular endothelial growth factor (VEGF) as well as inhibit tumor growth and metastasis in cancer treatments. In this work, a kind of multifunctional AIE NPs based on AIE-active PS (TTD) and decorated with cRGD peptide and siVEGF on the surface was fabricated. After being internalized into the cell, the disulfide bond between DSPE-PEG and siVEGF could be cleaved by the elevated GSH level in cellular interior, which could trigger the release of siVEGF, thereby boosting the treatment effect. Guided by the cRGD peptide, this cRGD-siVEGF-TTD NPs could selectively kill the integrin $\alpha_v\beta_3$-overexpressed MDA-MB-231 cells with high efficiency owing to the excellent synergistic effect between PDT and RNA interference.

In contrast to PDT for ROS-induced tumor cell death via light activation of PSs, PTT as another phototherapy modality that utilizes photothermal conversion agents to generate heat for thermal ablation of tumor cells has emerged as a promising tool for cancer treatment. The cooperation of PDT and PTT would be a tactful strategy to achieve synergistic effects with enhanced therapeutic outcomes. For example, Wang et al. designed a nanocomposite named PMTi comprising polydopamine NPs as the photothermal conversion agents and AIEgen MTi as PS, which was decorated on the surface of polydopamine NPs through $\pi-\pi$ and hydrogen interactions for synergistic PDT and PTT in tumor treatment.\textsuperscript{133} Guided by FLI provided by AIEgen, the preferential accumulation of PMTi NPs in tumor tissues driven by EPR effect after intravenous injection was initially observed. Upon simultaneous irradiation of white light and NIR laser (808 nm), the PMTi NPs induced excellent antitumor outcomes confirmed by notably inhibited tumor growth and H&E-stained analysis of tumor slices attributing to the superior synergistic effect of the combined PDT and PTT. Furthermore, the in vivo biocompatibility and biosafety of PMTi NPs were also evaluated,
indicating no obvious toxicity for physiological function of organs.

At present, the all-in-one strategy is commonly used to build multi modality theranostic systems for enhanced tumor treatment. Although promising, this approach inevitably compromises the preparation reproducibility caused by the complicated composition, resulting in a low accessibility of clinical translation. By comparison, the development of one-for-all theranostic agents would be an ideal alternative of all-in-one ones. Tang et al. recently constructed a versatile phototheranostic agent based on single AIE component through regulating the balance between radiative and nonradiative energy dissipations (Figure 15). Owing to the abundant intramolecular rotators and vibrators, twisted conformation and high D-A strength, all those synthesized AIEgens (TI, TSI, TSSI) showed bright fluorescence emission, efficient ROS generation, and high photothermal conversion performance in the aggregate state within NPs. Notably, compared with the other two AIEgens, TSSI bearing the maximum D-A strength and intramolecular motion ability exhibited superior NIR-II emission with the longest emission wavelength, as well as the most prominent ROS and heat production inside the NPs, which also enable the photothermal imaging (PTI). In vitro cellular experiments showed that TSSI NPs could efficiently induce tumor cell death in a highly controlled way under a 660 nm NIR laser irradiation. In regard to in vivo experiments, the exact tumor size was first determined by NIR-II FLI and PAI. Thanks to the excellent synergistic therapeutic effect of PDT and PTT, only once injection and once irradiation were needed to entirely eradicate the tumors, and no relapse was observed in the 15-day treatment duration. Therefore, the as-prepared TSSI NPs presented remarkable versatility in terms of NIR-II FLI-PAI-PTI trimodal-imaging-guided PDT-PTT synergistic therapy. This study provided a new strategy on the construction of multi modality theranostic agents for potential clinical applications.

