Activity-based smart AIEgens for detection, bioimaging, and therapeutics: Recent progress and outlook

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Abstract
Activity-based approaches for designing AIEgens possess prominent advantages including high selectivity, sensitivity, and signal-to-noise ratio, and they have received more attention in recent years. Excellent activatable AIE probes have been reported for detecting toxic substances, imaging intracellular active molecules/biomolecules, as well as monitoring the activity of overexpressed enzymes in cancers. Moreover, the majority of activatable theranostic AIEgens can be specifically triggered in cancer cells and can kill these cells under light irradiation, while they have no distinct effect on normal cells, demonstrating satisfactory therapeutic selectivity that is superior to that of traditional chemotherapy. Thus, in this review, we systematically summarized the development of activatable AIE bioprobes in recent years from molecular design principles to biological applications. The challenges of activatable AIE probes and the corresponding solutions are described. We hope that the information provided in this review will facilitate the design of more activatable AIE probes to promote practical application of corresponding AIEgens.

KEYWORDS
activity-based, aggregation-induced emission, AIEgens, bioimaging, detection, therapeutics

INTRODUCTION
Fluorescent probe technology has been widely used to image biologically important substances (e.g., ions, signaling small molecules, and enzymes) in living cells and organisms for exploring the physiological and pathological processes of related diseases due to its intrinsic advantages of simplicity, convenience, high sensitivity, real-time, and noninvasiveness.[1–17] With the popularity of multiphoton technology (two-/three-photon) and development of near-infrared fluorescent dyes (especially near-infrared region II dyes, NIR II, 1000-1700 nm), we can directly observe the dynamic transformation process of some important active substances in deeper tissue and even in vivo, which can provide a comprehensive understanding of their important roles.[18–21] Interestingly, superresolution imaging of cells beyond the diffraction limit can be achieved through the combination of superresolution fluorescence microscopy and appropriate molecular probes.[22–26] Additionally, fluorescent probes can be transformed into theranostic tools with both imaging and therapeutic functionalities for personalized treatment.[27–30] In other words, with the aid of fluorescent probes, the microscopic cellular structure and deeper tissue framework can be more clearly visualized. In addition, the molecular mechanisms of diseases can be accurately identified and solved accordingly.

However, most fluorescent probes based on the conventional fluorophores (e.g., fluorescein, rhodamine, and cyanine) suffer from aggregation-caused quenching (ACQ) at high concentrations, significantly limiting the detection or labeling of analytes with high signal-to-noise ratio.[31] Activatable fluorescent probes can be divided into “activity-based” probes and “binding-based” probes. The former is activated by the specific chemical reaction with an analyte. And, the latter is composed of fluorophore and targeted ligand that is mainly dependent on the coordination of host and guest, resulting in the change of fluorescence properties. Up to now, a variety of fluorescent bioprobes have been developed based on a “lock-and-key” strategy for imaging intracellular analytes to reveal their function in the physiological and pathological processes. However, some of them are easily affected by cell micro-environmental parameters (pH, polarity, viscosity, etc.) with high background fluorescence, which cannot be ignored in practical use. Therefore,
some “false-positive” signals have been gathered, which can lead to misinterpretation of the results. Intriguingly, activity-based activatable fluorescent probes provide more reliable detection and imaging results by relying on the specific responsive reactions between probes and analytes, and have been favored by researchers in recent years.[32] In addition, activity-based fluorescent probes can be better adapted to existing operating room workflows due to their unique merits, including smaller doses, high signal-to-noise ratio, and well-defined pharmacokinetics.[33] It should be noted that, unless special stated, the activatable fluorescent probes described below are of the activity-based type.

In contrast to the ACQ phenomenon, in 2001, Tang and colleagues found an organic compound called 1-methyl-1,2,3,4,5-pentaphenylsilole that was almost nonfluorescent in ethanol solvent, but the fluorescence intensity gradually increased with an increase in the volume fraction of water in the mixture solvent.[34] After studying this anomaly phenomenon, Tang et al. innovatively advanced the concept of aggregation-induced emission (AIE), and many pioneering contributions in fields of AIE luminescence mechanisms and bioapplications have been made by his group.[35–38] In addition, a large number of studies have shown that AIE fluorogens exhibit higher photostability, extremely high signal reliability, and lower background signal compared with traditional fluorophores. As we have seen, tremendous activatable AIE probes designed on the principle of chemical reactivity for detecting analytes of interest and sensing biological analytes have been reported in the past decade.[31,39,40] Of note, a majority of theranostic AIE probes have demonstrated satisfactory efficiency in the treatment of diseases, especially tumors under fluorescence-guided.[41,42] Therefore, it is worthwhile to systematically summarize the recent development of activatable probes based on AIEgens.

This review is composed of five parts. In the first part, we introduced activity-based activatable AIE probes, followed by a brief discussion of the working and design principles of activatable probes based on AIEgens. In the third part, examples of specific activatable AIE probes for reactive sulfur species, reactive oxide species, toxic agents, hydrazine, and other small molecules were provided. In the subsequent section, we focused on development and application of enzyme-activatable AIE fluorescent probes in the field of diagnosis and treatment of tumors. Finally, a summary and outlook of AIE research were demonstrated for guiding the design of smarter AIE probes.

WORKING AND DESIGN PRINCIPLES OF ACTIVITY-BASED AIEGENS

Compared to traditional “lock-and-key” strategy, activity-based approach provides a reliable alternative by virtue of chemical reaction specificity.[32,39,43–45] That is, activatable fluorescent AIE probes are triggered by specific reactive targets or some parameters of the intracellular microenvironment, resulting in significant changes in fluorescence intensity, fluorescence shift, or fluorescence lifetime. Theories, including restriction of intramolecular rotation (RIR) and restriction of intramolecular vibration (RIV), have been put forward to explain the AIE luminescence mechanisms.[46–48] Among them, restriction of intramolecular rotation to regulate the release of excited state energy is often used to explain the AIE luminescence process. In addition, the fluorescence mechanisms of traditional probes highlighted in other reviews, including photo-induced electron transfer (PET), intramolecular charge transfer (ICT), Förster resonance energy transfer (FRET), and excited-state intramolecular proton transfer (ESIPT), are useful for construction of AIE bioprobes.[40–53] We are not going to talk about the sensing mechanism in detail and will focus on the design principles and applications of activity-based AIEgens in this review paper. Fluorophores with AIE luminescence are modified with a specific reactive group to obtain an activatable AIE probe. A strong fluorescence signal is observed by cleavage or addition with the reactive group of an AIE bioprobe to realize the detection of target analyte, as shown in Figures 1A and 1B, respectively. If two spectral matching fluorophores (at least one of them with AIE characteristics) are connected by an activatable linkage bond, a ratiometric AIE probe can be formed to detect a specific analyte. By conjugating an AIE fluorophore with a drug, it can be developed into a theranostic probe with dual functions of imaging and treatment (Figure 1C). Additionally, if the AIE fluorophore has the ability to produce reactive oxygen species under light radiation, construction of an activatable AIE probe is an effective tool for integration of diagnosis and treatment. Schematic illustrations of AIE probes for fluorescence activation and therapeutic responses are summarized and shown in Figures 1D and 1E. While these principles are commonly used to guide design of small molecule-based AIE probes, they also can be used as references for preparation of polymers or other forms of theranostic probes.

AIEGENS FOR ANALYSIS OF SMALL MOLECULES

AIEgens for hydrogen sulfide

Hydrogen sulfide (H2S) is widely recognized as a toxic gas and plays pivotal roles as a significant signal molecule in regulating a broad array of physiological processes to maintain cellular health, such as cardioprotection, angiogenesis, and vasodilation.[54–58] Thus, it is desirable to fabricate efficient AIE bioprobes for tracking H2S in living cells and in vivo for fully revealing its functions in different systems. Among the existing testing approaches, optical techniques including colorimetric and fluorescence methods are widely used for detection of H2S. For example, Tang’s group reported a new tetraphenylethylene (TPE)-based AIE bioprobe 1a, as shown in Figure 2[59] The azido functionalized 1a was nonfluorescent in both solution and aggregate states owing to the quenching effect of azido unit. However, the TPE decorated with an amino group emitted intense fluorescence in the aggregate state because of the RIR effect. The authors used the fact that azido groups are reduced to amino units by H2S to design molecule 1a for detection of H2S in aqueous solutions. The probe exhibited high selectivity and sensitivity toward H2S. Compared to molecule 2, probe 1a possessed a detection threshold different from the traditional fluorescent probes, which could be used to indicate the concentration of H2S simply by brighter AIE fluorescence. Using the same reaction mechanism, Kumar and Bhalla designed
and synthesized a supramolecular aggregate of hexaphenylbenzene 3 with AIE enhancement for detecting H$_2$S.[60] In the presence of H$_2$S, probe 3 underwent a reduction reaction to generate a product bearing dual amino groups, while its morphology was transformed from spherical to flower shape, resulting in bright fluorescence due to the restriction of intramolecular rotation. Additionally, the low toxicity, high photostability, good selectivity, and high two-photon action cross-section area enabled probe 3 to detect intracellular H$_2$S content with high signal-to-noise. In 2017, Wu's group developed an AIE/ESIPT-based fluorescent probe 4 with dinitrophenyl ether as the reaction group.[61] To improve its usability, modified nanoprecipitation of H$_2$S-responsive 4 and three phospholipids that were subsequently added by surface modification using a Tat peptide was adopted to obtain a nanoprobe for highly sensitive imaging of H$_2$S in living HeLa cells and zebrafish, which provided a new direction for the development of nanoprobes for tracking biological analytes. Tang and Li reported a dual signaling molecule probe 5 for detecting H$_2$S level through fluorescence, UV-visible, and
visual mode.[62] Without \( \text{H}_2\text{S} \), probe 5 was nonfluorescent because of the PET effect between TPE and dinitrophenyl ether. After the \( \text{H}_2\text{S} \)-triggered cleavage reaction, the product exhibited an intense fluorescence enhancement owing to the RIR mechanism. The low detection limit was as low as 12.8 nM, showing the high sensitivity of the probe toward \( \text{H}_2\text{S} \) in vitro. In addition, the changes in \( \text{H}_2\text{S} \) during starvation of Caenorhabditis were successfully observed using probe 5.

In 2019, Wu et al. designed an AIE bioprobe 6a based on the triphenylamine benzopyridine platform for \( \text{H}_2\text{S} \) assay.[63] Probe 6a was composed of four parts: a triphenylamine part as the electron donor, a quinolinium part serving as the electron acceptor, a \( \beta \)-oxynbenzyl moiety as the cleavable linker, and the dinitrophenoxynbenzyl segment as both the electron-withdrawing and recognition groups. The fluorescence signal of the probe was quenched due to the strong electron-withdrawing capability of quinolinium and dinitrophenyl segments. Upon addition of \( \text{H}_2\text{S} \), the recognition group was cleaved rapidly and spontaneously underwent 1,6-elimination of \( p \)-quinone-methide (6b) to generate the hydrophobic product 6c, resulting in brighter AIE fluorescence. Moreover, only \( \text{H}_2\text{S} \) could induce the obvious fluorescence enhancement, displaying high selectivity of the probe in response to \( \text{H}_2\text{S} \). The detection limit of the probe toward \( \text{H}_2\text{S} \) was calculated to be 0.17 \( \mu \text{M} \), and the probe was applied to detect \( \text{H}_2\text{S} \) in food samples, cells, and tumor tissues. For accurate detection of endogenous \( \text{H}_2\text{S} \), Zhao and coworkers fabricated a FRET-based fluorescent probe 7 for ratiometric detection of \( \text{H}_2\text{S} \) level.[64] For molecule 7, a TPE moiety served as an energy donor, and a monochlorinated BODIPY fluorophore was used as an energy acceptor and \( \text{H}_2\text{S} \)-responsive unit. Efficient FRET emission of probe occurred due to a spectral match between TPE and BODIPY. However, upon addition of \( \text{H}_2\text{S} \), the nucleophilic substitution reaction resulted in a significant red-shift of the absorption peaks of BODIPY, which inhibited the FRET process and produced a new fluorescence peak at 920 nm with the fluorescence tail extending to 1300 nm. More importantly, probe 7 was successfully used to track \( \text{H}_2\text{S} \) level in cancer cells and identify \( \text{H}_2\text{S} \)-rich tumors in the NIR II region with high resolution. A turn-on fluorescent probe 8 based on an ESPT fluorophore bearing AIE properties was developed by Yoon and Kim for sensing \( \text{H}_2\text{S} \).[65] Probe 8 was almost nonfluorescent because of the suppression of ESPT process. Addition of \( \text{H}_2\text{S} \) resulted in ring opening and formation of a 2-(2'-hydroxyphenyl)-benzothiazole (HBT) derivative, restoring the ESPT process to emit an intense fluorescence. Additionally, HBT derivatives had considerable two-photon cross-sections. Thus, the authors used probe 8 to image intracellular \( \text{H}_2\text{S} \) with the aid of one-/two-photon microscopy.

