Mini-Review

Type I AIE photosensitizers: Mechanism and application

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 21788102;
Natural Science Foundation of Guangdong Province, Grant/Award Number: 2019B030301003

Abstract
Photodynamic therapy (PDT) with plenty of advantages is expected to become a promising modality for cancer treatment, but challenges still remain. In the past decade, abundant photosensitizers (PSs) with aggregation-induced emission (AIE) property make the development of PSs enter upon a new phase, offering incomparable merits. Recently, Type I AIE PSs with capability of generating radical reactive oxygen species (ROS) have emerged as strong candidates to overcome the inherent hypoxia nature of solid tumors. In this review, detailed discussions on the mechanisms of PDT are drawn to highlight the basic advantages of Type I pathway over Type II one in hypoxic PDT, followed by a summary of frequently-used detection methods for the accurate distinguishing of the nature of ROS. Finally, the latest representative advances are summarized, and future perspectives of Type I AIE PSs are discussed.

Keywords
aggregation-induced emission, cancer treatment, photodynamic therapy, photosensitizer, reactive oxygen species

1 | Introduction

Cancer, as a major public health issue, is now one of the world’s leading causes of mortality.[1] The search for cancer treatment has never stopped. Surgery, chemotherapy, and radiotherapy are the most common therapeutics for cancer,[2] whose treatment courses and outcomes are always limited by their shortcomings including invasiveness, multidrug resistance, severe adverse effects, immunosuppression, and poor patient compliance.[3] As one of the recently developed therapeutics, immunotherapy owns highly admirable features, but it also has its complexity and uncertainty.[4] It is urgent to develop a high-efficacious treatment modality with less side effects. Photodynamic therapy (PDT), emerging as a noninvasive option for cancer treatment, does have its distinguished advantages.[5] Essentially, PDT process contains three non-toxic elements: light, photosensitizer (PS) and oxygen. Activated by light, PS converts tissue oxygen to cytotoxic reactive oxygen species (ROS) (i.e., singlet oxygen [¹O₂], superoxide anion radical [O₂•−], peroxyl radical [H₂O₂], and hydroxyl radical [HO•]), which can result in oxidative...
damage to the cellular substrates, like amino acids, proteins and DNA, and thereby effectively causes anti-tumor effects of cell death, vasculature damage and immune response.[6] In this process, the controllable distribution of light and selective delivery of drug empower PDT spatiotemporally precise operability with low side effects. To date, PDT has been clinically applied for the treatment of several diseases, such as lung, esophageal, bladder, gastric, skin, head, and neck cancers,[7] accompanied by various PSs being approved, for example, Photofrin (hematoporphyrin derivative, HpD), Hexpix, Cysview, Lutrin, Photolons (chlorin e6, Ce6), etc.[8] However, there still exist 'Achilles’ heels’ that hinder the preclinical and clinical progress of PDT:[9]

1. Limited tissue penetration depth of excitation light invalidates PDT in deep-seated tumors. Excitation light penetrates deeper into living tissues with the increase of its wavelength, yet whose energy accordingly shrinks. To fulfill the minimal energy requirement for ROS generation, the excitation wavelength is recognized to be capped at 850 nm[7,10] which determines the light penetration depth is less than 3 mm.[11]

2. High oxygen dependence makes PDT self-limited in the hypoxic (not anaerobic) microenvironment of solid tumors.[12] Aggressive proliferation of cancer cells and insufficient blood supply of solid tumors lead to severe hypoxia at some interior sites (oxygen pressure < 5 mm Hg),[13] which is not only inconducive to the production of ROS, but also further aggravates the oxygen shortage, leading to an unsatisfactory anti-tumor outcome.