In order to construct effective theranostic protocols for the treatment of deep-seated tumors, upconversion nanoparticles (UCNPs) were introduced. By integrating UCNPs whose emission spectrum perfectly matches with the absorption spectrum of AIEgens, robust NIR light excitable theranostic platforms can be constructed. The first example of achieving an NIR triggered photodynamic cancer therapy in vivo through the combination of UCNPs with AIE PSs was reported by Xu and coworkers. As shown in Figure 16, the NIR-activated multifunctional nanoplatform was obtained by co-encapsulating the hydrophobic AIEgen TTD and UCNPs using DSPE-PEG and further decorating with cRGD for targeted FLI and PDT under NIR laser irradiation. As the emission of the UCNP rightly matches the absorption of the TTD, efficient ROS generation of the synthesized UCNP@TTD-cRGD NPs could be measured under a 980 nm laser illumination, even in the presence of a 6 mm thick tissue. Beyond that, the NPs could selectively and efficiently kill the targeted MDA-MB-231 cells both in two-dimensional level and three-dimensional in vitro tumor models. Additionally, the growth of tumors in MDA-MB-231 tumor bearing mouse was significantly inhibited after administration of intratumoral injection of NPs and 980 nm laser illumination, suggesting an excellent PDT efficacy of the NPs in the treatments of deep-seated tumor. Moreover, the UCNP@TTD-cRGD NPs could also preferentially...
accumulate at the tumor site for tumor imaging and significantly restrict tumor growth after intravenous administration.

In addition to being aided by UCNPs, developing AIEgens with multiphoton absorption features is another effective tactic to address the problem resulted by the short-wavelength excitation. Through adjusting conjugation length, degree of coplanarity as well as extent of polarizability, the two-photon absorption cross-section (2PACS) or three-photon absorption cross-section (3PACS) of organic luminogens could be modulated, which can potentially be implemented in the design of two-photon excitable AIEgens. On the other hand, AIEgens are also promising choice for two-photon or three-photon imaging-guided PDT, as both the 2PACS and 3PACS of AIE NPs can be elevated by simply enhancing the loading amount of AIEgens inside the NPs, showing aggregation-enhanced nonlinear optical effect. TPEDC, which possessed large 2PACS and outstanding ROS generation efficiency endowed by its high D-A strength, was the first AIEgen developed for two-photon excited PDT (Figure 17A). After being formulated into NPs, TPEDC dot was further functionalized with peptide sequence TAT on the surface. The average 2PACS of the dots were measured to be 3500 GM at 850 nm and 1700 GM at 800 nm, respectively. Upon two-photon excitation using femtosecond (fs) laser at 800 nm, the dots showed bright red emission at 620 nm which was used for two-photon brain blood vessels imaging, giving a high imaging contrast up to a depth of 200 μm. Meanwhile, the dots were also capable of continuously producing ROS under the NIR fs laser irradiation, which could be used for the ablation of tumor cells and the closure of brain blood vessels. Furthermore, a good resolution of 5 μm at depth of 100 μm was achieved in the precise blood vessel closure experiments with the help of fluorescence visualization.

Afterward, the some group synthesized conjugated polymers named PTPEDC based on TPEDC through Suzuki coupling polymerization (Figure 17B). Since the large conjugated structures of conjugated polymers are very conducive to two-photon absorption, polymerized PTPEDC is highly promising to show both improved photosensitization and 2PACS than TPEDC. After modification with TAT peptide, the PTPEDC dots were obtained for further in vitro and in vivo applications. The ROS generation efficiency of PTPEDC dots was 5.48 and 6.37 times higher than those of TPEDC dots and Ce6 dots, respectively. The 2PACS value of PTPEDC dots was tested to be 7.36 × 10^5 GM, which was about 6.5-fold than that of TPEDC dots. With the 820 nm fs laser irradiation, the PTPEDC dots incubated HeLa cells were almost completely eliminated, much superior to TPEDC dots. Moreover, the PTPEDC dots nicely located in the liver tissue of zebrafish liver tumor model and led to a 20% reduction in liver tumor size after the fs laser illumination, while 45–55% increase appeared in the other control groups.