Jiang et al. reported a novel AIE molecule 9a for detection of \( \text{H}_2\text{S} \) that consisted of a TPE segment and a positively charged indolium moiety, as seen in Figure 2.[66] The nucleophilic addition reaction between H2S and the probe resulted in fluorescence signal transformation, which was confirmed by \( ^1\text{H-NMR} \) titration, mass spectrometry characterization, and fluorescence spectra. Among common biological analytes including thiols, various metal ions, and anions, only \( \text{H}_2\text{S} \) induced the fluorescence quenching of probe 9a, indicative of high selectivity. In addition, probe 9a was successfully employed to image \( \text{H}_2\text{S} \) in the mitochondria. Salicylaldazine consists of two salicylaldimine units containing intramolecular hydrogen bonds, which improve its AIE characteristics in a poor solvent due to the inhibition of the free rotation of the N-N bond. Therefore, Huang and coworkers reported an amphiphilic polymer 10 for detection of \( \text{H}_2\text{S} \) by introduction of methyl polyethylene glycol and a long alkyl chain into the salicylaldazine skeleton.[67] Interestingly, small (sub-20 nm) micelles were obtained in water via self-assembly of 10 and exhibited a high stability, favorable for applications in biological systems. The fluorescence of micelles of 10 was first quenched by chelating with Cu\(^{2+}\). Upon addition of \( \text{H}_2\text{S} \), precipitation of CuS occurred due to the high affinity between Cu\(^{2+}\) and S\(^{2-}\), leading to recovery of fluorescence, which was utilized for monitoring mitochondrial \( \text{H}_2\text{S} \) in HeLa cells. Inspired by AIEgens widely used in fluorescence bioimaging and theranostics, in 2019, Li and coworkers rationally developed a low-cost AIE-active fluorescent probe 11 for highly selective sensing of \( \text{H}_2\text{S} \).[68] In aqueous solution, a chemical-derivation-triggered AIE approach by \( \text{H}_2\text{S} \) induced a specific AIE fluorescence at 475 nm, which sharply red-shifted compared with monomer probe 11 (375 nm). Probe 11 displayed a high sensitivity toward \( \text{H}_2\text{S} \) with a detection limit of 0.25 nM in a wine-beer sample and 0.30 nM in a red wine sample. Using high-performance liquid chromatography with fluorescence detection, probe 11 was successfully employed to determine \( \text{H}_2\text{S} \) content in wine matrices. Lee and Kang reported a disulfide-conjugated AIEgen 12 that could significantly improve aggregability via \( \text{H}_2\text{S} \)-selective disulfide cleavage.[69] Under physiological conditions, probe 12 emitted weak fluorescence. However, in the presence of \( \text{H}_2\text{S} \), intense fluorescence at 480 nm was observed due to formation of TPE dimer. In addition, other common biological interferences including glutathione, homocysteine, and cysteine had little effect on the fluorescence spectra of probe 12, clearly demonstrating a high selectivity toward \( \text{H}_2\text{S} \). Additionally, the detection limit was calculated to be 84 nM, which enabled practical ability to image \( \text{H}_2\text{S} \) in HeLa cells.

Tang et al. developed a novel fluorescent probe 13 based on the skeleton of tetraphenylpyrazine functionalized with a malonitrile group.[70] In the aggregate state, probe 13 emitted green or orange emission, much redder than in most previous reports due to the donor-acceptor structure. The results of in vitro experiments exhibited that the probe had good selectivity and fast response to \( \text{H}_2\text{S} \) with a detection limit of 0.5 \( \mu \text{M} \). Probe 13 showed an abnormal and reversible mechanochromism with a hypsochromic effect, which provides huge potential for development of AIEgens for bioapplications in the future.

**AIEgens for common biological thiols**

Cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) are the major biological thiols in human physiology, and they play vital roles in synthesis of important biological molecules, maintenance of intracellular redox balance, and signal transduction.[71–75] Abnormal levels of these thiols are closely related to many human diseases including hair depigmentation, lethargy, cardiovascular diseases, Alzheimer’s disease, and cancers.[76–78] Thus, detection of biological thiols in samples and organisms has received much attention.[79,80] For instance, in 2015, the group of Yoon reported a new fluorescent probe 14a based on a salicylaldazine fluorophore, for
sensing of biothiols.\[81\] As shown in Figure 3, the ESIPT process of probe 14a was blocked when the two hydroxyl groups were decorated with two acrylate moieties as the recognition site, resulting in weak fluorescence emission in aqueous solution. Upon reaction with biothiols, probe 14a was converted to product 14c, exhibiting a strong fluorescence signal for distinguishing Cys from other biothiols via its reaction kinetics, which was also employed for imaging intracellular Cys. Wang and Li developed a TPE-based probe 15a bearing a 2,4-dinitrobenzenesulfonyl group, which served as the reaction site and fluorescence quencher. Biothiols could trigger the cleavage of a probe’s sulfonamide through nucleophilic aromatic substitution to generate 15b. Then corresponding aggregates formed in aqueous solution, releasing an intense AIE fluorescence owing to the RIR effect. Based on the kinetics of the reaction with various biothiols, probe 15a could selectively identify Cys with high sensitivity in solution and in living HeLa cells.

In 2019, Huo, Zhang, and Ji developed two isomer fluorescent probes 16 and 17 for distinguishing Hcy/Cys from GSH, as shown in Figure 3.\[83\] There probes were composed of a pyrene decorated with a TPE derivative as an AIE luminescent core and a maleimide group as the recognition moiety and were nearly nonfluorescent due to the PET effect. Obvious fluorescence signals were observed upon interaction with Cys or Hcy (not GSH), which was ascribed to termination of the PET effect via Michael addition of maleimide group with biothiols and the stereo-hindrance effect. The reaction mechanism of the probe toward Cys was confirmed by DFT calculations and mass data. Moreover, both probes were successfully applied to detect biothiols in living H1299 lung cancer cells. To detect Hcy specifically, a new AIE bioprobe 18a containing the α,β-unsaturated ketone moiety as a recognition site was designed by Zhao and coworkers.\[84\] Interestingly, only Hcy induced a significant ratiometric fluorescence change from yellow to blue in aqueous solution, which enabled probe 18a to efficiently distinguish Hcy from Cys and GSH. A case of AIE probe 19a developed by Tang and Sun showed good performances (such as fast response, high selectivity, and sensitivity) for discriminating between Cys and Hcy.\[85\] As shown in Figure 3, the selective property of probe 19a was demonstrated in a biocompatible medium by the chemical reaction between the aldehyde group on the probe molecule and Cys/Hcy to form the rate of thiazolidine/thiazolidane (compounds 19c and 19d), which enabled probe 19a to detect Cys accurately in human plasma. In 2018, Wang and coworkers developed a new AIE-active Schiff base 20a based on tetraphenylethene-diketopyrrolopyrrole fluorophore for detecting different concentration ranges of GSH through two distinct reaction mechanisms.\[86\] Probe 20a emitted a weak fluorescence due to active intramolecular rotation of TPE and the PET process of the Schiff base. Promisingly, the Schiff base moiety was hydrolyzed to release 20b and diketopyrrolopyrrole (20c) fluorophores with turn-on fluorescence signal in the presence of a low concentration of GSH. A ratiometric mode of fluorescence was observed due
to the nucleophilic addition reaction of excess GSH and a probe for generating compound 20d. Moreover, probe 20a exhibited high selectivity for GSH over other biological interference substances including Cys and Hcy, which had been used to image GSH in living cells. Mitochondrial biothiols play important roles in maintaining oxidative stress and exerting physiological functions. In 2019, Tang and coworkers reported a ratiometric probe 21a for imaging mitochondrial biothiols by introducing a para-dinitrophenoxy benzylpyridinium segment (as recognition site) to a TPE skeleton.[87]

Upon addition of biothiols, the dinitrophenyl ether bond was cleaved, followed by the self-immolation reaction to generate compound 21c in aqueous solution, while the fluorescence intensity at 500 nm gradually increased and a concomitant decrease at 631 nm was observed, enabling detection of biothiols in ratiometric mode. Additionally, probe 21a exhibited high selectivity, good biocompatibility, a large two-photon absorption cross-section, and outstanding sensitivity toward biothiols detection and was employed for ratiometric targeting biothiols in living cells and fish larva under a two-photon microscopic platform. These results showed that probe 21a is a potential tool for clinical diseases diagnosis.

In 2017, by integrating a dicyanovinyl unit with a tetraphenylsilole derivative, Tang and coworkers fabricated two fluorescent probes 22 and 23 with AIE features.[88] The reactive dicyanovinyl unit enabled above-mentioned iso-mers the ability to specifically recognize thiol species. Spectral results showed that probe 22 could differentiate Cys and Hcy from GSH depending on significant differences in kinetics. Additionally, probe 23 exhibited higher sensitivity toward Cys with a detection limit lower than 0.5 μM. Moreover, probes could be designed as test strips for detection of biothiols. Utilizing the same recognition unit, Xie et al. reported an NIR turn-on fluorescent probe 24 for Cys and Hcy, which was composed of a triphenylamine analogue as the electron-donor and a dicyanovinyl unit as the electron-acceptor and the reactive site.[89] Probe 24 emitted weak fluorescence because of severe intramolecular rotations of the donor segment and intramolecular charge transfer effect. After cysteine or homocysteine reacted with dicyanooethylene, the intramolecular charge transfer property was weakened, and the products were aggregated in aqueous solution, resulting in obvious fluorescence at 651 nm and 656 nm, respectively. Benefiting from its excellent performances (including high selectivity, rapid response, and low cytotoxicity), probe 24 was successfully applied to image intracellular biothiols with a high signal-to-noise ratio.

It is an indisputable fact that there are more biological thiols (e.g., GSH and Trx) in tumor cells than in normal cells. Thus, Kim’s group has pioneered a series of disulfide-based fluorescent drug delivery conjugates to integrate tumor diagnosis and treatment, which have received more attention recently.[90,91] The selected fluorophores (coumarin, naphthalimide, BODIPY, and cyanine dyes) in their work are susceptible to aggregation-caused quenching at high concentrations, which reduces fluorophore brightness and affects the sensitivity in bioimaging. Thus, the nature of AIE materials has become a powerful and considered strategy for constructing new activatable bioprobes. For instance, Liu and Tang reported a water-soluble AIE bioprobe 25, as shown in Figure 4A.[92] Probe 25 consists of a TPE fluorogen, a disulfide liker that could be cleaved by GSH, a hydrophilic peptide containing five aspartic acids, and a cyclic RGD peptide serving as a targeting group. Introduction of the strongly hydrophilic moiety allowed probe 25 to behave in a monomolecular state in aqueous media. The probe could be selectively taken up by tumor cells mediated by receptor \( \alpha_\text{v}\beta_3 \) and activated by intracellular GSH, releasing insoluble AIE fluorophore accompanied by strong fluorescence owing to the formation of aggregates restricting of intramolecular rotation. The authors showed a general strategy for constructing functionalized AIE bioprobes for detection of thiols in living cells. Later, Liu et al. developed AIE photosensitizer 26 with the ability to self-report apoptosis, which consisted of a red-emissive photosensitizer and a built-in apoptosis sensor for in situ, real-time monitoring of therapeutic response.[93] Since the probe 26 contains a cyclic RGD peptide-targeting group, it can be selectively taken up by cancer cells with overexpressed \( \alpha_\text{v}\beta_3 \) integrin, and disulfide bonds can be cleaved by high level of GSH, liberating an efficient photosensitizer with red AIE fluorescence and an apoptosis sensor. Under light irradiation, ROS are generated by the photosensitizer to trigger cell apoptosis and activate caspase-3/-7. Meanwhile, the upregulated caspases were tracked in real time by an apoptosis sensor with green AIE fluorescence. The integrated probe strategy provides a new avenue for future research of tumor treatments and early evaluation feedback.