Enormous efforts have been devoted to explore effective approaches to break through these inherent bottlenecks. Except for the synergistic strategies and some auxiliary means, optimizing and upgrading the performances of PS are one of the most important outlets, because PS is the critical factor that directly determines the therapeutic efficacy of PDT.[9] External near infrared (NIR) light/X-ray-triggered PSs, internal self-luminescing PSs, and Förster resonance energy transfer-based PSs offer a promising platform on deep-tissue PDT.[14] Oxygen-replenishing PSs and Type I PSs represent a developing prospect for hypoxic PDT.[15] Although these advanced PSs have made significant progress in recent years, there are still several drawbacks impeding their clinical translation. For nanocomposite PSs containing inorganic and/or metallic components, it seems to remain an uphill battle to address their severe cytotoxicity, limited reproducibility, low biodegradability, and complicated pharmacokinetics.[16] Comparatively, organic PSs have the advantages of flexible preparation, tunable properties, favorable biosafety, and robust reproducibility to meet the requirements for an ideal PS.[17] However, most traditional organic PSs built with a planar π-conjugated structure suffer from aggregation-caused quenching effect. The hydrophobic PSs tend to form aggregates in a hydrophilic bio-environment and deactivate excited states through some competitive channels, ultimately causing the reduction of ROS yield.[18]

Fortunately, various PSs with aggregation-induced emission (AIE) attributes have been flourishing in recent decade, which have placed great expectations for overcoming the shortcomings mentioned above.[19] AIE luminogens (AIEgens) are non-emissive or weakly emissive in good solvents, but have strong luminescence in aggregates or solid state, due to the mechanism of restriction of intramolecular motion (RIM) that diminishes the competitive non-radiative process. From the quantum-chemical perspective, the RIM mechanism can be revealed by the following four modes: restriction of vibronic coupling, restriction of access to conical intersection, restriction of access to dark state, and suppression of photochemical reaction.[20] With additional optical merits of high signal-to-noise ratio, no self-absorption, and robust photobleaching resistance, AIE PSs can be competent as both therapeutic and fluorescence-imaging contrast agents at the same time, which allow the integration of diagnosis, treatment, and post-monitoring. Up to now, there are several reviews focusing on the development of AIE PSs with their different emphases including: molecular design strategy, deep-tissue application, and pathogen theranostics.[21]

However, AIE PSs through Type I mechanisms against tumor hypoxia have not received deserved attention until recently, which may be once limited by the past negligence of PDT mechanism from biomaterial scientists and various elusive ROS indicators. To get out of this trouble, this review is devoted to give a detailed introduction on the PDT mechanism and summarize the latest advances on AIE PSs from the perspective of PDT mechanism, with an emphasis on the Type I ones. We wish to arouse people’s attention on the underlying photophysical and photochemical mechanism of PSs, which is of great importance to the subsequent pharmacologic and pharmacokinetic analysis for a clinical purpose and the optimization of treatment options by combining with appropriate inducers, inhibitors, and other therapeutics.

2 MECHANISM OF PHOTODYNAMIC THERAPY

2.1 Photophysical and photochemical process of PDT

PDT can be classified as Type I and Type II based on the mechanisms involving photophysical and photochemical reactions (Figure 1).[21] Upon absorbing a photon, the PS can be excited from its ground state (S0) to the
Schematic illustration of the photophysical and photochemical mechanisms of PDT (PS = photosensitizer)

short-lived excited singlet state ($S_1$), where the unstable excited electron may either relax back to the stable $S_0$ by fluorescent radiation or internal conversion (IC), or it may undergo intersystem crossing (ISC) to transform the PS into the excited triplet state ($T_1$). Among these three competitive processes, the relatively long-lived $T_1$ allows the $3PS^*$ to undergo the photochemical reaction with neighboring oxygen (and some participating substrates) to produce ROS, via Type I and/or Type II pathways. These two pathways generate different kinds of ROS, whose nature directly determines the PDT performance.