In addition to two-photon excitation features, AIE NPs also serve as a good option for three-photon excitation theranostics, because of the aggregation-enhanced nonlinear optical effect. Nevertheless, the relevant reports of AIEgens with a large 3PACS value are still fairly rare. Recently, an AIE-active luminogen namely BTF was facilely synthesized. The obtained BTF molecules possessed a high quantum efficiency of up to 42.6% and ultra-bright FR/NIR emission in powders. Through a simple nanoprecipitation procedure using F127 as the encapsulation matrix, BTF dots were yielded, which exhibited high brightness, large Stokes shift, good biocompatibility, satisfactory photostability, and large 3PACS. At 1550 nm, BTF dots showed much higher 3PACS value of 2.56 × 10^-79 cm^6 s^2 than commonly used organic dye Rh6G (6 × 10^-81 cm^6 s^2). Moreover, the excitation (1550 nm) power-dependent three-photon imaging intensity of the BTF dots evidently demonstrated the main nonlinear optical process of three-photon fs laser. Upon the NIR-II laser excitation, they could be utilized as efficient fluorescent nanoprobes for in vivo three-photon brain vascular imaging through the intact skull. This work represents the first example of using AIEgen-based imaging agent for the noninvasive visualization of cerebral stroke process in mice through three-photon microscopy imaging.

Although upconversion strategy and multiphoton excitation technology have solved the problem in terms of the...
penetration depth of excitation light source to a large extent, they are still enslaved to the external light. Chemiluminescence is a unique fluorescence phenomenon relying on the energy release of chemical reaction rather than light excitation. This favorable light-free excitation process can fundamentally push through the limitation of light source penetration depth. The first chemiluminescence-based AIE PS system was designed by Liu’s group in 2017. As demonstrated in Figure 18A, C-TBD NPs were prepared by co-encapsulating TBD, CPPO, and soybean oil into the nanomicelles formed by amphiphilic matrix F127, where TBD acted as the AIE-active PS, CPPO served as a chemical excitation source to generate the chemiluminescence by reacting with H2O2 and subsequently excite TBD for ROS generation, and soybean oil worked as the retarder to slow down the energy release rate to favor in vivo circulation. Since certain types of cancer tissues have proven to exhibit a higher expression level of H2O2 than that of normal tissues, C-TBD NPs would be spontaneously activated for FLI-guided PDT once they accumulated at H2O2-overexpressed tumor site via EPR effect after systematic administration into the 4T1 breast tumor bearing mice. After injection of C-TBD NPs intravenously into intraperitoneal 4T1 metastasis tumor-bearing mouse model for 1.5 h, intense FR/NIR chemiluminescence signal was found in the tumor region, whereas no obvious fluorescence signal was observed, indicating the significantly deep imaging capacity of chemiluminescence over traditional fluorescence. Moreover, further removal of the skin and peritoneum revealed that the fluorescence and chemiluminescence colocalized well in the abdominal metastatic breast tumor sites, which was also demonstrated by further H&E staining. After combining with FEITC, an antitumor drugs that can increase the amount of H2O2 produced by the tumors, the chemiluminescence image contrast and PDT efficacy could be further enhanced.

More recently, another new AIEgen-based chemiluminescence system was developed to emit ultralong afterglow luminescence for promoted imaging-guided cancer surgery by Ding and coworkers (Figure 18B). They first synthesized an enol ether precursor of Schaap’s dioxetane (AGL) and a NIR-emissive AIE PS of TPE-Ph-DCM, and prepared afterglow-luminescent AIE NPs (AGL AIE dots) by encapsulating AGL and TPE-Ph-DCM using DSPE-PEG. Based on rational molecular design, TPE-Ph-DCM owned stronger AIE effect and higher 1O2 generation efficiency. Upon white light excitation, 1O2 would be generated by TPE-Ph-DCM in AGL AIE dots to oxidize some of AGL to form Schaap’s adamantylidene-1,2-dioxetane (a chemiluminescent compound showing chemiluminescence at 540 nm), which could subsequently excite TPE-Ph-DCM through chemiluminescence resonance energy transfer. The excited TPE-Ph-DCM exhibited NIR emission and 1O2 generation, further
FIGURE 18 Enhanced theranostics with external light source-free features by utilizing chemiluminescence tactics. (A) Schematic illustration of the preparation of C-TBD NPs, the principle of chemiluminescence and $^1$O$_2$ generation, as well as the corresponding application in chemiluminescence-activated FLI and PDT triggered by H$_2$O$_2$-enriched tumor microenvironment. Reproduced with permission: Copyright 2017, Elsevier.[133] (B) Schematic illustration of the AGL AIE dot formation, mechanism for amplified NIR afterglow luminescence and the afterglow imaging-guided tumor resection. Reproduced with permission: Copyright 2018, American Chemical Society[134]

