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# Bright red aggregation-induced emission nanoparticles for multifunctional applications in cancer therapy†

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Developing multifunctional photosensitizers (PSs) is needed to effectively simplify cancer treatment, but it remains a big challenge. Here, two red-emitting AIE-active, donor–acceptor (D–A) PSs with small  $\Delta E_{ST}$  and their AIE nanoparticles, are rationally designed and synthesized. The PS1 NPs exhibit bright red-emission with high quantum yield, appropriate  $^1O_2$  generation ability and good biocompatibility. More importantly, PS1 NPs can strongly light up the cytoplasm by gently shaking the cells for only 5 s at room temperature, indicating ultrafast staining and mild incubation conditions. *In vitro* and *in vivo* cell tracing demonstrate that PS1 NPs can track cells over 14 days, and effectively inhibit tumor growth upon irradiation. To the best of our knowledge, this work is the first example of a PS that integrates image-guided PDT, ultrafast staining and long-term tracing functions, demonstrating the “all-in-one” concept which offers great advantages for potential clinical applications.

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## Introduction

Image-guided photodynamic therapy (PDT) has recently gained increasing attention. This technique simultaneously achieves real-time molecular diagnosis and concurrent light-triggered therapy, and has surpassed traditional surgery, such as radiotherapy and chemotherapy.<sup>1–8</sup> Noninvasive fluorescence imaging has emerged as a very powerful tool for visualizing clinical diagnostics and for biological research.<sup>9–14</sup> Unfortunately, discontinuous and short-term cellular tracing is unable to provide continuous real-time dynamic information, thereby limiting the insights into a variety of complex biological processes.<sup>15–21</sup> Long incubation times and harsh incubation conditions are critical barriers for cellular fluorescence imaging

in clinical applications.<sup>22–25</sup> Cancer treatments mainly include three essential steps: ultrafast staining, therapy, and long-term tracing. The former two play important roles for the discovery and excision of tumors, and the last is to further monitor the metastasis of residual tumor.<sup>15,21</sup> The current scope of photosensitizers (PSs) is far from ideal, and until now, only a few PSs can achieve partial procedures, whereas there are no reports on simultaneous multiple applications, namely: (i) image-guided PDT, (ii) ultrafast staining and (iii) long-term tracking. Is it possible to design a single “all-in-one” PS that embraces all the above functions at the same time?

PSs with efficient generation of singlet oxygen ( $^1O_2$ ) and bright red-emission are crucial to realize imaging-guided PDT.<sup>26</sup> An effective strategy to improve the efficiency of  $^1O_2$  generation is to accelerate the intersystem crossing (ISC) and to reduce  $\Delta E_{ST}$  (the energy gap between  $S_1$  and  $T_1$  states).<sup>27–31</sup> A small  $\Delta E_{ST}$  is obtained by constructing a conjugated donor–acceptor (D–A) structure, which is beneficial for strong intramolecular charge transfer (ICT) with efficient separation of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), leading to red-shifted emission.<sup>32–35</sup> Red emission is important because of its minimized autofluorescence interference, increased penetration depth, and less damage to tissue.<sup>36–38</sup> However, traditional organic molecules tend to form aggregates in aqueous media, for example by  $\pi$ – $\pi$  stacking, which directly leads to reduced  $^1O_2$  production, non-radiative pathways and quenched fluorescence.<sup>27,39–41</sup>

To overcome this challenge, the concept of aggregation-induced emission (AIE) has been exploited, notably by Tang's

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group.<sup>42–45</sup> A series of AIEgen-based PSs have been reported to improve both the fluorescence intensity and the <sup>1</sup>O<sub>2</sub> production ability in the aggregated state, due to the restriction of intramolecular motions (RIMs) which lead to energy dissipation.<sup>46–54</sup> Recently, our group obtained three red-emitting AIEgen nanoparticles (NPs) with higher brightness, enhanced <sup>1</sup>O<sub>2</sub> generation, and better biocompatibility compared to the pure iridium(III) complexes.<sup>55</sup> These precedents make AIE NPs with purely organic D–A units particularly suitable as smart PSs to successfully obtain an “all-in-one” (image-guided PDT, ultrafast staining and long-term tracing) system.

In this contribution new PSs are designed and easily synthesized in high yield *via* a three-bladed propeller-like triphenylamine (TPA) as the strong donor and the dicyanovinyl (DC) groups as the acceptor, in which TPA also acts as a rotor to realize AIE.<sup>56</sup> The two red-emitting AIE D–A and D–A–D molecules are **PS1** and **PS2** (Fig. 1A) and their corresponding polymer-encapsulated NPs are **PS1 NPs** and **PS2 NPs**. **PS1 NPs** possess bright red-emission, a large Stokes shift, appropriate <sup>1</sup>O<sub>2</sub> generation ability, good biocompatibility and excellent image-guided PDT activity. More importantly, **PS1 NPs** achieve ultrafast staining of cells in only 5 s at room temperature, with excellent long-term imaging of more than 14 days *in vitro* and *in vivo*.