For Type I process, the $3PS^*$ interacts with the substrates (e.g., vitamin C, riboflavin, reduced coenzymes, amino acids, and nitrogenous bases) to convert into radicals (or radical ions), who further transfers the electron to surrounding oxygen to generate $O_2^{•−}$. $O_2^{•−}$ can be further transformed into $H_2O_2$ through spontaneous dismutation or superoxide dismutase (SOD)-catalyzed dismutation. Furthermore, the accumulated $H_2O_2$ can sequentially react with $O_2^{•−}$ to generate HO• by Haber-Weiss reaction or Fenton reaction, in which the Fenton reaction takes place in the presence of iron ions. $O_2^{•−}$ and $H_2O_2$ have limited biological toxicity that oppositely endows them broad diffusion range to generate the most reactive HO•. HO• is extremely destructive to almost all biological molecules, which enables the limited oxygen in the hypoxic tumor to be fully utilized. That is why Type I PDT processes a better anti-hypoxia outcome. But it is still oxygen-dependent. The term ‘oxygen-independent’ should not be used. As for Type II pathway, the $3PS^*$ transfers its energy to surrounding oxygen, forming the $1O_2$ with the decent cytotoxicity but short action range. A blind pursuit on high $O_2$ yield may cause excessive consumption of $O_2$ but with little enhancement of efficacy.

Generally, Type I and Type II pathways can occur simultaneously in the PDT process, which are in a competitive relationship. Their proportions depend on the PS property as well as several other factors, such as solvent polarity, PS concentration, local oxygen concentration, and substrate property. One of the most common views is that Type II pathway dominates at high oxygen concentration, whereas Type I pathway prevails at low oxygen concentration. However, the Type II mechanism is still universally accepted to predominate in most cases. This fixed mindset always makes researchers overlook the reaction pathway that PDT actually works through.

### 2.2 Detection methods of ROS

Distinguishing the nature of ROS is the critical process to investigate the PDT reaction pathway, which is meaningful for accurately assessing and efficiently optimizing the performance of a PS. In the past few years, many methods have been developed for detecting and distinguishing various ROS.

The only widely accepted direct detection method is by means of infrared spectroscopy to examine the luminescence of $1O_2$ at $\sim$1270 nm. Recently, a novel detection method called field-induced droplet ionization mass spectrometry has been reported, which can accurately determine the nature of ROS by mass spectrometry without using any chemical media.

The other indirect detection methods include quencher methods and indicator detection ones to detect and identify the types of ROS. The former ones perform through restraining the functions or indicator signals of a specific ROS to reversely and roughly verify its present, but without a precise conclusion. Vitamin C (Vc), D-mannitol, and $N$-acetyl-$L$-cysteine (NAC) are known as scavengers of Type I ROS. Sodium azide (NaN$_3$) is widely employed to quench $1O_2$. The latter ones are so reliable and quantifiable that utilizing some spectroscopic techniques, like absorption spectrometer, fluorescence spectrometer, fluorescence microscope, flow cytometer, or electron spin resonance/electron paramagnetic resonance (EPR) spectrometer, can measure the signals of indicators before and after reacting with specific ROS.
Several commonly used indicators for ROS detection

| Nature of ROS | Indicators |
|---------------|------------|
| General ROS   | DCFH-DA    |
| Type I        | \( \text{O}_2^{--} \), DHE, MitoSOX Red, HKSOX-1 |
| Type I        | \( \text{HO}^- \), HPF, APF |
| Type I        | \( \text{H}_2\text{O}_2 \), DHR123, Amplex Red |
| Type II       | \( ^1\text{O}_2 \), SOSG, ABDA |