oxidizing AGL. Under optimized conditions, the AGL AIE dots could emit persistent NIR emission more than 10 days upon single light excitation through a series of processes of this cycle occurring in the AIE dots, which had been used for imaging at high penetration depth on a peritoneal carcinomatosis bearing mice model. In addition, in vivo animal experiments uncovered that the afterglow signal of AGL AIE dots quenched quickly in normal tissues such as liver, leading to a rather-high tumor-to-liver signal ratio of 100-fold. Ultimately, the ultralong NIR afterglow luminescence, ultrahigh tumor-to-liver signal ratio, as well as low afterglow background noise, enabled AGL AIE dots to performance outstandingly in precise imaging-guided cancer surgery in living mice.

4.1.2 Loading of AIEgens in liposomes and protein carriers

Except for loading AIEgens into nanomicelles, entrapping AIEgens in liposomes through a coassembly approach has also been used to construct theranostic AIE NPs.[135] For example, Luo et al. reported that the hydrophobic AIE-active TPCI and chemotherapy agent (PTX) could be co-encapsulated into the lipid bilayers of liposomes to yield liposome-based theranostic nanoparticles (TPCI/PTX@Lipo) (Figure 19A).[136] The obtained liposomes exhibited high fluorescence brightness and efficient ROS production after excitation by white light as the intramolecular motions of TPCI were block in
FIGURE 19 Enhanced theranostics via loading AIEgens in liposomes or protein carriers. (A) Molecular structure of TPCI and schematic illustration of PTX-potentiated TPCI-based photodynamic theranostics for synergistic ablation of large tumors and self-reporting of the anticancer effect. Reproduced with permission: Copyright 2020, American Chemical Society.[136] (B) Schematic illustration of preparation of the binary AIE NPs named BONAPs by using the mixture of TPE-Br and TBDTT, and their application in mouse deep-brain three-photon imaging. Reproduced with permission: Copyright 2020, American Chemical Society[140]

the lipid bilayers due to its typical AIE nature. Markedly, TPCI/PTX@Lipo showed excellent synergism in killing a series of human carcinoma cells upon irradiation with significantly low combination index values (below 0.5). The potency of the combined therapy had been boosted for up to 30-fold compared with sole PDT or CHT. More strikingly, the released TPCI from TPCI/PTX@Lipo could light up the nuclei of dead cells induced either by PDT or CHT through binding with the chromatin and activating its AIE property, therefore self-reporting the antitumor effect of the combination treatment in real time. In addition, this outstanding synergistic effect was further demonstrated by the effective ablation of established large prostate tumor models with initial sizes of 200 mm³.