In 2020, Kim and coworkers developed a new activatable probe 27a for imaging-guided photodynamic therapy.[94] As shown in Figure 4C, the AIE fluorescence and singlet oxygen generating ability of the probe were inhibited by ferrocene owing to the PET effect. GSH could cleave the disulfide bonds to generate compound 27b with AIE characteristic as well as producing singlet oxygen under light irradiation. Moreover, phototherapeutic evaluation of probe 27a was successfully carried out in living CT-26 cells. To improve the efficacy of cancer chemotherapeutics, in 2019, Ding and Zheng reported a fluorescence and photocactivity dual-activatable prodrug 28a, which was composed of a 4-dimethylamino-2'-hydroxychalcone fluorogen with AIE characteristics, a disulfide bond, and the anticancer drug hydroxycamptothecin.[95] Utilizing an amphiphilic copolymer as the encapsulation carrier, 28a nanoparticles (about 126 nm in size) in aqueous solution were obtained, and these were favorable for in vivo applications. Upon addition of GSH, the disulfide bonds were cleaved, followed by release of active anticancer drug 28d and compound 28e, which could emit brighter AIE fluorescence and generate ROS under light irradiation for synergistic treatment of tumors.

To avoid damage to normal cells during photodynamic therapy, Liu and coworkers presented a dual-targeted activatable photosensitizer 29a for selectively recognizing and killing cancer cells.[96] Cell experiments demonstrated that probe 29a was efficiently taken up by tumor cells with overexpressed \( \alpha_\text{v}\beta_3 \) integrin and activated by intracellular over-expression of GSH, releasing a red-emission photosensitizer for ablating cancer cells under light irradiation. This work provided guidance for design of activatable photosensitizers with low intrinsic photocytotoxicity.

**AIEgens for highly toxic agent**

Highly toxic agents are dangerous to human life and health[97,98] Among them, phosgene (a colorless gas) was
employed as a chemical weapon agent, causing nearly 80% of deaths during World War I.\[99\] Therefore, developing a rapid and portable approach for detecting highly toxic agents is an urgent requirement for monitoring unexpected leakage in industry and avoiding unnecessary harm. In this regard, Wu and Zeng reported the first ratiometric fluorescent test-strip probe 30a with AIE characteristics for highly selective and sensitive determination of diethyl chlorophosphate (DCP), a gaseous nerve agent analog.\[100\] As shown in Figure 5A, a diphenylamine segment as the electron–donor and a pyridine group as the electron–acceptor were mounted on opposite ends of the TPE fluorophore matrix to fabricate a
smart sensor for responding to DCP. The pyridine unit was involved in nucleophilic attack of the strong electrophilic phosphonyl group of DCP, leading to a change in the fluorescence signal. Based on the above response mechanism, probe 30a was deposited onto filter paper to obtain a probe test strip for rapid detection of DCP with a high sensitivity of 1.82 ppb, which is below the reported IDLH concentration. To solve the issue of traditional probes for phosgene caused by aggregation-caused quenching, the same group later designed the first portable ratiometric fluorescent probe 31a for determination of gaseous phosgene. As shown in Figure 5B, this was accomplished by introducing an o-phenylenediamine segment and a malononitrile moiety into a typical AIE fluorophore to build a phosgene-responsive sensor 31a. Upon addition of phosgene, the o-phenylenediamine group in the sensor could react with phosgene to generate a new molecule skeleton with tunable AIE fluorescence from blue to green. The probe-loaded test-strip was employed for ratiometric determination of gaseous phosgene with a detection limit of 1.87 ppm, far lower than the “harmless” level.

In 2020, Wang and Hu reported a new ratiometric and colorimetric fluorescent probe 32a, which was composed of an AIE-based TPE fluorophore and a TICT-based 3-benzo[d]imidazole-chromen-2-imine segment. Probe 32a could detect phosgene in solvent and in the solid state through a luminescence mechanism conversion. Moreover, it could be prepared a test paper for rapid and highly selective identification of trace phosgene in solvent in less than 6 seconds and in an air atmosphere within 2 minutes. The detection limit was calculated to be 0.36 μM in solvent and 0.27 ppm in air. These satisfactory results demonstrated that this AIE + TICT strategy can provide an efficient approach for constructing portable solid-state sensors for identification of gaseous nerve agent.
AIEgens for hydrazine

Hydrazine (N₂H₄) is a well-known reducing agent that is widely used in the synthesis of various organic synthetic processes (e.g., emulsifiers, pharmaceutical intermediates, and textile dyes). However, overexposure to highly poisonous hydrazine can lead to many diseases in humans because it is associated with mutations, carcinogenesis, and malformations. Hydrazine is defined as a potential carcinogen by the US Environmental Protection Agency (EPA), and the exposure threshold value is suggested to be as low as 10 ppb. Thus, it is important to develop a reliable analytical method for detection of hydrazine in the environment and biological systems. Lee and coworkers reported a turn on fluorescent probe for hydrazine. As shown in Figure 5D, probe 33a was composed of a TPE as a typical AIE fluorophore and a dicyanovinyl unit as the recognition site for hydrazine as well as a fluorescence quencher. Upon addition of hydrazine, the fluorescence quencher was removed from the probe, generating compound 33b, which had a brighter AIE yellow fluorescence. The particle size changes of the probe before and after the reaction with hydrazine, which exhibited that product 33a rapidly aggregated to further reveal the AIE characteristics. Probe 33a was also prepared as a test strip sensor for sensing hydrazine vapor.

In 2020, Lin’s group developed the first AIE bioprobe 34a based on an α-cyanostilbene derivative for detection of hydrazine by integrating ICT and AIE mechanisms. Upon reaction with hydrazine in the mixture solution of 1,4-dioxane and H₂O, the probe fluorescence peak at 635 nm gradually decreased under excitation of 470 nm, while a new fluorescence peak at 500 nm was significantly enhanced. The detection limit of probe 34a toward hydrazine was 0.101 μM based on 3σ/k. Finally, probe 34a was successfully employed for detection of hydrazine in TLC plates, living cells, and zebrafish. Hung and coworkers developed a series of fluorescent probes 35-38 based on a triphenylamine Schiff-base derivative to accurately detect endogenous hydrazine in living systems. In vitro experimental results demonstrated that probes 35 and 37 displayed brilliant AIE yellow fluorescence in DMSO/H₂O solution. In the presence of hydrazine, a chemical reaction occurred such that –CH₂NH₂, resulting in weak fluorescence in aqueous solution due to the more hydrophilic reaction product. Additionally, probes 35 and 37 exhibited high selectivity and good bio-compatibility, suitable for tracking of endogenous hydrazine in living cells.

AIEgens for reactive oxygen species

AIEgens for hydrogen peroxide

Hydrogen peroxide (H₂O₂) is an omnipresent intracellular second messenger in signaling pathways that it is involved in regulation of many physiological processes. However, overgeneration of H₂O₂ in living cells is associated with serious diseases including diabetes, neurodegenerative disease, and cancers. Thus, developing efficient methods for in situ monitoring intracellular of H₂O₂ content are vital for biological studies and clinical diagnoses. To resolve the problem of slow response between the probe and H₂O₂, Tang and Li reported a novel AIE bioprobe bearing a boronate ester moiety as the recognition group, as shown in Figure 6. By increasing the polarity of the C-B bond, probe 39 could recognize H₂O₂ more quickly with high selectivity in the solution. In addition, the detection limit was estimated to be 0.52 μM, showing the high sensitivity of the probe toward H₂O₂. Overgeneration of H₂O₂ in RAW 264.7 cells induced by phorbol-12-myristate-13-acetate (PMA) was observed using probe 39. Using the reaction mechanism of boronate with H₂O₂ to generate phenol, Wang et al. described a H₂O₂-activatable fluorescent probe 40 for tracking lipid droplets in real time in living cells. Using an interface-targeting strategy and electrostatic interaction, probe 40 selectively aggregated on the surface of lipid droplets. After a H₂O₂-mediated reaction, a hydrophobic compound was generated in aqueous solution with bright AIE fluorescence. The probe exhibited higher photostability compared with the commercial dye Nile red. Cells imaging results demonstrated that probe 40 could be an efficient tool for dynamic movement monitoring of lipid droplets. In 2019, based on triphenylamine derivative, Li and coworkers developed a novel colorimetric and fluorescent probe 41 for detection of H₂O₂. In the absence of H₂O₂, probe 41 was nonfluorescent in solution and the aggregate state, thoroughly overcoming the interference of background signal. However, the presence of H₂O₂ triggered the oxidation reaction of the boronate group, followed by a 1,6-rearrangement elimination reaction, liberating the hydrophobic AIEgen with a strong fluorescence of 606 nm. Additionally, the detection limit was calculated to be 160 nM, superior to most previous findings. Chemiluminescence technology has received more attention due to the advantages of high sensitivity and signal contrast. However, most chemiluminescence probes cannot be used for long-term dynamic monitoring of organisms because the photons released after being activated by the analyte are not controllable. To overcome this issue, in 2020, Guo et al. adopted a sequential dual-lock strategy for constructing a chemiluminescent probe 42, which could be employed for detection of H₂O₂ with dual-modes of a bright AIE fluorescence and a chemiluminescent signal in vitro and in vivo. Tang and colleagues reported a theranostic drug delivery system 43, which was composed of TPE as an AIE fluorophore, a benzyl boronic ester as the reaction site for H₂O₂, and doxorubicin as an anticancer drug. Overexpression of H₂O₂ in cancer cells and tumors initiated the reaction of boronate to phenol, and the subsequent 1,6-rearrangement elimination reaction, which promoted simultaneous release of an AIE fluorophore and an anticancer drug. Ultimately, this strategy achieved integration of diagnosis and treatment. Wu’s group designed and synthesized a nanoprobe for detection of H₂O₂, which was formed through boronate ester groups between probe 44 and hydrophilic polyphenol tannic acid as well as the hydrophobic interaction. The boronate ester was cleaved in the presence of H₂O₂, and the hydrophobic product was generated with bright AIE fluorescence. This nanoprobe was utilized to detect endogenous and exogenous H₂O₂ in living cells. More importantly, overexpression H₂O₂ in acute abdominal, ankle inflammation, and renal ischemia mice model were monitored in situ using this nanoprobe. To overcome the dilemma of fluorescence quenching and ROS
weakening caused by aggregation of traditional photosensitizers. Wang and coworkers fabricated a H₂O₂-activatable AIE-active photosensitizer 45 for fluorescence-guided photodynamic therapy.⁴⁵ After molecule 45 was activated by H₂O₂ overexpressed in cancer cells, the product accumulated in lipid droplets with bright AIE fluorescence and high O₂ generation under light irradiation. In addition, the probe exhibited prominent features including good photostability, a large Stokes shift, and high sensitivity. The results of cell experiments demonstrated that probe 45 is a powerful tool for accurate treatment of cancer cells under mediation of H₂O₂.

AIEgens for hypochlorous acid/hypochlorite

Hypochlorous acid/hypochlorite (HClO/ClO⁻) is generated in activated phagocytes by peroxidation of H₂O₂ and Cl⁻ mediated by myeloperoxidase, which acts as a powerful antimicrobial agent in the immune system to protect against infections.¹²⁸–¹³¹ However, uncontrolled production of HClO/ClO⁻ from phagocytes is associated with many human diseases including osteoarthritis, rheumatoid arthritis, and cancers.¹³²–¹³⁵ Thus, detecting cellular HClO/ClO⁻ concentration is vital for revealing its role in physiological and pathological processes. For instance, Wu and colleagues
designed an AIE-active probe 46 bearing a dicyanovinyl group, which was further constructed into a nanoprobe with surfactant micelles for sensing of ClO\(^{-}\).\[136\] The nanoprobe displayed strong fluorescence at 595 nm owing to the intrinsic AIE aggregates luminescence. ClO\(^{-}\) reacted with the nanoprobe in aqueous media, resulting in a new structural transition from a dicyanovinyl group to an aldehyde group. This resulted in a new fluorescence around 498 nm, allowing ratiometric detection of ClO\(^{-}\). In living cells and zebrafish, endogenous ClO\(^{-}\) was imaged with a dual-channel using this nanoprobe. To improve the selectivity and sensitivity of ClO\(^{-}\) detection, Zhang et al. developed turn-on fluorescent probe 47 based on a TPE derivative.\[137\] The aggregates of 47 were almost nonfluorescent because of the PET between the 1-methyl-1,2-dihydropyridine-2-thione unit and the TPE core. Upon addition of ClO\(^{-}\), the recognition moiety was transformed into a 2-sulfate substituted pyridinium unit, releasing a strong emission at 620 nm owing to the suppression of the PET process. Additionally, the probe exhibited high selectivity and sensitivity (DL, 92 nM) toward ClO\(^{-}\) and was applied for detection of ClO\(^{-}\) in drinking water and living cells.