It is worth mentioning that dihydrolipoamide 123 (DHR123) has been once misunderstood as a specific probe for \( \text{O}_2^{--} \) to demonstrate the formation of Type I ROS in some reported works.\(^{[39]}\) But actually, DHR123 is an indicator for \( \text{H}_2\text{O}_2 \) with very low sensitivity to \( \text{O}_2^{--} \).\(^{[40]}\) Although \( \text{H}_2\text{O}_2 \) also a secondary product from \( \text{O}_2^{--} \) during Type I process, it is inappropriate to consider DHR123 as a direct \( \text{O}_2^{--} \) probe. Besides, N-acetyl-3,7-dihydroxyphenoxazine (Amplex Red) is another commercially available indicator for \( \text{H}_2\text{O}_2 \), which can be oxidized by \( \text{H}_2\text{O}_2 \) to form resorufin with high fluorescence at the catalysis of horseradish peroxidase.\(^{[41]}\) There are already a large number of products of Hydrogen Peroxide Assay Kit based on Amplex Red for cell-free application. But precisely speaking, Amplex Red can be activated by total peroxides in the present of catalysis, resulting in inaccurate detection results. Worse still, high concentration of peroxides will further quench the fluorescent resorufin that limits its detection.

As for the \( \text{OH}^- \) detection, 2-[6-(40-hydroxy) phenoxy-3H-xanthen-3-0n-9-yl] benzoic acid (HPF) and 2-[6-(40-amino) phenoxy-3H-xanthen-3-0n-9-yl] benzoic acid (APF) are widely used as highly selective fluorescent probes for detecting \( \text{HO}^- \).\(^{[42]}\) Better yet, the HPF and APF have high resistance to light-induced autoxidation, which can be applied in both cell and cell-free systems.

EPR is a powerful tool for detecting ROS with the help of spin trappers. 5,5-Dimethyl-l-pyrroline N-oxide (DMPO) and 5-tert-butoxycarbonyl-5-methyl-1-pyrroline N-oxide (BMPO) can be used to detect and identify \( \text{O}_2^{--} \) and \( \text{HO}^- \),\(^{[43]}\) which can produce different spin trappers/ROS adducts with distinguished EPR signals. Both of them are suitable for cell and cell-free systems. Relatively, the ROS adducts of BMPO have longer half-life than those of DMPO. Besides, 5-(dimethoxyphosphoryl)-5-methyl-1-pyrroline N-oxide and 5-ethoxycarbonyl-5-methyl-1-pyrroline N-oxide (EMPO) are also commercially used to detect the Type I ROS.\(^{[43a]}\)

### 2.2.2 Detection methods of Type I ROS

Firstly, fluorescence detection method for Type I ROS can be carried out easily, that, yet, limited by the respective peculiarity of available fluorescent probes, including specificity, stability, and applicability. For detecting the primary product, \( \text{O}_2^{--} \), dihydroethidium (DHE) and MitoSOX Red are extensively used membrane permeable fluorescent probes, which are only applied in bio-system.\(^{[37]}\) DHE can be oxidized by \( \text{O}_2^{--} \) to form 2-hydroxyethidium (2-OH-E\(^+\)), which can be incorporated into chromosomal DNA to emit red fluorescence. MitoSOX Red is a cationic derivative of DHE that is specially designed to detect mitochondrial \( \text{O}_2^{--} \) in living cells with high specificity. A cell-free \( \text{O}_2^{--} \) fluorescent probe with high specificity was commercially scarce for a time. Recently, HKSOX-1 has commercially emerged as an outstanding \( \text{O}_2^{--} \) fluorescent probe, which is a trifluoromethyl sulfonate ester-functional 5-carboxy-

### 2.2.3 Detection methods of Type II ROS

For the assay of Type II ROS (\(^1\text{O}_2\)), there are many commercially available probes based on absorption and fluorescence spectroscopies, among which, Singlet Oxygen Sensor Green (SOSG)\(^{[44]}\) and 9,10-anthracenediylbis(methylene)-dimalonic acid (ABDA)\(^{[45]}\) are two most popular probes. SOSG, as a fluorescent probe, has a faint blue light and emits green fluorescence after reaction with \(^1\text{O}_2\). Although it is in the commercial instruction that SOSG is a non-cellular permeable probe, some SOSG

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\(^{[36]}\) Reference to further reading or discussion.

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\(^{[42]}\) Reference to further reading or discussion.

\(^{[43]}\) Reference to further reading or discussion.