Besides the synthetic polymers and lipids, natural carbohydrates or proteins have also been demonstrated effective encapsulation matrices to load luminogens into NPs.[137–139] For example, addition of AIEgens (TPE-Br and TBDTT) in DMSO to the large amount of aqueous solution of BSA followed by simple vortex for 10 s could afford the binary AIEgens-loaded NPs named BONAPs (Figure 19B).[140] After filtration, BONAPs with a diameter around 50 nm could be obtained. TPE-Br as the diluting molecule was introduced in BONAPs in order to increase the intermolecular distance of TBDTT inside the NPs and thus modulate the luminescence QY. By increasing the molar ratio of TPE-Br/TBDTT, the fluorescence of TBDTT within NPs was shifted to a shorter wavelength accompanied by increased QY from 0.01 to 0.23. The enhancement of fluorescence QY further increased the 3PACS value of TBDTT, which was determined as \(1.92 \times 10^{-81}\) cm⁶ s² at 1600 nm with a TBE-Br to TBDTT molar ratio of 25:1, 28-fold higher than that of standard dye SR101, suggesting the great potential of BONAPs for three-photon FLI. The optimized BONAPs with the brightest luminescence were applied for mouse deep-brain vessel imaging, which could attain an imaging depth of 1.68 mm upon excitation at 1610 nm. Without changing the molecular structures, the strategy of creating BONAPs provided a simple method for elevating the performance of multiphoton contrast agents. By using another natural protein of mini-ferritin protein (Dps) as scaffold, a theranostic AIE NPs named Dps–AIE nanodots were also constructed for simultaneous imaging and PDT by Li and coworkers. The as-prepared Dps–AIE nanodots showed high biocompatibility, good stability, and relatively long absorption and emission wavelengths. Under white light irradiation, the nanodots exhibited strong fluorescence
emission and efficient $^1\text{O}_2$ generation, achieving effective elimination of HeLa cells via PDT.

4.2 Self-assembly of AIEgens into NPs

Supramolecular assemblies have been proved to be the available strategies to construct intelligent nanomaterials for theranostics.\cite{141,142,149} Through rational molecular design, hydrophobic AIEgens could be chemically decorated with hydrophilic chains (e.g., hydrophilic neutral polymers, charged chains, biomolecules) to yield amphiphilic AIE conjugates. In aqueous medium, these structurally amphiphilic AIE conjugates can spontaneously self-assemble into hierarchical nanostructures without assistance of any encapsulation matrix for theranostic applications (Figure 12B).

On the basis of an iconic AIEgen TPE, Liang et al. developed for the first time an AIEgen-based self-delivery system for drug tracking by simply introducing a carboxyl group into TPE.\cite{143} Owing to the AIE effect of carboxylated TPE (TPE-COOH), the obtained TPE NPs could emit bright fluorescence and easily be monitored in cells. The TPE NPs were superior to conventional drug delivery systems and quantum dots because they were ACQ-free, did not interfere with drug function, showed good cytocompatibility, and were easy to be fabricated. Upon the incorporation of an antitumor drug DOX into the TPE NPs, a new therapeutic system named TD NPs was formulated. Due to the fluorescence resonance energy transfer (FRET) effect of TPE-COOH and DOX, the cellular delivery and distribution of DOX could be observed in detail. In addition, the TD NPs were also effective in suppressing the cancer cells proliferation ascribing to chemotherapy activity of DOX. On the other hand, the supramolecular self-assembly strategy has also been proved to be an available tool to construct stimuli-responsive AIE NPs for theranostics.\cite{144}

Recently, a novel pH-responsive carrier-free AIE NPs self-assembled from an amphiphilic AIE PS (HD-APNNA) was constructed by Chang and coworkers.\cite{145} This carrier-free AIE NPs overcame the safety problems of nanocarriers, and the protonation and deprotonation of carboxyl groups of HD-APNNA endowed the AIE NPs pH-responsibility. Meanwhile, The ROS production efficiency of AIE NPs was as high as 56.7%, superior to the commonly used PSs in clinic. As a result, the AIE NPs showed a remarkable photodynamic therapeutic effect in vitro and in vivo with the aid of external white light.