By exploiting the advantages of aggregation-induced emission and through-bond energy transfer, Sun and colleagues reported a fluorescent probe 48 for HClO detection with high signal-to-noise ratio and sensitivity.\[138\] Probe 48 was composed of a TPE moiety as the energy transfer cassette, and a rhodamine B thiohydrazide as the energy-acceptor. Probe 48 emitted blue AIE fluorescence due to formation of aggregates in aqueous solution restricting of intramolecular rotation. After adding HClO, the spirilactam ring of rhodamine was opened through HClO-mediated cyclization of monothiol-bishydrazide to 1,2,4-oxadiazole, resulting in red emission at 589 nm due to the mechanism of dark through-bond energy transfer. The detection limit was 1.29 nM, demonstrating the high sensitivity of the probe for detecting HClO. Lastly, the exogenous and endogenous HClO in living cells was successfully imaged using probe 48. In 2020, a similar strategy was adopted by Huang and coworkers to develop a ratiometric fluorescent probe 49 for sensing HClO in aqueous media.\[139\] By virtue of its high selectivity, sensitivity, and prominent photostability, probe 49 could be used to identify tumor cells in cocultured models through HClO detection. Chen et al. designed a fluorescent probe 50 based on a TPE platform that was made into AIE dots by a coprecipitation strategy for ratiometric detection of ClO\(^{-}\).\[140\] The response mechanism of the probe toward ClO\(^{-}\) was oxidation of olefinic carbon-carbon double bonds, which was further confirmed by assays of \(^1\)H-NMR titration, mass spectra, and theoretical calculation. Additionally, the AIE dots were applied to detect ClO\(^{-}\) in real samples.

Based on TPE-functionalized diarylamine derivatives, Li and coworkers developed three new AIEgens 51-53 with different functions depending on the decoration of end-groups.\[141\] Both 51 and 52 were easy to crystallize because of the symmetric structure, which possessed reversible mechanochromic properties with significant changes of fluorescence emission. In contrast, asymmetric probe 53 was equipped with indolenine as the end-capping subunit and did not exhibit mechanochromic characteristics, but it could serve as an efficient tool for detection of hypochlorite with high selectivity and sensitivity (DL, 105 nM). To expand its biological applications, nanoprobe 53 was obtained using an amphiphilic polymer as a carrier and exhibited good ability to detect hypochlorite in living cells with high photostability.

**AIEgens for superoxide radicals**

Superoxide radical (O\(_2\)\(^{•−}\)), as the precursor of other ROS species, can generate peroxynitrite by reacting with nitric oxide and undergoing disproportionation to produce hydrogen peroxide.\[142-145\] Therefore, it is vital to develop an efficient tool for revealing the relationship between superoxide radical and diseases. Tang and coworkers described a two-channel AIE bioprobe for highly specific imaging of O\(_2\)\(^{•−}\) in living cells, which was composed of a pyridinium-modified TPE derivative as the fluorescent reporter, a phosphinate group as the recognition group, and a methenyl-quinone as the linker.\[146\] The presence of a strong “push-pull” electronic structure caused red fluorescence of the probe. Upon addition of O\(_2\)\(^{•−}\), the diphenyl phosphinate group of probe 54 was selectively remove to generate a hydrophobic product, emitting green fluorescence. Of note, the elevated O\(_2\)\(^{•−}\) in cells with inflammation induced by lipopolysaccharide was observed using probe 54. To improve the accuracy of detection, in 2017, Tang and Wang rationally designed a novel fluorescent probe 55a for sensing of O\(_2\)\(^{•−}\) with simultaneous turn-on fluorescence/chemiluminescence signals.\[1\] As shown in Figure 6, imidazopyrazinone was chosen as the reaction group in the TPE fluorophore with AIE nature. Probe 55a exhibited a high sensitivity toward O\(_2\)\(^{•−}\) with a detection limit of 0.21 nM for fluorescence and 0.38 nM for chemiluminescence. Accordingly, native O\(_2\)\(^{•−}\) in RAW 264.7 cells, elevated O\(_2\)\(^{•−}\) in stimulated inflamed mice, and endogenous O\(_2\)\(^{•−}\) in HL-7702 cells induced by acetaminophen were successfully monitored in real time using probe 55a with chemiluminescence sensing and fluorescence imaging, displaying a novel example of dual signal detection of a single analyte in complex biological systems.

**AIEgens for peroxynitrite**

Peroxynitrite (ONOO\(^{−}\)), one kind of reactive nitrogen species (RNS), is produced through coupling of superoxide radicals (O\(_2\)\(^{•−}\)) and nitric oxide (NO) in living organisms.\[144,148,149\] It takes part in many pathologic processes and cellular signaling pathways.\[150-152\] Abnormal generation of ONOO\(^{−}\) is associated with many diseases such as cardiovascular disease, Alzheimer’s disease, and cancers.\[153-155\] As a consequence, powerful tools for selectively detecting ONOO\(^{−}\) are needed for discovering its deeper physiological functions. For example, based on an imine-functionalized TPE derivate, Tang and Ding rationally designed and fabricated a turn-on nanoprobe 56 for detection of ONOO\(^{−}\). As seen in Figure 7A, probe 56 consisted of three segments: a phenylboronate as the reaction group for ONOO\(^{−}\), an imine unit as the emission mediator, and a TPE derivative as the AIE emission fluorogen. Nanoprobe 56 obtained using lipid-PEG200 as the encapsulation matrix was nonfluorescent, but emitted a brighter yellow fluorescence in aqueous solution after adding ONOO\(^{−}\). In contrast, the contrast nanoprobe 57 and 58 did not yield responsive fluorescence changes. Additionally, nanoprobe 56 exhibited high selectivity toward ONOO\(^{−}\),
overcoming the interference of other reactive oxygen species including ClO\(^-\), ROO\(^-\), •OH, H\(_2\)O\(_2\), and O\(_2\)\(^{-}\). The elevated ONOO\(^-\) in the constructed inflammatory mouse model was in-situ observed using nanoprobe 56.

In 2020, Ye, Zhang, and Cao reported a new activatable fluorescent probe 59a based on a quinolone-malononitrile (QM) fluorogen platform for sensing of ONOO\(^-\). In vitro experimental results demonstrated that probe 59a possessed a fast response time, high sensitivity (DL, 27.5 nM), and a large Stokes shift (190 nm) for detection of ONOO\(^-\). Moreover, owing to its good biocompatibility, probe 59a was successfully used to image endogenous ONOO\(^-\) in living EC1 cells.

Presently, there are few fluorescent probes for sensing of ONOO\(^-\) with AIE features. To resolve this issue, the first ratiometric AIE bioprobe 60a was designed by Wang et al. for visualization of ONOO\(^-\) with superior photostability. Probe 60a was composed of a benzothiazolyl derivative as the AIE-active fluorophore and a diphenylphosphinate segment as the recognition site. In the absence of ONOO\(^-\), the probe emitted red fluorescence in aqueous solution owing to the molecular aggregation restricting the C = C isomerization. Upon reaction with ONOO\(^-\), the diphenylphosphinate group was cleaved, resulting in a phenate intermediate, which generated compound 60b with green AIE fluorescence. The detection limit was calculated to be as low as 30 nM, indicating the high sensitivity of the probe toward ONOO\(^-\). Additionally, real-time visualization of ONOO\(^-\) in living cells and mice was successfully realized using bioprobe 60a.

**FIGURE 7** (A) Schematic illustration of nanoparticles 56, 57, and 58 toward ONOO\(^-\). Reproduced with permission: Copyright 2016, Wiley-VCH GmbH.[156] (B) Proposed reaction mechanism of probe 59a with ONOO\(^-\). (C) Schematic representation of probe 60a for detection of ONOO\(^-\). Reproduced with permission: Copyright 2020, Elsevier[158]

**AIEgens for other small molecules**

Thanks to the characteristic of phenyl boronic acid reacting with diols rapidly in aqueous media, many biomedical sensors for glucose have been developed, however, were rather limited because of their high affinity with isomeric saccharides (D-fructose, D-galactose, D-mannose). As shown in Figure 8A, Tang et al. designed TPE-cored diboronic acid biosensor, 61a, using aggregation induced emission property and restriction of intramolecular rotation for the specific detection of D-glucose (Glu). The light emission of 61a started to decrease with increasing pH. Since the pKa of phenylboronic acid is about 9, 61a was ionized by the alkaline solution and became soluble in the buffer and nonfluorescent. With increasing concentration of Glu, the fluorescence intensity of 61a was gradually intensified, as expected. However, the concentration of Glu over 5 mM promoted the decreased of fluorescence intensity. To analyze this unique phenomenon, the working mechanism of the binding 61a to Glu was proposed in Figure 8A. In addition, application of 61a was conducted by the measurement of Glucose in urine, which proved the potential of 61a for real-world biomedical applications.

Sulfur dioxide (SO\(_2\)) is recognized as a toxic gas or pollutant and it becomes a new member of gaseous signaling molecules. Inhalation of SO\(_2\) produces various sulfur-centered radicals and oxygen-centered radicals leading to formation of superoxide anion radicals. Therefore, developing an effective probe for monitoring SO\(_2\)-induced...
oxidative stress is crucial. Ye and coworkers described a mitochondrion-targeted fluorescent probe $62a$ for detection of SO$_2$, which combined with TPE and benzopyrylium. In the presence of SO$_2$ derivatives, 1,4-conjugate Michael Addition reaction of benzopyrylium took place which induced strong green fluorescence of $62b$. Additionally, probe $62a$ exhibited high selectivity and sensitivity in response to SO$_2$ in vitro. Furthermore, cell imaging results demonstrated that upregulation of SO$_2$ in mitochondria may be dependent on the oxidative stress induced by heat shock for the first time.

Numerous evidences demonstrated that NO is associated with so many diseases (such as stroke, heart disease, hypertension, neurodegeneration, and erectile dysfunction). Efforts to design fluorescent probe for detection of NO have been made remaining not enough quantum yield and fluorescence interference from the biological matrix. Tian et al. synthesized silica nanoparticles ($63a$@SiO$_2$ NPs) based on the TPE derivative for highly sensitive and selective ratiometric detection of NO in aqueous buffer. Probe $63a$ was rationally designed and synthesized, which was composed of AIE chromophore and NO-sensitive fluorescence modulator. In the presence of NO, $o$-phenylenediamine of $63a$ was converted to $63b$ with benzotriazole moiety, an electron acceptor group inducing emission red-shift. Then, silica nanoparticles were fabricated for the dispersibility and stability of the probe. In addition to inherent property of $63a$, its aggregation in silica even more enhance the fluorescence intensity by the restriction of intramolecular motions. Remarkable red-shift (about 100 nm) of NPs treated with DEA-NONOate enabled NO to be evaluated via the ratiometric change in two emission bands. Furthermore, Cell imaging results displayed that $63a$@SiO$_2$ could be applied to detect intracellular NO in real time.

The inhalation of formaldehyde (FA), a reactive carbonyl compound, leads to headache, tingling sensation in the throat, dyspnea, and even cancer with long-term exposure. Therefore, developing a facile and sensitive method for gaseous FA detection is imperative. For better sensitivity and
selectivity than conventional methods for FA detection, Yin and coworkers designed a new probe, 64a, using an AIE fluorophore. In the absence of FA, due to PET effect, 64a had weak fluorescence. After reacting with FA, 64a turned into highly emissive compound by 2-aza-Cope sigmatropic rearrangement and was also easy to observe with naked eye. Furthermore, by simply soaking high performance thin-layer chromatography silica gel plate in probe solution, solid sensor was prepared which made FA detection more probable and convenient.

Because it is one of the significant biological regulators which naturally modulates inflammatory responses, carbon monoxide (CO) has received great attention nowadays. Finding out physiological and pathological functions relies on powerful tools to selectively visualize CO in living organisms. Tang et al. synthesized a ratiometric fluorescent probe, 65a, for sensing CO via Pd0-mediated Tsuji-Trost reaction. In the presence of CO, removal of the allyl group generated phenolate intermediate which underwent rapid cyclization. As a result, restriction the rotation of the C-C double bond gave the highly fluorescent 65c. The potential application of 65a for imaging of CO in living cells and animals were investigated, indicating its good permeability and fluorescence response. This work opened a new way to develop reaction-based AIE probe for real-time visualization of CO in a living system.