\(^{[43a]}\) Reference to further reading or discussion.
Chemical structures and ROS types of several typical AIE photosensitizers reported by literatures can incorporate into the living cell, which may be due to its efficient binding capacity with membrane proteins. Indeed, many evidences have been given that fluorescence signal of SOSG can be captured from inside of cells in the PDT process, yet, which is also probably because the membrane permeability of cells has been disrupted by the produced 1O2. Hence, we consider that when using SOSG to indicate no generation of 1O2, a corroboration of the alteration of membrane permeability is necessary (i.e., co-staining with propidium iodide [PI]). As for ABDA, it can be bleached by 1O2 to its corresponding endoperoxide, whose attenuation can be monitored by absorption spectroscopy. As for EPR trapper for 1O2, 2,2,6,6-tetramethylpiperidine (TEMP) is the most classical one, which can form 1:1 triplet signal for TEMP/1O2 (namely, 2,2,6,6-tetramethyl-1-piperidinyloxyl, TEMPO) adduct under the action of 1O2.

Of course, the selectivity, accuracy, and applicability of indicators mentioned above are relative, especially in the complex bio-environment. In practical use, it is highly suggested to use more than one method to corroborate the types of ROS, which also needs a concrete analysis of a specific situation.

3 AIE PSs

3.1 PSs based on Type I mechanism

Owing to the unique advantages, Type I PDT has become a research focus in recent years, in which pure organic Type I PSs with AIE property are emerging as the shining stars. Recently, we reported a new kind of Type I AIE PSs with high specificity (α-TPA-PIO and β-TPA-PIO, Figure 2). In this work, a phosphindole oxide (PIO) core was proposed as an ideal Type I core that could not only promote the ISC process based on the ‘heavy atom’ effect of phosphorus, but also accept and stabilize an external electron to form radical anion intermediates (Figure 3A).

To investigate the ROS generation capacity of these new PSs, DCFH and HPF and ABDA/SOSG were used to detect the formation of general ROS, HO•, and 1O2 in aqueous media, respectively, where crystal violet (CV) and methylene blue, known as classical Type I and Type II PS, respectively, were employed as references (Figure 3B). Besides, bovine serum albumin was added to work as the additional electron donor, which can elevate the formation of radical intermediate. In addition, EPR measurements of the two molecules further confirm the generation of Type I ROS under irradiation using BMPO as the spin-trap agent. Intracellular ROS generation detection using confocal laser scanning microscopy were also conducted (Figure 3C), where DCFH-DA, DHE, and HPF served as the indicators of universal ROS, O2•− and HO•, respectively. The fluorescence intensities increased with the prolong of radiation time to indicate the generation of ROS. All the series of experimental results consistently demonstrated that these PIO-based isomers were capable as the superior pure Type I PSs over CV, and β-TPA-PIO possessed better Type I ROS generation ability that α-TPA-PIO.

Quantum mechanical calculation revealed β-TPA-PIO held a more efficient ISC process owing to its higher
spin-orbit coupling interaction from $S_1$ to $T_1$ and the additional hyperfine coupling interaction from $S_1$ to $T_2$ (followed to $T_1$ by IC), relative to $\alpha$-TPA-PIO (Figure 3D). Under irradiation of white light, $\beta$-TPA-PIO showed anti-hypoxia activity to cause severe cell death in hypoxic environment (ambient oxygen content is 8%) and achieved effective tumor growth inhibition of solid tumor in vivo.

Noticeably, this work also focused on the internal cytological mechanism during the $\beta$-TPA-PIO-mediated PDT process. A series of experiments including co-localization, Western blot, and immunohistology analyses indicated ROS-based ER stress was triggered during this process, which subsequently activated both death modes of apoptosis and autophagy. These physiological processes signified the PIO-cored Type I PSs were promising as immunogenic cell death inducers to cause anti-cancer immunostimulatory effect.