In addition, the AIEgen-based self-assembling nanosystem was also demonstrated as an effective template for efficient noncionic gene delivery.\cite{146} As shown in Figure 20A, a core-shell nanotheranostics based on AIEgens for efficient and synergistic gene/photodynamic therapies was fabricated by Liu’s group, through taking advantage of the spherical nucleic acid (SNA) and AIE-active PS. In this system, anti-apoptosis protein (Bcl-2) antisense oligonucleotide (OSA) was conjugated onto the surface of self-assembled AIE NPs by click reaction. After the Bcl-2 SNA was taken up by tumor cells, the AIE PS could produce $^1\text{O}_2$ inside cells under white light irradiation. Then the lysosomal structure would be ruptured by the accumulative $^1\text{O}_2$, followed by the escape of Bcl-2 OSAs, which was able to degrade the corresponding mRNA, and further down regulate the expression of Bcl-2 in tumor cells, favoring tumor cell apoptosis. Moreover, Bcl-2 is also a typical PDT-resistant related protein, the downregulation of which could remarkably reverse intrinsic oxidative
resistance and significantly promote PDT effect. Thus, synergistic gene and photodynamic therapies with enhanced therapeutic efficacy could be obtained by cooperating the advantages of SNA and AIE PS in the integrated core-shell nanotheranostics.

Furthermore, construction of AIE-active nanotheranostics via supramolecular self-assembly strategy are also endowed with the stimuli-activated performance and exhibit promising potentials in clinical applications. In 2018, Liu’s group reported a bacterial infection-activatable nanotheranostic system based on the supramolecular self-assembly of amphiphilic AIEgen (TBD-PEG) and NIR IR786S, which exhibited typical ONOO− and ClO− responsive properties for specific imaging-guided photodynamic bacterial ablation.[147] As shown in Figure 20B, TBD-PEG, serving as the highly effective PS and an energy donor, self-assembled into NPs with good biocompatibility, and IR786S dye, serving as the energy acceptor, was encapsulated within the lumen of the as-formed NPs, in which the FRET process was facilitated to excite the NIR emission of IR786S and quench the fluorescence emission as well as the $^{1}\text{O}_2$ generation of TBD-PEG for elimination of phototoxicity in normal tissues. Significantly, the overexpressed ONOO− and ClO− in bacterial infection sites could induce the decomposition of IR786S, recovering the red fluorescence and $^{1}\text{O}_2$ generation of TBD-PEG and realizing the detection of bacterial infection and photodynamic bacterial ablation. Considering the negligible in vivo dark toxicity, the constructed nanotheranostics demonstrated great potentials in antibacterial applications.

Hybrid materials with AIE effect could also be developed via supramolecular self-assembly for enhanced therapeutic performance. Liu et al. integrated TBD-PEG with metal-organic frameworks (MOFs) to achieve the synergistic imaging-guided photodynamic-chemo therapy, which could be efficiently modulated by the tumor microenvironment.[148] AIE-active PS of PBD was conjugated to PEG for encapsulating the Pt(IV)-loaded MOFs, in which the Pt(IV) could escape from the nanoplatform upon activating by tumor microenvionment. The released Pt(IV) could not only act as chemotherapy drug but also generate $^{1}\text{O}_2$ to relieve the hypoxic tumor, which would further promote PDT potency. Thus, the nanotheranostics based on AIE PS with the integration of MOFs could realize bright tumor imaging, synergistic and enhanced chemo-photodynamic therapy, exhibiting the promising potential to break the limitation for clinical application.

4.3 | Macrocycle-guided assembly via host-guest interaction

Other than physical loading and self-assembly strategies, host–guest interaction is also playing a crucial role in supramolecular chemistry.[141,149] Supramolecular nanotheranostics fabricated from host–guest interaction has promised the development of integrated nanosystems for enhanced diagnosis and therapeutics by cooperating with AIEgens. As the fundamental building blocks in supramolecular chemistry, synthetic macrocycles including cyclodextrins (CDs), cucurbit[n]urils (CBs), calix[n]arenes (CAs), and pillar[n]arenes (PAs) with different structural properties and host–guest interactions have been used to construct supramolecular nano-materials with reversible, stimuli-responsive, and controllable functions. With the aid of host–guest interaction, AIE-active nanotheranostics for synergistic therapies can be successfully fabricated with tunable and controllable characteristics to enhance the theranostic efficiency and minimize the side effects (Figure 12C).[142]