The analytical parameters of activatable AIE probes discussed in the subsections are summarized and shown in Table 1.
| AIE probe | Analyte       | Excitation/emission      | Detection limit | Application            |
|-----------|---------------|--------------------------|-----------------|------------------------|
| 1a        | H₂S           | 340 nm/493 nm            | No data         | In solution            |
| 3         | H₂S           | 300 nm/380, 465 nm       | 0.33 μM         | Cells                  |
| 4         | H₂S           | 452 nm/550 nm            | 0.09 μM         | Cells and zebrafish larvae |
| 5         | H₂S           | No data/480 nm          | 12.8 nM         | Cells and C. elegans   |
| 6a        | H₂S           | 390 nm/468 nm            | 0.17 μM         | Cells and mice         |
| 7         | H₂S           | 360 nm/438, 598 nm; 760 nm/920 nm | 0.65 μM   | Cells and mice         |
| 8         | H₂S           | 350 nm/540 nm            | 41 nM           | Cells                  |
| 9a        | H₂S           | 450 nm/700 nm            | 0.57 μM         | Cells                  |
| 10        | H₂S           | 408 nm/530 nm            | No data         | Cells                  |
| 11        | H₂S           | 330 nm/475 nm            | 0.25 nM; 0.30 nM | In solution            |
| 12        | H₂S           | 320 nm/480 nm            | 84 nM           | Test strips and cells  |
| 13        | H₂S           | 372 nm/429, 565 nm       | 0.5 μM          | In solution            |
| 14a       | Cys and GSH   | 340 nm/505 nm            | No data         | Cells                  |
| 15a       | Cys           | 341 nm/490 nm            | 0.18 μM         | Cells                  |
| 16        | Cys and Hcy   | 362 nm/500 nm            | No data         | Cells                  |
| 17        | Cys and Hcy   | 361 nm/500 nm            | No data         | Cells                  |
| 18a       | Hcy           | No data/455, 550 nm      | 0.346 μM        | In solution            |
| 19a       | Cys and Hcy   | 343 nm/422, 501 nm       | No data         | In solution            |
| 20a       | GSH           | 340 nm/411, 578 nm       | 0.05 μM         | Cells                  |
| 21a       | Cys, Hcy, and GSH | 365 nm/500, 631 nm     | 0.61 μM         | Cells and tissues      |
| 22        | Cys and Hcy   | No data/516, 602 nm      | No data         | Test strips            |
| 23        | Cys, Hcy, and GSH | No data/490, 550 nm    | <0.5 μM         | Test strips            |
| 24        | Cys and Hcy   | 530 nm/651 nm            | 8.4 μM; 5.7 μM  | Cells                  |
| 25        | GSH           | 312 nm/470 nm            | 1.0 μM          | Cells                  |
| 26        | GSH and caspase-3/-7 | 430 nm/650 nm       | 2.3 pM          | Cells                  |
| 27a       | Cys, Hcy, and GSH | 430 nm/620 nm          | 0.57 μM; 0.6 μM; 0.77 μM | Cells                  |
| 28a       | GSH           | 405 nm/560 nm            | No data         | Cells and mice         |
| 29a       | GSH           | 430 nm/650 nm            | No data         | Cells                  |
| 30a       | DCP           | 465 nm/546, 624 nm       | 1.82 ppb        | Test strips            |
| 31a       | COCl₂         | 365 nm/435, 496 nm       | 1.87 ppm        | Test strips            |
| 32a       | COCl₂         | 365 nm/503 nm; 470 nm/495, 570 nm | 0.36 μM; 0.27 ppm | Test strips            |
| 33a       | N₂H₄         | 400 nm/575 nm            | 0.11 μM         | Test strips            |
| 34a       | N₂H₄         | 470 nm/500, 635 nm       | 0.101 μM        | Test strips and cells  |
| 35/37     | N₂H₄         | 330 nm/560 nm            | 55.1 nM         | Cells                  |
| 39        | H₂O₂         | 400 nm/500 nm            | 0.52 μM         | Cells                  |
| 40        | H₂O₂         | 420 nm/590 nm            | 0.083 μM        | Cells                  |
| 41        | H₂O₂         | 495 nm/606 nm            | 160 nM          | In solution            |
| 42        | H₂O₂         | No data/600 nm           | No data         | Cells and mice         |
| 43        | H₂O₂         | 330 nm/480 nm            | No data         | Cells                  |
| 44        | H₂O₂         | 580 nm/725 nm            | 117 nM          | Cells and mice         |
| 45        | H₂O₂         | 450 nm/625 nm            | 0.13 μM         | Cells                  |
| 46        | ClO⁻         | 340 nm/498, 595 nm       | 0.47 μM         | Cells and zebrafish larvae |
| 47        | ClO⁻         | 400 nm/620 nm            | 92 nM           | Test strips and cells  |
| 48        | ClO⁻         | 350 nm/477, 589 nm       | 1.29 nM         | Cells                  |
| 49        | ClO⁻         | 355 nm/485, 595 nm       | 0.05 μM         | Cells                  |
| 50        | ClO⁻         | 360 nm/467, 536 nm       | 89 nM           | Test strips            |
to track ALP activity in stem cells during osteogenic differentiation. This design approach revealed that the ratio relationship between the recognition group and AIE fluorophore determined the properties of the probe. A new sensing strategy based on a micelle was developed by Lee and coworkers for quantitatively detecting the activity of ALP in human serum.\textsuperscript{[180]} Compared with the control probe 70, introduction of a tetraethylene glycol unit on AIEgen 71 resulted in formation of micelles of the ALP-catalyzed products in the solution, resulting in a ratiometric monitoring behavior with a detection limit of below 0.034 mU/mL. Based on a chalcone derivative, Tang and Liu designed a ratiometric AIEgen 72 for tracking ALP activity.\textsuperscript{[181]} The probe itself emitted greenish-yellow fluorescence in solution. With addition of ALP, the phosphate group of AIEgen 72 was cleaved, generating an insoluble enzymatic product that could form aggregates accompanied by red fluorescence due to the AIE process and the intramolecular hydrogen bonding formation of the ESIP mechanism. The detection limit was calculated to be 0.15 mU/mL. In view of its excellent biocompatibility, AIEgen 72 was successfully applied to monitor the ALP activity in HeLa cells. Recently, Yoon and Peng adopted a matched amphiphilic strategy to devise the activated AIEgen 73 for sensing ALP activity.\textsuperscript{[182]} The amphiphilic property of 73 meant that it did not fluoresce in aqueous solution owing to the loosely packed nanoAIEgens. After enzymatic reaction with ALP, the phosphate group was cleaved from probe 73, and an insoluble quinolone-malononitrile (QM) chromophore was generated, inducing in situ aggregation and releasing its intrinsic bright AIE fluorescence. This probe exhibited a high sensitivity toward ALP in aqueous solution. The upregulation of ALP activity in HeLa induced by sodium butyrate and cortisol was first imaged using an AIE probe with high spatiotemporal resolution. Relying on overexpression APL in cancer cells, AIEgen 73 was delivered into 3D-structured tumor spheroids to map the distribution of ALP and was successfully employed for imaging-guided resection of cancerous tissue. Zeng’s group constructed a novel probe 74 for detection of ALP.\textsuperscript{[183]} In the absence of ALP, the probe emitted weak blue fluorescence because the ESIP process was hindered. Upon addition of ALP, the phosphate group on AIEgen was rapidly hydrolyzed, yielding a yellow fluorescence owing to recovery of the ESIP process and AIE features. Additionally, the AIE probe did not respond to other common biologically relevant species, showing high selectivity. Probe 74 was successfully used to track the activity of ALP in living cells. By introducing a cyano group (strong electron-withdrawing) in the middle and a phosphate group (recognition moiety), Wu and Zeng developed an AIE-active probe 75 for detecting ALP activity, showing a good conjugation degree and water solubility.\textsuperscript{[184]} DLS characterization demonstrated that the catalytic products of the probe formed aggregates after addition of ALP, accompanied by AIE fluorescence. Considering its good properties, such as excellent cell permeability, high selectivity, and sensitivity, the probe has been successfully used to differentiate cancer cells (HeLa and HepG-2) from normal cells (L929). Utilizing enzyme-instructed self-assembly strategy, Ding, Bao, and Liu designed a new theranostic AIE probe 76 for fluorescence imaging-guided photodynamic treatment of cancer cells.\textsuperscript{[185]} Activation of the probe by overexpressed ALP on the surfaces of Saos-2 cells induced the catalyzed products to self-assemble into nanoparticles, which restricted the intramolecular free rotation and blocked the nonradiative decay of the excited states, restoring AIE fluorescence and high ROS generation capability, finally resulting in specific identification and ablation of cancer cells. This study provides a new idea for the design of activatable AIE probes for integration of diagnosis and treatment of diseases.

Inspired by these pioneering studies, Sun et al. described a new bioprobe, 77, for monitoring ALP activity with the combined advantages of AIE and ESIP mechanisms.\textsuperscript{[186]} In vitro experiments showed that the probe had high selectivity and sensitivity for detecting ALP with a detection limit of 0.012 U/L as well as a large Stokes shift of 180 nm. Additionally, from cell imaging, probe 77 demonstrated the ability to differentiate cell lines via imaging intracellular ALP activity. Most enzyme-reactive fluorescent probes cannot provide in situ information because the liberated fluorophores tend to diffuse away from the reaction site. To address the above issue, Zhang and coworkers designed a smart AIEgen 78 based on their previously developed solid-state fluorochrome.\textsuperscript{[187]} It showed robust sensing ALP activity with a high signal-to-noise ratio. After breaking the phosphate ester bond by ALP, soluble probe 78 could be transferred into highly insoluble products, subsequently precipitating in situ and emitting intense AIE fluorescence. This probe was applied to track the levels of ALP in cancer cells.

### Table 1 (Continued)

| AIE probe | Analyte   | Excitation/emission | Detection limit | Application          |
|-----------|-----------|----------------------|-----------------|----------------------|
| 53        | ClO\(^-\) | 529 nm/560 nm        | 105 nM          | Cells                |
| 54        | O\(_2^{2-}\) | 390 nm/525, 615 nm    | No data         | Cells                |
| 55a       | O\(_2^{2-}\) | 350 nm/500 nm        | 0.21 nM; 0.38 nM | Cells and mice       |
| 56        | ONOO\(^-\) | No data/538 nm       | 100 μM          | Cells and mice       |
| 59a       | ONOO\(^-\) | 430 nm/620 nm        | 27.52 nM        | Cells                |
| 60a       | ONOO\(^-\) | 440 nm/525, 632 nm    | 30 nM           | Cells and mice       |
| 61a       | Glu       | 365 nm/485 nm        | No data         | In solution          |
| 62a       | SO\(_2^\)  | 400 nm/455 nm        | 27.22 μM        | Cells                |
| 63a       | NO        | No data/519, 655 nm   | No data         | Cells                |
| 64a       | FA        | 335 nm/480 nm        | 0.036 mg/m\(^3\) | Test strips          |
| 65a       | CO        | 465 nm/546, 710 nm    | 30.8 nM         | Cells                |
and tissues. More importantly, the authors showed the ability to visualize enzyme activity in situ. Previously reported ALP fluorescent probes were mainly used in cells and in vivo. However, developing new fluorescent probes for sensing bacterial ALP activity remains challenging. In 2020, Yang et al. designed the first probe based on a self-assembly-controlled AIEgen-peptide structure for detecting bacterial ALP activity, and it showed a detection limit of 6.6 mU/mL. Dephosphorylation of the probe by bacterial ALP could significantly reduce its hydrophilicity, triggering the products to form a fibrous structure on the surface of *E. coli* owing to peptide self-assembly. This generated AIE fluorescence, and the proposed approach provide a new avenue for bacterial ALP detection.

**AIEgens for esterase**

In numerous animal species, esterase plays an important role in drug metabolism and activation or chemical toxin clearance by catalyzing the hydrolysis of substrates. Therefore, it is particularly important to study the activity and distribution of esterase. However, most esterase fluorescent probes are affected by ACQ in high-concentration solution or in cells. Thus, developing AIE-active probes for detecting esterase has become urgent. For instance, Liu et al. developed probe 80 based on a salicylaldazine fluorogen for detection of lysosomal esterase activity, as shown in Figure 10. Reaction of the probe with esterase, resulted in acetyl ester cleavage to form phenolic hydroxyl fluorophores with AIE properties, followed by recovery of the ESIPT process to emit an intense green fluorescence. Further, lysosomal esterase in cells and lysosomal movements could be in situ visualized using 80, showing its great potential for diagnosis of Wolman disease. Li and Chen also described a new fluorescent “light-up” probe 81, in which a carboxylic ester group acted as the reactive site. The probe itself showed negligible fluorescence in aqueous solution. Addition of carboxylesterase led to generation of a relatively hydrophobic tetraphenylethene derivative that further formed supramolecular microfibers by self-assembling, resulting in strong fluorescence. This AIE probe exhibited high selectivity for carboxylesterase over other proteins with circa 29 pM detection limit. Later, Tong and coworkers rationally developed a red-emission bioprobe 82 with AIE and ESIPT characteristics by attaching diethylamine (electron donor) and a maleonitrile group (electron acceptor) to salicylaldazine. In this contribution, the authors revealed the vital role of interplanar spacing among molecules in realizing AIE fluorescence. In addition, biological experiments displayed that AIE bioprobes possessed potential for use in the monitoring of mitochondrial esterase in MCF-7 and HeLa cells.