3.2 Converting PSs from Type II to Type I mechanism

Recently, molecular engineering has been conducted to turn PSs from Type II to Type I mechanism by Wang et al.\cite{50} Rational molecular design strategies for developing Type I AIE PSs were proposed: 1. The improved electron-donating ability is conductive to suppressing nonradiative IC channel and promoting radiative and ISC processes, further resulting in higher performance in imaging-guided PDT; 2. “more ICT leading to better free radical ROS generation.” In this work, four electron-rich anion-$\pi^+$ AIEgens (TBZPy, MTBZPy, TNZPy, and MTNZPy) were prepared (Figure 2). Firstly, DCFH and ABDA/SOSG were used to measure the formation of total ROS and $^{1}\text{O}_2$, respectively (Figure 4A). Strangely, the trend for the total ROS generation was
TBZPy < MTBZPy < TNZPy < MTNZPy, but the \( \text{^1O}_2 \) generation was MTNZPy < TNZPy < MTBZPy < TBZPy which were totally contradictory. These findings inspired the authors to consider the generation of Type I ROS using DHR123 as fluorescent indicator and Vc as radical quencher (Figure 4B). EPR measurements using BMPO as spin trap were also conducted (Figure 4C). These measurements revealed that MTNZPy and TNZPy showed better Type I ROS generation capacity with negligible \( \text{^1O}_2 \) yield. In addition, these PSs showed low-efficient ROS generation in pure DMSO, but high ROS generation in aggregated state (\( \nu_{\text{PBS}}/\nu_{\text{DMSO}} = 99:1 \)). For intracellular
performed, the Type I ROS generations of MTNZPy and TNZPy were further verified by DHE as $O_2^{•−}$ indicator (Figure 4D). MTNZPy and TNZPy achieved good PDT outcomes under hypoxic situation (ambient oxygen content is 8%) and in vivo solid tumor suppression.

Zhang et al presented an effective strategy to improve the performances of PSs by cationization. TPAN, TPAPy and their corresponding cationic products, TPANPF$_6$, and TPAPyPF$_6$, were prepared (Figure 2), among which TPAPy and TPAPyPF$_6$ were AIE-active. DCFH and ABDA were employed to detect the generation of total ROS and $1^O_2$ in aqueous system, respectively (Figures 5A and 5B). In addition, the $1^O_2$ generation ability in different water fractions was measured to confirm that aggregation can lead to the increase of ROS production capacity. The decomposition rate of ABDA enhanced with the increase of water fraction, indicating that aggregation may promote ROS production for TPANPF$_6$ (Figure 5C). Similar to the last work, the inconsistent results of the total ROS and $1^O_2$ generation abilities of TPAPy and TPAPyPF$_6$ prompted the authors to dig into the Type I ROS generation. Hence, flow cytometry was used to detect the intracellular $O_2^{•−}$ generation using DHE as probe (Figure 5D). The enhanced fluorescence intensity implied the effective production of $O_2^{•−}$. All the results showed that ionized TPANPF$_6$, and TPAPyPF$_6$ could generate two types of ROS. However, the nonionized TPAPy could only produce the $1^O_2$ with negligible PDT efficiency in cells due to targeting ability toward lipid droplet rather than mitochondria.

### 3.3 AIE PSs based on Type I and Type II mechanisms

There are many works which did not strictly distinguish the nature of ROS, where only $1^O_2$ indicators or nonspecific indicators were used to demonstrate the ROS generation capacity. In addition, a majority of works have been summarized in previous reviews, which are not mentioned in this review to keep the paper reasonably concise. In this part, several representative and advanced works as to PSs possessing both Type I and Type II ROS generation abilities are reviewed.

Fabricating AIE PSs into nanoparticles (NPs) with special functional modification can achieve high performance of biomaterials. Lou et al developed an AIE PS (TTB), which showed excellent ROS ($O_2^{•−}$ and $1^O_2$) generation ability. The NPs (RGD-MPD/TTB NPs, MPD/TTB NPs and RGD-4R-MPD/TTB NPs) were fabricated by encapsulating TTB within polymeric matrix (Figure 6A) to further improve the hydrophilicity, targeting ability and membrane penetration capacity. DCFH, ABDA, and DHR123 were used to detect the general ROS, $1^O_2$, and free radical, respectively. EPR measurement using BMPO as trapping further confirm its good $O_2^{•−}$ generation capacity (Figure 6B). The results demonstrated that RGD-4R-MPD/TTB NPs had the most excellent performance, which could highly target $\alpha_v\beta_3$-overexpressed cells with enhanced internalization ability to realize superior PDT efficiency.