CDs possess excellent biocompatibility, which facilitates their applications in the biomedical fields. AIE-active supramolecular nanomaterials for drug/gene delivery and bioimaging have been fabricated, with which the drug delivery process can be monitored and the therapeutic functions can be regulated upon the external stimuli.[150] Besides, theranostic prodrugs and hybrid materials based on the host–guest interactions of CDs and AIEgens can realize the multifunctional and synergistic therapies with the guidance of FLI, which can satisfy the different clinical demands.[151,152] Recently, Ding et al. reported the supramolecular AIE nanomaterials based on the host–guest interaction of α-CDs and PEG, which exhibited high performance for imaging-guided orthotopic pancreatic cancer therapy.[153] Two kinds of α-CDs were first synthesized via covalently conjugating AIE-active TPR and anticancer drug GEM to α-CDs, respectively, whose structures were illustrated in Figure 21A. Supramolecular nanomaterials were fabricated through the coassembly of α-CDs and PEG-peptide with MMP-2 sensitivity, driven by the host–guest interaction. The supramolecular AIE NPs with elaborated tailoring exhibited versatility, such as long blood circulation, effective tumor accumulation, selective GEM release upon triggering by tumor overexpressed MMP-2, accurate tumor imaging, and enhanced therapeutic efficiency, achieving the imaging-monitored cancer therapy for both subcutaneous and orthotopic pancreatic tumors.

Except CDs, CBs also exhibited remarkable potentials to construct AIE-active supramolecular nanomaterials with enhanced theranostic efficacy due to their rigid structures and good biocompatibility. For example, Tang et al. reported the multifunctional supramolecular assemblies based on the host-guest inclusion between CB[8] and AIEgens to identify cell line, evaluate the cell contamination, and discriminate the cancer cells.[154] CAs are one kind of synthetic macrocycles with bowl-like shape and electron-rich cavity. To achieve the ultimate goal of precise imaging-guided cancer surgery, Ding et al. fabricated the supramolecular AIE dots by the co-assembly of CAs and AIEgens for ultrasensitive FLI-guided cancer surgery therapy.[155] Collaborated with CBs, CAs were also utilized to fabricate supramolecular assemblies with NIR emission for lysosome-targeted imaging.

As the newest macrocycles, PAs have attracted increased attentions due to their symmetric structures, facile functionalization and typical host–guest interaction. In recent years, PA-based supramolecular nanotheranostics with AIE effect have been developed, contributing a lot to the systems of enhanced and integrated diagnosis and therapeutics.[156,157] As shown in Figure 21B, an adaptive AIE PS system was successfully developed via PA-based host–guest interaction by Liu and co-workers.[158] The host–guest complex was prepared from the water-soluble pillar[n]arene (WP5) and the AIE-active PS (G) with binding site toward WP5, in which the fluorescence and ROS generation could be regulated in response to acidic environment. The formation of the host–guest interaction quenched the fluorescence and inhibited ROS generation of G. After internalization into tumor cells, the binding
site between G and WP5 would change through a shuttle movement of WP5 on the AIE-active stalk upon triggering by lysosomal acidity (pH 5.0), accompanied by turn-on fluorescence and ROS generation. This study thus paved a new way to construct supramolecular theranostics with intelligent and controllable performance via host–guest interactions.

5 CONCLUSION AND PERSPECTIVES

As shown here, AIEgen-based theranostic systems have been extensively investigated and demonstrated to be promising candidates in improving the treatment efficacy. Highly efficient theranostics based on AIEgens are successfully achieved through controlled in situ aggregation as well as pre-aggregation via supramolecular assembly. Due to the well-designed in situ aggregation process, AIE-active theranostics are endowed with turn-on feature showing improved controllability and specificity as well as minimized side effects. On the other hand, AIE NPs based on pre-aggregation exhibit not only enhanced fluorescence and ROS generation, but also the feasibility in construction of multi modality theranostics based on single-component AIEgens allowing all of theranostic modalities in terms of FLI, PAI, PDT, and PTT simultaneously through precise molecular design. Moreover, the modification of AIE NPs with stimuli-responsiveness and targeting properties as well as the ingeniously combined therapeutics with drugs further improve the diagnostic accuracy and therapeutic efficacy. Consequently, the concept of AIE indeed sparkles in biomedical field.