**Full understand the complex interactions between various chemical species and organelles are of great significance for unravelling the secrets of life. To address this challenge, in 2020, Tang’s group rationally devised a single AIEgen 83, in which an acetyl group acted as the esterase-hydrolysis site, delocalized cation served as the mitochondria-targeting moiety, and the hydrophobic triphenylamine (TPA) part was employed as an AIE core.** Cell imaging results demonstrated that probe 83 was first located in the mitochondria with red fluorescence and partially hydrolyzed by esterase. Then, it migrated to the lipid droplets with blue fluorescence owing to the strong lipophilicity of the enzyme-catalyzed products. This dual localization and fluorescence spectroscopically distinguishable property enabled the probe to distinguish various cell physiological stages (including live, early apoptotic, late apoptotic, and dead cells) through laser confocal microscopy and flow cytometry, respectively.

**AIEgens for β-galactosidase**

Some experiments have shown that β-galactosidase (β-gal) is both a common reporter enzyme used to detect transcription and transfection efficiency and a participant
in important physiological and pathological processes in organisms.\cite{197,198} Deficiency of \( \beta \)-gal can lead to Morquio B syndrome or \( \beta \)-galactosidosis.\cite{199,200} Its overexpression is associated with malignant tumors and cell senescence.\cite{201,202,203} Therefore, sensitive detection and imaging of \( \beta \)-gal are of great importance. Given that conventional probes of \( \beta \)-gal suffered from the ACQ effect, Liu and coworkers described an AIE-active fluorescent probe \( 84 \) bearing a \( \beta \)-galactopyranoside group as the activation site.\cite{205} With addition of \( \beta \)-gal, the hydrophilic \( \beta \)-galactopyranoside group on probe \( 84 \) was cleaved, generating salicylaldehyde azines (SAs) with poor water solubility as AIE luminescence cores. This AIE bioprobe was capable of detecting \( \beta \)-gal with a low detection limit of 0.014 U/mL in aqueous buffer and monitoring \( \beta \)-gal activity in cells with high contrast. Using the same recognition mechanism, with a TPE skeleton, AIE light-up sensor \( 85 \) was reported by Wang and coworkers, for detection of \( \beta \)-gal activity in vitro and in cells.\cite{206} Upon reaction of \( 85 \) with \( \beta \)-gal in aqueous solution, the \( \beta \)-galactopyranoside group was cleaved and spontaneously underwent 1,6-elimination of \( p \)-quinone-methide to obtain a hydrophobic product, generating AIE fluorescence because of the RIR effect. Other common biomolecules caused a weak change in the fluorescence intensity, clearly showing the high specificity of probe \( 85 \) for \( \beta \)-gal. Additionally, \( \beta \)-gal activity in living cells could be imaged using \( 85 \), which demonstrated its potential for biological applications. In 2018, Zhu and Guo fabricated a \( \beta \)-gal-activatable AIEgen \( 86 \) capable of high-fidelity on-site sensing and long-term tracking.\cite{207} The probe was composed of a hydrophobic fluorophore as the AIE fluorescence reporter and a hydrophilic \( \beta \)-galactopyranoside group as the triggered moiety. The fluorescence increase of 15-fold was observed, which was attributed to in situ nanoaggregate formation of enzyme-catalyzed product induced by \( \beta \)-gal. Notably, probe \( 86 \) was successfully applied to monitor the activity of \( \beta \)-gal in SKOV-3 cells for long time with high resolution. Later, the same group developed a smart near-infrared AIE-active bioprobe \( 87 \) by extending \( \pi \)-conjugated structure.\cite{208} Addition of \( \beta \)-gal to \( 87 \) in aqueous buffer resulted in intense fluorescence at 650 nm. Owing to good cell penetrability and a nontoxic nature, \( \beta \)-gal activity could be monitored by \( 87 \), clearly exhibiting its potential in preclinical applications. The selective recognition and removal of senescent cells are of great significance for prolonging life and improving the treatment efficacy of tumor therapy. Yang, Ding, and Wang adopted an enzyme-triggered self-assembly strategy to construct fluorescent probe \( 88 \) with AIE character and efficient ROS generating properties.\cite{209} High level of \( \beta \)-gal converted probe \( 88 \) to an insoluble product. Subsequently, formation of nanomaterials in senescent cancer cells resulted in brighter fluorescence and generated more ROS under light irradiation for removal of senescent cancer cells.

### AIEgens for lipase

Patients with acute pancreatitis are at risk for enduring pain and high mortality. Early diagnosis of pancreatic diseases is difficult, mainly because the organ is relatively hard to reach.\cite{210,211,212} Therefore, the necessary treatment is not administered in time. In clinical applications, lipase in serum has been regarded as an important biochemical indicator for early medical diagnosis of diseases.\cite{213,214,215} So, a simple and convenient method of detecting lipase levels in human serum is critical. In 2017, utilizing the heterogeneous catalytic character of lipase, Tang and coworkers developed a “turn-on” AIE probe \( 89 \) by attaching two glutamate moieties to the TPE core.\cite{216} The TPE core of \( 89 \) tended to insert into the oil layer, while two glutamine units of \( 89 \) could be inserted into the water layer, ensuring that \( 89 \) was distributed at the oil-water interface, and that the glutamine group contacting lipase was fully in the aqueous phase. Accordingly, two glutamate groups on the probe were cleaved with high efficiency, releasing an insoluble product in the solution and emitting a strong blue fluorescence. The detection limit of the probe toward lipase was as low as 0.13 U/L. This was superior to previous reports and the probe was successfully used to detect the lipase content in serum. Based on the same AIE fluorogen, Huang’s group presented another “light-up” probe \( 90 \) for detecting lipase activity. In the presence of lipase, the \(-\text{COOC}_6\text{H}_{13}\) unit was rapidly cleaved, which prompted transformation from the probe to the relatively insoluble enzymatic product in the same solution.\cite{217} The self-assembled aggregates subsequently formed with “turn-on” AIE fluorescence. There was an excellent liner relationship between fluorescence intensities and various lipase concentrations (0.1-1.3 mg/mL). Furthermore, other relative bioenzymes and proteins such as pepsin, caspase-3, glucose oxidase, acylase, lysozyme, trypsin, and amylase induced negligible fluorescence changes of probe \( 90 \), indicating its high selectivity for lipase.

### AIEgens for cathepsin B

Cathepsin B, belonging to one of lysosomal cysteine proteases, is overexpressed in many malignant tumors.\cite{218,219} Thus, it plays a vital role in the regulation of angiogenesis and invasion during tumor progression.\cite{219,220} Substrates with the \(-\text{Gly-Phe-Leu-Gly}\) (GFLG) peptide sequence can be specifically recognized and cleaved, and they are widely employed for guiding the design of enzyme-activatable fluorescent probes as well as for drug delivery.\cite{221} For instance, Liu’s group designed and synthesized a dual-targeted enzyme-responsive probe \( 91 \).\cite{222} As shown in Figure 11A, this system contains a cyclic RGD peptide as the tumor-targeting unit, three Asp elements as a hydrophilic linker, a GFLG peptide sequence substrate as a cleavable subunit, and a newly developed AIE fluorogen as a vital fluorescent reporter and photosensitizer. As illustrated in Figure 11B, the substrate containing a GFLG peptide sequence can be cleaved by cathepsin B overexpressed in the tumors under a physiological environment. The released AIE core produced a strong fluorescence signal in aqueous solution concomitant with activated photodynamic activity for ablation of cancer cells. The therapeutic effect of the probe was further evaluated through a standard MTT method using MDA-MB-231, MCF-7, and 293 cells. Conjugate \( 91 \) exhibited the ability to selectivity kill MDA-MB-231 cancer cells (Figure 11D), overexpressing cathepsin B under light irradiation compared to the group without light (Figure 11C). Additionally, the cell viability results of MDA-MB-231 cells preincubated with cRGD, CA-074-Me, or vitamin C were consistent with apoptosis studies. This observation verified
that the αvβ3 integrin overexpressed in the outer member of cancer cells played an important role in uptake of molecules (Figure 11E). Furthermore, probe 91 reduced the proliferation of MDA-MB-231 cells under different light irradiation times. Compared with the existing traditional photosensitizer, Liu et al. provided a simple but effective design strategy for constructing dual-targeted activatable photosensitizers for highly selective killing of cancer cells. In 2015, Ji and Jin developed a smart multifunctional bioprobe 92a (Figure 11G) for imaging-guided therapy, which was composed of TPE as a fluorescent reporter, a GFLG peptide sequence responsive to cathepsin B, five Asp units as a hydrophilic peptide to increase the hydrophilicity of probe, a cRGD moiety as a tumor-targeting ligand, and an antitumor drug (gemcitabine) conjugated to the cleavable disulfide bond.\(^\text{[223]}\) In physiological condition, probe 92a was expected to have no fluorescence owing to its high aqueous solubility. More probes could be internalized into BxPC-3 cells using cRGD-mediated endocytosis. Accordingly, the disulfide bond was activated by GSH overexpression in cancer cells, resulting in release of gemcitabine to inhibit the viability of pancreatic cancer cells. The cleavage of the CFLG subunit of the probe by cathepsin B led to an intense AIE fluorescence signal output due to the RIR effect. This prodrug design approach successfully achieved the goal of imaging-guided chemotherapy of tumors.

To overcome the issue of tumor multidrug resistance (MDR), in 2018, Zhang’s group proposed to construct a transformable chimeric peptide (named probe 93, Figure 11H) encapsulated with DOX by self-assembly, to target and self-assemble on cell membranes for encapsulating cells and further blocking drug efflux.\(^\text{[224]}\) Its molecular structure consists of five parts: (1) a TPE fluorogen as typical AIE fluorescence reporter, (2) hydrophobic 16-carbon alkyl chain as a cell membrane-anchor, (3) Gly-Gly-Gly-His sequence and polyethylene glycol (PEG) as the hydrophilic groups, and (4) the GFLG sequence as a cathepsin B-activatable unit. The probe formed nanomicelles in aqueous solutions by self-assembly and then encapsulated DOX as a drug delivery system (denoted as 93@DOX) with high stability. When 93@DOX accumulated at a tumor site through the EPR effect, nanoparticles disassociation was triggered by cathepsin B. Then, nanoparticles formed to tightly encapsulate the cell to restrict DOX efflux and increase anti-MDR ability.
FIGURE 12  (A) Chemical structure of probe 94a and the activatable mechanism of the probe toward NQO1. (B) Construction of responsive polymeric AIE nanoaggregates via self-assembly in aqueous solution. Reproduced with permission: Copyright 2019, Wiley-VCH GmbH. (C) Proposed mechanism of the reaction of probe 96a with reductase under a physiological hypoxic condition. Reproduced with permission: Copyright 2019, Wiley-VCH GmbH. (D) Activatable mechanism of probe 97a with NTR. Reproduced with permission: Copyright 2016, Wiley-VCH GmbH. (E) Chemical structures of 98a and 98b and schematic illustration of lymphatic metastasis imaging. Reproduced with permission: Copyright 2019, Wiley-VCH GmbH. (F) Schematic illustration of the reaction of nanoprobe 99a with NTR in a tumor (top) and two image scenarios of breast tumor metastasis (bottom). Reproduced with permission: Copyright 2019, Wiley-VCH GmbH. (G) Chemical structure of probe 100a and its response to NTR in vivo. Reproduced with permission: Copyright 2020, American Chemical Society. (H) Chemical structures of bioprobes 101-103 for sensing furin.