Li et al encapsulated an AIE PS (TPE-PTB) with lipids to form NPs (Figure 6C), which had high two-photon activity and ROS generation ability. The NPs could efficiently generate $1^O_2$ and HO• under two-photon laser irradiation, which was testified by using ABDA and HPF as fluorescent probe for $1^O_2$ and HO• detection, respectively, and using TEMP and DMPO as EPR trappers for $1^O_2$ and HO• detection, respectively (Figure 6D). Being different from other works, the flow cytometry analysis was used to confirm the intracellular ROS generation quantitatively under NIR I excitation (DCFH-DA as a ROS indicator) (Figure 6E).
FIGURE 6  (A) Chemical structures of TTB, MPD, and RGD-4R and schematic illustration of the preparation of RGD-4R-MPD/TTB NPs. (B) Plots of relative PL intensity of DHR123 in the presence of TTB versus irradiation time; plots of absorption spectra of ABDA after light decomposition by ROS generated from TTB; EPR signals of BMPO in the presence of TTB. Copyright 2020, American Chemical Society. (C) Design and preparation of Lipid-Encapsulated AIE NPs. (D) The Plots of the relative PL intensity of HPF versus irradiation time under different conditions; the plots of the relative absorbance of ABDA versus irradiation time under different conditions; EPR signals of TEMP in the presence of AIE NPs; EPR signals of DMPO in the presence of AIE NPs. (E) Average fluorescence intensity of intracellular ROS measured by a flow cytometer after A375 cells treatment with or without AIE NPs before and after two-photon laser irradiation for 5 min. Copyright 2020, American Chemical Society
4 | CONCLUSION AND OUTLOOK

Hypoxia in solid tumors still remains as a major challenge of current PDT applications. One of the most effective strategies is to develop Type I PSs to enable the limited oxygen to be fully utilized, in which Type I AIE PSs have received considerable attention due to their eminent advantages and recent significant advances. In this review, we give a detailed introduction on the PDT mechanism to demonstrate the superiority of Type I pathway over the Type II pathway. Under this circumstance, a summary of common detection methods is presented to distinguish Type I AIE PSs are still at the infant stage of development. The design strategy for Type I PSs needs to be further explored. Although external factors can impact the nature of ROS, there are still some tips useful for designing Type I PSs: 1. Promoting the ISC process to obtain enough triplets, which is necessary for both Type I and Type II PSs; 2. Increasing the electron affinity of PSs, which facilitates electron capture to form stable free radical anion intermediates; 3. Inhibiting the Type II pathway by reducing $T_1$ energy level, which improves the competitiveness of Type I pathway.

Besides universal strategies to obtain high-performance Type I PSs, there are several issues need to be addressed to substantially accelerate the development of Type I PDT, including: 1. The quantitative evaluation method and criterion of the yield of Type I ROS need to be established. 2. The assessment of tumor hypoxia (e.g., the overexpression of hypoxia inducible factor $\alpha$) in vivo is required. 3. Combining with appropriate agents to intervene the PDT pathway would be an effective approach for further improvement of Type I PDT. 4. The physiological processes during Type I PDT are also an notable issue that might cause positive or negative systemic responses.

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (grant number: 21788102) and the Natural Science Foundation of Guangdong Province (grant number: 2019B030301003). The authors declare no conflict of interest.

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How to cite this article: J. Li, Z. Zhuang, Z. Zhao, B. Z. Tang, VIEW 2022, 3, 20200121.
https://doi.org/10.1002/VIW.20200121