Although significant advancements of versatile AIEgens in disease theranostics has been achieved and exciting potential in preclinical research and clinical application is clearly foreseen in recent years, there are still challenges and perhaps future opportunities should be considered to further promote the translation of AIEgens into clinical practice. First, accurate diagnosis and precise treatment of deep-seated disease are significantly important for disease theranostics, which, however, are severely hindered by light attenuation, auto-fluorescence interference and nonspecific signals. Under this predicament, long wavelength fluorescence (e.g., NIR-II) imaging has pointed out a promising solution, and the development of AIEgens emitting in the NIR-II window remains to be further explored. Thanks to the negligible auto-fluorescence, RTP represents another appealing option and is very promising for “zero background” lifetime imaging favorable in the imaging-guided surgery. Consequently, the further development of pure organic RTP fluorophores with more diverse chemical structures, longer lifetime, and higher phosphorescence QY in NIR region is expected. Moreover, theranostics based on on-site chemiexcitation or sonodynamic with no requirement for real-time external light source open up new avenues for advanced theranostics. Second, more precise and tailored design as well as modification with optimized performances of AIEgens or AIE NPs that can potentially or eventually meet the demands of clinic applications is urgently needed. For example, theranostic systems equipped with high specificity and selectivity toward biomarkers as well as with high controllability of stimuli-responsive turn-on features in the lesion region awaits to be strengthened. Additionally, the wide development of multi modality theranostic systems with one-for-all feature based on single-component multifunctional AIEgens is highly desired for improved diagnostic and therapeutic outcomes. Moreover, comprehensive combination between AIEgens with clinical imaging (e.g., CT, MRI, PET, and US) and therapy (CHT, RT, GT, and IMT) methods is favored for multimodal clinical theranostics. Third, although most studies have preliminarily suggested the good biocompatibility of AIEgens, systematic and comprehensive in vivo research data such as pharmacokinetic, biodistribution, metabolic, and excretion, as well as comprehensive assessment of their long-term safety are essentially required on the way to clinical translation, which relies on the concerted collaborations of

FIGURE 21 Enhanced theranostics based on macrocycle-guided assembly via host–guest interaction. (A) Schematic illustration showing the synthetic route to TPR, chemical structures of α-CD-TPR and α-CD-GEM, preparation of α-CD-TPRGEM-mmp(+) NPs via host–guest assembly and programmed tumor-microenvironment-responsive targeting and drug release. Reproduced with permission: Copyright 2020, American Chemical Society.[153] (B) Schematic illustration of chemical structures of AIEgen and macrocycles, as well as resulting pH-responsive host-guest nanomaterials for turn-on theranostics. Reproduced with permission: Copyright 2020, John Wiley and Sons[158]
a broad range of participants. Last, in terms of enhanced biosafety for clinical transition, the recent exploration of clusteroluminescence from nonconjugated AIEgens or even natural products may represent promising options,\textsuperscript{159,160} the overall quality of which, however, remains limited at present. Therefore, the development of more nonconjugated or natural AIEgens as well as corresponding in-depth investigation are still required. Moreover, it should be reconsidered that how to achieve the balance between sufficient surgical time window and fast body clearance when designing theranostic agents for imaging-guided tumor surgery.

With these promising possibilities and sparking opportunities, it is reasonably to anticipate the future clinical transformation of AIEgens. We are expecting, through this review, to arouse collaborative research interests from related interdisciplinary areas to work on such an enthusiastic and transformative AIEgens. We are expecting, through this review, to arouse collaborative research interests from related interdisciplinary areas to work on such an enthusiastic and transformative AIEgens.
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