AIEgens for reductase

Hypoxia is the condition of oxygen deficiency at solid tumor sites. It leads to overexpression of several reductases, such as nitroreductase (NTR), NAD(P)H:quinone oxidoreductase-1 (NQO1), and azo reductase (AZO), which participate in relevant physiological functions or pathological activities. Many elegant fluorescent probes and prodrug have been developed for imaging-mediated diagnosis and treatment of tumors. Kim and colleagues reported a mitochondria-targeting theranostic agent 94a, which was composed of an AIE scaffold (TPE), a quinone-based trigger, and a triphenylphosphonium group. In the aqueous buffer, probe 94a was almost nonfluorescent owing to existence of the PET effect between an AIE fluorogen and a quinone unit. However, upon addition of sodium dithionite, the quinone moiety in probe 94a was rapidly reduced, followed by the cyclization reaction, as shown in Figure 12A, resulting in the release of AIE dyes accompanied by an intense fluorescence output. Cells experiments showed that...
probe 94a was preferentially internalized in the mitochondria of cancer cells, and it was subsequently activated by NQO1, resulting in cell apoptosis through the caspase pathway. Aided by probe 94a, tumor growth was efficiently inhibited, providing a new direction for developing anticancer drugs. In 2019, He and Du synthesized a hypoxia-activatable fluorescent probe 95a equipped with amphiphilic PEGylated azobenzene, as shown in Figure 12B.230 This probe could form a micelle structure by self-assembly, and its fluorescence was quenched efficiently due to the FRET process. After adding azoreductase into the aqueous solution, the azo bond was enzymatic hydrolyzed, which led to micelle disruptions to release AIE fluorogen with a strong fluorescence output. Additionally, fluorescence imaging data suggested that the probe could detect the azoreductase in hypoxia cancer cells under a one/two-photon fluorescence microscopy platform. To realize the diagnosis of early tumor, Tang’s group presented a new strategy to design AIE bioprobes for in vitro hypoxia imaging.231 As seen in Figure 12C, probe 96a exhibited excellent solubility owing to existence of zwitterionic N-oxide groups, which also could be rapidly reduced in vitro. Interestingly, cancer cells in hypoxic circumstances were successfully illuminated via cleavage of N-O covalent bonds of probe 96a to form corresponding amine 96b by the overexpressed reductase.

To evaluate the hypoxic status of cancer cells, Zhang and coworkers reported a novel fluorescent probe 97a for monitoring the activity of NTR by taking advantage of the AIE signal of tetraphenylethylene.232 The recognition mechanism of probe 97a for NTR in aqueous buffer is shown in Figure 12D and involves emission of a weak yellow fluorescence. With addition of NTR, the nitro group of the probe was rapidly reduced into an amino product, followed by 2,5-rerarrangement and elimination, resulting in formation of pyridine-substituted 97b in aqueous solution with strong blue emission. In addition, the probe exhibited high selectivity for sensing NTR as well as an excellent detection limit of 5 ng/mL. Owing to its good biocompatibility, probe 97a was successfully utilized to image intracellular NTR level to show its anaerobic status. Based on unsymmetrical squaraine dye with a high molar extinction coefficient, in 2019, Wu and Zeng developed an activatable dual-mode nanoprobe for detection of NTR in mouse model.233 In aqueous solution, a nanoprobe formed by self-assembly of molecule 98a, specifically responded to NTR and transformed into product 98b, accompanied by output of fluorescence and optoacoustic signals, which were further employed for visualization of tumor sites and the metastatic route with higher resolution. This approach offers a strategy for constructing multifunction AIE bioprobes for preclinical studies and clinical practice. Additionally, the same group fabricated a new multifunction AIE probe 99a with nitrobenzoxylidiphenylamino as the recognition group, quinolinium as the electron acceptor, and a dihydroxanthenium unit as the electron donor, as illustrated in Figure 12F.234 The nitro element was reduced rapidly to an amino group by the NTR-mediated enzymatic reaction, followed by a self-elimination reaction, producing compound 99b with hydroxy group as an electron-donating unit and generating a strong optoacoustic signal as well as fluorescence in NIR-I/II regions as a result of aggregate formation. The detection limit was as low as 0.052 μg/mL, showing the high sensitivity of the nanoprobe toward NTR. With this nanoprobe, metastases from orthotopic breast tumors to lymph nodes and then to the lungs in mice bearing 4T1 cancer cells were monitored through NIR-I/II fluorescence imaging and multispectral optoacoustic tomography imaging. Notably, the chemotherapy effect of the tumor could be imaged in real time using the nanoprobe. Later, Wu et al. described another activatable fluorescent probe 100a for intraoperative navigation during tumor resection.235 The nanocomposite probe can be formed by self-assembly between probe 100a and bovine serum albumin (BSA). Upon the NTR-mediated reaction, the activated probe with AIE character in aqueous solution emitted an intense NIR-I/II fluorescence signal and strong optoacoustic signal. In view of its high sensitivity, selectivity, and cell penetrability, the nanocomposite probe could be capable of accurately imaging orthotopic liver tumors via 3D multispectral optoacoustic tomography, and providing reliable NIR-I/II fluorescence signal for tiny tumors resection.

AIEgens for furin

Furin plays an important role in maturation of proprotein substrates and embryogenesis.236–238 However, high overexpression of furin in vivo is associated with many malignant tumors and diseases, such as glioblastoma, nonsmall cell lung carcinoma, Ebola fever, and Alzheimer’s disease.239,240 In addition, the peptide sequences Arg-X-Lys/Arg-Arg↓X (X indicates any amino acid residue and ↓ represents the cleavage site) were selectively responsive for furin.241 Fluorescent probes have been developed for imaging the activity of furin in cells and in vivo based on the previously mentioned specific peptide sequences. Inspired by pioneering research, Liang’s group rationally constructed an AIE bioprobe 101 for enhanced fluorescence tracking of furin activity in aqueous and cells.242 As shown in Figure 12H, the disulfide bond on probe was reduced upon addition of GSH and furin, while the Ac-Arg-Val-Arg-Arg peptide sequence could be specially cleaved to produce the reactive intermediate. Immediately, the CBT-Cys condensation reaction occurred to obtain the hydrophobic dimer, which self-assembled into nanoparticles, emitting the enhanced AIE fluorescence. Furthermore, smart probe 101 was utilized to sense the furin distribution in MDA-MB-468 cells.

To image furin activity in situ, in 2018, Zhang, Huan, and Liu described a novel fluorescent probe 102, which was composed of a solid-state fluorophore, a specific peptide substrate, and a self-immolative linker.243 Probe 102 was barely fluorescent in aqueous solution owing to the presence of the hydrophilic Ac-Arg-Val-Arg-Arg peptide. After reacting with furin, the specific peptide could be cleaved to release insoluble product, precipitating near or at the enzyme activity site, and emitting intense solid-state fluorescence. For control probe 103 that has a different peptide sequence (Ac-Arg-Arg-Val-), there was no obvious response to furin under the same conditions, showing the specificity of enzyme recognition of substrates. In addition, probe 102 had outstanding sensitivity and selectivity toward furin. Finally, probe 102 was applied to in situ monitor the variation of furin activity in cells and tissues of MDA-MB-468.
AIEgens for aminopeptidase

Aminopeptidases, which are known as vital enzymes, catalyze the cleavage of amino acids from the amino terminus of peptide substrates and protein.\cite{244-247} The high-accuracy detection and visualization of tracking aminopeptidase activity in living cells and in vivo are beneficial for early detection and prognosis evaluation of related diseases.\cite{248-250} For example, Zhao and Wang reported a novel AIE bioprobe 105a for detection of γ-GGT, as observed in Figure 13B.\cite{252} The γ-GGT could cause the probe to transform from hydrophilic to hydrophobic, accompanied by AIE fluorescence. CTAB enhanced the fluorescent response of the probe toward γ-GGT in PBS-buffered solution. The probe had excellent photostability and could be utilized for intracellular γ-GGT imaging and γ-GGT assay in serum samples. Leucine aminopeptidase (LAP) is regarded as a vital biomarker for liver injury and cancers. In 2018, a turn-on fluorescent probe 106a with AIE characteristics was reported by Wu and Zeng for LAP detection.\cite{253} Probe 106a contained an L-leucine amide site as the recognition group, a diphenylamine (DPA) as the electron-donor, and a tetraphenyl ethylene moiety as the AIE core. The probe emitted a weak fluorescence signal owing to its good water solubility. After adding LAP into the aqueous solution, the L-leucine amide group was cleaved from the probe molecule and underwent 1,6-elimination to generate a hydrophobic product with enhanced fluorescence signal because of self-assembly of nanoparticles. This probe featured a large Stokes shift (194 nm), outstanding photostability, and high sensitivity (DL, 0.16 U/L), offering a practical tool for monitoring LAP activity in living cells and mice for revealing LAP-associated diseases.

**AIEgens for caspases**

Caspases belong to a family of cysteine proteases that play critical roles in apoptosis pathways.\cite{254-256} Among them, caspase-3 is a key mediator for execution of apoptosis and is commonly chosen as a target for apoptosis imaging.\cite{257-260} For example, Liu and Tang reported a novel cell-permeable fluorescent probe 107 for imaging cell apoptosis, which was comprised of a TPE as a typical AIE core and a hydrophilic Asp-Glu-Val-Asp (DEVD) peptide as a recognition segment, as shown in Figure 14A.\cite{261} In vitro spectral studies demonstrated that the probe was almost nonfluorescent in aqueous solution, while a distinct fluorescence signal was observed in presence of caspase-3/-7, clearly indicating specific cleavage by caspase-3/-7. Common proteins, such as pepsin, trypsin, and bovine serum albumin, did not cause significant changes in fluorescence behavior, showing the good selectivity of probe 107 toward caspase-3/-7. The distribution of caspase-3 in living MCF-7 cells induced by staurosporine could be imaged in real-time using a probe, which is consistent with the immunofluorescence staining results, further confirming its ability to track caspase-3/-7 activity as well as image cell apoptosis. To evaluate the therapeutic efficacy, Liu et al. developed a novel cell-permeable chemotherapeutic Pt(IV) prodrug 108 (Figure 14B) whose two axial positions were decorated with a hydrophilic cyclic tripeptide (arginine-glycine-aspartic acid, cRGD) as a targeting group for αvβ3 integrin overexpressing cancer cells and an apoptosis probe that consisted of a hydrophobic tetraphenylsilole as an AIE core and a specific DEVD peptide serving as an activation site for caspase-3 enzyme.\cite{262} Using αvβ3 integrin, the Pt(IV) precursor 108 was more easily and efficiently internalized by cancer cells, and converted to an active Pt(II) drug accompanied by release of an apoptosis probe. The reduced Pt(II) activated upregulation of caspase-3 enzyme coupling of a hydrophilic γ-glutamyl amide site onto a TPE fluorophore. Wu’s group designed a light-up AIE probe 105a for detection of γ-GGT, as observed in Figure 13B.\cite{252} The γ-GGT could cause the probe to transform from hydrophilic to hydrophobic, accompanied by AIE fluorescence. CTAB enhanced the fluorescent response of the probe toward γ-GGT.

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**FIGURE 13** (A) Schematic illustration of 104 for DPP-4 detection in living cells. Reproduced with permission: Copyright 2016, Royal Society of Chemistry.\cite{251} (B) Chemical structure of 105a and its reaction mechanism for γ-GGT. C) Chemical structure of 106a and its fluorescence switching triggered by LAP. Reproduced with permission: Copyright 2018, Elsevier.\cite{251}
when it induced cancer cells apoptosis, which cleaved the DEVD peptide, liberating the tetraphenylsilole fluorophore with enhanced fluorescence owing to formation of aggregates in the aqueous physiological environment. Cell imaging results demonstrated that the precursor 108 was capable of in situ and real-time imaging of drug-induced apoptosis, meaning that it could be employed as an early indicator for monitoring therapy with anticancer drug. Later, Liu’s group designed a self-validated AIE bioprobe 109a for accurately tracking caspase-3 activity. As observed in Figure 14C,
probe 109a was composed of a coumarin fluorophore as the donor part and a typical TPE derivative as the acceptor segment conjugated through a specific peptide Asp-Glu-Val-Asp substrate. Unlike a traditional FRET probe, probe 109a emitted a very weak fluorescence signal because of the energy transfer and energy dissipation of the acceptor AIEgen. Upon addition of caspase-3, the DEVD substrate was cleaved to generate Cou-DEVD (109b) with intense green fluorescence due to disjunction of the donor-acceptor system and a TPETP residue (109c) with strong red fluorescence because of aggregation in aqueous solution. Considering its good biocompatibility, probe 109a was successfully applied to in situ monitor the activity of caspase-3 using dual fluorescent signal in living cells, which provided a new direction for designing and developing the next-generation of self-validated FRET bioprobes.

Continuing in this area, Liu and coworkers fabricated a smart AIE probe 110 (Figure 14D) for tracking multiple enzyme activities in biological process. This probe was composed of a red AIE core, a hydrophilic peptide as the substrate of caspase-8 and caspase-3, and a green AIE fluorogen. In the absence of caspase-3/-8, probe 110 was nonfluorescent in the aqueous solution due to the energy dissipation of unrestricted molecule rotation. However, after adding the caspase-3 and caspase-8, intense green (TPS) and red fluorescence (TPETH) signals were observed due to the cleavable peptide substrate. H2O2 induced early apoptosis of HeLa cells to generate overexpressed caspase-8 and caspase-3, which could be trapped in real-time by probe 110 through a dual fluorescence signal. More importantly, the property of sequential fluorescence activation gave probe 110 the ability to evaluate the therapeutic efficiency of anticancer drugs.

### AIEgens for other enzymes

Development of smart AIEgens for accurate detection of disease-related enzyme activity is vital in early disease diagnosis and treatment. In 2016, Xia et al. designed a matrix metalloproteinase-2 (MMP-2) activatable prodrug 111 (Figure 15A) with AIE characteristics for controlling drug delivery and tracking drug release in living cells. This prodrug is highly water-soluble due to the existence of arginine residues, and only DOX emits a red fluorescence signal. In the presence of MMP-2, the prodrug 111 was cleaved into two segments. As observed in Figure 15B, the hydrophilic part includes cell-penetrating peptides decorated with DOX, which can easily enter the cell by interacting with the cell membrane, resulting in high kill efficiencies for tumor cells. The other part is the hydrophobic TPE derivative, which can form nanoparticles by self-aggregating with an intense yellow fluorescence that allows monitoring of the drug delivery process in MCF-7 cells. In dual fluorescence signal monitoring mode, AIEgen 111 was applied to distinguish cell lines by tracking the activity of MMP-2, and it demonstrated higher cytotoxicity for cells overexpressed MMP-2. Lysosomal β-N-acetyhexosaminidase (Hex) is a widely distributed glycoside hydrolase that plays an important role in physiological processes. The abnormal content of Hex in vivo is closely related to some diseases, such as cancers and neurodegenerative diseases. Therefore, developing a specific AIE fluorescent probe for tracking the activity of Hex in the lysosome is urgent. Inspired by Shin’s group work, Wang and coworkers fabricated the first lysosome-targeting AIE probe 112a, based on a TPE fluorophore, for detecting Hex activity in living cells and in vivo. As shown in Figure 15C,
| AIE probe   | Analyte | Excitation/emission | Detection limit | Application                      |
|------------|---------|----------------------|-----------------|-----------------------------------|
| 66a ALP    | ALP     | 350 nm/480 nm        | 18 mU/mL        | Cells                             |
| 67 ALP     | ALP     | 335 nm/470 nm        | No data         | Cells                             |
| 68 ALP     | ALP     | 335 nm/470 nm        | No data         | Cells                             |
| 71 ALP     | ALP     | 330 nm/475 nm        | < 0.034 mU/mL   | In solution                       |
| 72 ALP     | ALP     | 430 nm/539, 641 nm   | 0.15 mU/mL      | Cells                             |
| 73 ALP     | ALP     | 465 nm/550 nm        | 0.15 mU/mL      | Cells, tumor spheroids, and mice   |
| 74 ALP     | ALP     | 365 nm/550 nm        | 1.21 U/L        | Cells                             |
| 75 ALP     | ALP     | 440 nm/543 nm        | 14.2 U/L        | Cells                             |
| 76 ALP     | ALP     | 405 nm/600 nm        | No data         | Cells                             |
| 77 ALP     | ALP     | 356 nm/536 nm        | 0.012 U/L       | Cells                             |
| 78 ALP     | ALP     | 410 nm/550 nm        | 1.36 U/L        | Cells and tissues                 |
| 79 ALP     | ALP     | 405 nm/600 nm        | 6.6 mU/mL       | Bacteria                          |
| 80 Esterase| Esterase| 356 nm/532 nm        | 2.4 mU/mL       | Cells                             |
| 81 Esterase| Carboxylesterase| 375 nm/475 nm| 29 pM          | In solution                       |
| 82 Esterase| Esterase| 500 nm/650 nm        | 5 mU/mL         | Cells                             |
| 83 Esterase| Esterase| 405 nm/520 nm; 530 nm/660 nm| No data | Cells                             |
| 84 ß-gal   | ß-gal   | 387 nm/545 nm        | 14 mU/mL        | Cells                             |
| 85 ß-gal   | ß-gal   | 344 nm/512 nm        | 0.33 U/mL       | Cells                             |
| 86 ß-gal   | ß-gal   | 434 nm/560 nm        | 1.0 mU/mL       | Cells                             |
| 87 ß-gal   | ß-gal   | 460 nm/650 nm        | No data         | Cells                             |
| 88 ß-gal   | ß-gal   | No data              | No data         | Cells                             |
| 89 Lipase  | Lipase  | 360 nm/453 nm        | 0.13 U/L        | In solution                       |
| 90 Lipase  | Lipase  | 350 nm/420 nm        | No data         | In solution                       |
| 91 Cathepsin B| Cathepsin B| 405 nm/615 nm| No data | Cells                             |
| 92a Cathepsin B| Cathepsin B| 320 nm/475 nm| No data | Cells                             |
| 93 Cathepsin B| Cathepsin B| 370 nm/480 nm| No data | Cells and mice                    |
| 94a NQO1   | NQO1    | 340 nm/470 nm        | No data         | Cells and mice                    |
| 95a Azoreductase| Azoreductase| 365 nm/465 nm| No data | Cells and tumor spheroids          |
| 96a Reductase| Reductase| 380 nm/510 nm        | No data         | Cells                             |
| 97a NTR    | NTR     | 450 nm/525 nm        | 5 ng/mL         | Cells                             |
| 98a NTR    | NTR     | 690 nm/762 nm        | No data         | Mice                             |
| 99a NTR    | NTR     | 680 nm/780 nm; 808 nm/922 nm| 0.052 µg/mL | Mice                             |
| 100a NTR   | NTR     | 680 nm/791 nm; 808 nm/923 nm| 0.048 µg/mL ; 0.053 µg/mL | Mice |
| 101 Furin  | Furin   | 320 nm/470 nm        | No data         | Cells                             |
| 102 Furin  | Furin   | 365 nm/500 nm        | No data         | Cells                             |
| 104 DDP-4  | DDP-4   | 320 nm/450 nm        | No data         | Cells and zebrafish               |
| 105a ß-GGT | ß-GGT   | 360 nm/472 nm        | 0.59 U/L        | Cells                             |
| 106a LAP   | LAP     | 359 nm/505 nm        | 0.16 U/L        | Cells and tissues                 |
| 107 Caspase-3/-7| Caspase-3/-7| 312 nm/470 nm| No data | Cells                             |
| 108 Caspase-3| Caspase-3| 365 nm/480 nm        | 1 pM            | Cells                             |
| 109a Caspase-3/-7| Caspase-3/-7| 405 nm/465, 665 nm| No data | Cells                             |
| 110 Caspase-3/-8| Caspase-3/-8| 430 nm/650 nm; 360 nm/480 nm| No data | Cells                             |
| 111 MMP-2  | MMP-2   | 405 nm/565 nm        | No data         | Cells                             |
| 112a Hex   | Hex     | 360 nm/612 nm        | 1.4 nM          | Cells                             |
| 113a GUS   | GUS     | 370 nm/519 nm        | No data         | Bacteria                          |
introduction of an N-acetyl-β-D-glcosaminide group (as a responsive moiety) made the AIE probe highly water-soluble and almost nonfluorescent. After being cleaved by Hex, probe 112a produced a phenolate intermediate, which underwent 1,6-elimination spontaneously to generate the hydrophobic product 112b, resulting in bright emission owing to aggregation in a water environment. Probe 112a exhibited high selectivity and sensitivity (DL, 1.4 nM), outstanding photostability, and large Stokes shift (252 nm) toward Hex. Additionally, cell-permeable AIE probe 112a was used to track the activity of Hex in real-time in living cells and a tumor bearing mouse model.

To address the issue of visual detection and isolation of both O157:H7 and non-O157:H7 Escherichia coli, based on β-Glucuronidase (GUS), Wu et al. reported a novel AIE bio-probe 113a modified with a solid-state fluorophore and a GUS-triggered recognition segment, as demonstrated in Figure 15D. Upon addition of GUS, the recognition segment was cleaved to generate water-insoluble 113b accompanied by strong fluorescence, due to the ESIP and AIE effect. In addition, probe 113a was successfully employed to detect the activity of GUS in living E. coli and to develop a novel chromogenic-fluorogenic culture medium as a simple and efficient tool for detecting and isolating both O157:H7 and non-O157:H7 E. coli in milk.

The analytical parameters of activatable AIE probes discussed in the subsections are summarized and shown in Table 2.

**SUMMARY AND OUTLOOK**

In this review, many representative AIEgens using the activity-based strategy have been systematically summarized and demonstrated, focusing on molecular design and applications, especially in the fields of imaging and diseases therapy. These smart AIE probes rely on selective chemical reactions to achieve specific detection and imaging of analytes of interest (e.g., signaling molecules, biomolecules, and enzymes), contributing to a deeper understanding of their disease-related physiological and pathological functions. With aid of two-photon microscopy, higher-resolution imaging of cells and tissues with a deeper penetration depth was obtained using activity-based AIEgens. Additionally, modified AIE fluorophores produced reactive oxygen species when they fluoresce under light irradiation. Thus, activatable AIE probes triggered by tumor biomarkers have been constructed for accurate tumor ablation without affecting normal cells, attracting the attention of numerous research groups in recent years. For toxic gases, a test strip loaded with AIE probes was more convenient for nonprofessional use. Of note, as observed in the second section, most working mechanisms for constructing traditional fluorescent probes including ICT, PET, and FRET are suitable for designing AIE biosensors.

Considerable evidence shows that substantial progress of activity-based AIEgens in this area of research. However, there are some weaknesses that limit clinical transformation of activatable AIE materials. For instance, (1) absorption wavelengths of most AIE fluorescent probes are relatively short (usually in the visible region) due to the existence of rotor-shaped structures, which greatly affect application of AIEgens in vivo. (2) High background fluorescence of AIE biopros cannot be ignored for accurate detection of biologically related species. (3) Activatable AIE probes are rarely used in the multimode evaluation of biological analytes. (4) It is difficult to evaluate the activation efficiency of AIE probes in cells or in vivo, and the corresponding therapeutic effect cannot be guaranteed. (5) Until now, there have been relatively few AIE fluorophores that can be utilized to construct activatable AIE fluorescent probes.

Faced with these challenges, the following strategies address the above deficiencies to some extent. For example, (1) two-/three-photon technology or upconversion processes are adopted to achieve an NIR biological window in AIE probes and improve penetration depth of tissues. (2) Amphiphilic AIE probes can reduce the influence of background signal introduced by aggregation of probes before activation. (3) Combined with other technologies, including photoacoustic imaging, magnetic resonance imaging and stimulated Raman imaging, multimode functional AIE probes should be constructed as part of the research of AIE. (4) AIE probes constructed based on a FRET mechanism possess the ability to quantitatively detect biological analytes due to their self-calibration properties. (5) More AIE fluorophores should be developed to meet different needs of probe design, further enriching their usability in practical applications.

We provided a systematic overview of the current design and application of activity-based activatable AIE probes, and discussed their shortcomings and proposed corresponding solutions to provide guidance for future development in diagnosis and treatment of diseases. We anticipate that this review will stimulate more novel ideas for research on AIE materials.

**ACKNOWLEDGMENTS**

J.Y. thanks the National Research Foundation of Korea (NRF) funded by the Korean government (MSIP) (No. 2012R1A3A2048814). X.P. acknowledges support from the National Natural Science Foundation of China (No. 22090011).

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**REFERENCES**

1. D. Wu, A. C. Sedgwick, T. Gumlaugsson, E. U. Akkaya, J. Yoon, T. D. James, Chem. Soc. Rev. 2017, 46, 7105
2. H.-W. Liu, L. Chen, C. Xu, Z. Li, H. Zhang, X.-B. Zhang, W. Tan, Chem. Soc. Rev. 2018, 47, 7140
3. H. Li, Q. Yao, F. Xu, N. Xu, X. Ma, J. Fan, S. Long, J. Du, J. Wang, X. Peng, Anal. Chem. 2018, 90, 4641
4. K. Gu, Y. Xu, H. Li, Z. Guo, S. Zhu, S. Zhu, P. Shi, T. D. James, H. Tian, W.-H. Zhu, J. Am. Chem. Soc. 2016, 138, 5334
5. J. Mei, H. Tian, Aggregate 2021, https://doi.org/10.1002/agt2.32
6. M. Chiba, Y. Ichikawa, M. Kamiya, T. Komatsu, T. Ueno, K. Hanaoka, T. Nagano, N. Lange, Y. Urano, Angew. Chem., Int. Ed. 2017, 56, 10418
7. Q. Yao, H. Li, L. Xian, F. Xu, J. Xia, J. Fan, J. Du, J. Wang, X. Peng, Biomaterials 2018, 177, 78
8. M. Vendrell, D. Zhai, J. C. Er, Y.-T. Chang, Chem. Rev. 2012, 112, 4391
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How to cite this article: Li H, Kim H, Han J, et al. Activity-based smart AIEgens for detection, bioimaging, and therapeutics: Recent progress and outlook. Aggregate. 2021;e51. https://doi.org/10.1002/agt2.51