## Results and discussion

To gain an insight into the molecular properties of **PS1** and **PS2**, time-dependent density theory (TD-DFT) calculations were performed (Fig. 2A). The HOMOs are mainly localized on TPA units, whereas the LUMOs are located on DC units, with significant HOMO–LUMO separation. The energy gap between the HOMO and LUMO of **PS1** and **PS2** is 2.449 and 2.425 eV, respectively. These relatively narrow band gaps induce the red-shifted absorption. Furthermore, the additional triphenylamine unit in **PS2** results in a slight reduction of  $\Delta E_{ST}$  (**PS2** 0.204 eV;

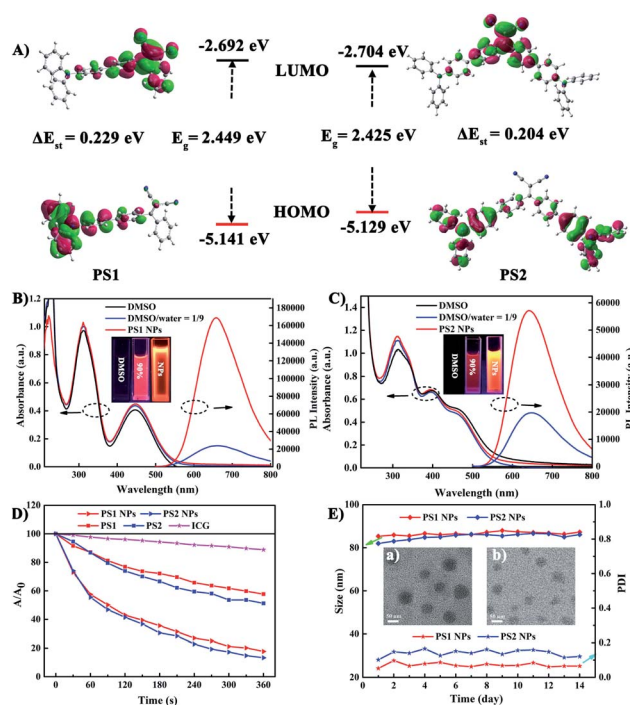


Fig. 2 (A) HOMO and LUMO distributions of **PS1** and **PS2** based on TD-DFT. UV-vis and emission spectroscopies of (B) **PS1** and (C) **PS2** in DMSO, DMSO/water (*v/v*) = 1/9, and the corresponding PS NPs in water ( $\lambda_{\text{ex}}$  = 488 nm), insert: the fluorescent image of PSs and their NPs under 365 nm UV light. (D) The decomposition rates of ICG with different PSs under white irradiation (20 mW cm<sup>-2</sup>). (E) Size measurement of different PSs in 14 days, insert: the TEM images of (a) **PS1 NPs** and (b) **PS2 NPs**.

**PS1** 0.229 eV), owing to a smaller spatial overlap of the Frontier orbitals. The small  $\Delta E_{ST}$  values of **PS1** and **PS2** are beneficial for ISC, which will facilitate highly efficient <sup>1</sup>O<sub>2</sub> generation.<sup>29</sup>

The photophysical properties of the PSs were investigated by absorption and photoluminescence (PL) spectroscopy. In Fig. 2B and C the absorption bands at about 210–315 nm are assigned to the  $\pi$ – $\pi^*$  transition of the conjugated backbone. The bands located in the 380–600 nm region belong to the intense ICT process between the D–A units. The absorption band covers most of the visible light range, which is beneficial for matching the white light spectrum.<sup>28</sup> In Fig. S9 (ESI<sup>†</sup>), both **PS1** and **PS2** show negligible fluorescence in DMSO, because the rotational motions of the triphenylamine moieties increase the dissipation of energy. However, the PL intensities of **PS1** and **PS2** gradually increase upon raising the water content, when intramolecular rotation is restricted by the formation of aggregates, revealing a typical AIE effect. Compared with **PS1** ( $\lambda_{\text{max}}$  660 nm) and **PS2** (645 nm), their NPs show similar emission peaks but with significantly enhanced intensities. The PL intensities of **PS1 NPs** and **PS2 NPs** are 6.9 and 2.86 times higher than those of **PS1** and **PS2**, respectively, with **PS1 NPs** 2.9 times higher than **PS2 NPs** in the same conditions. The fluorescence spectra of these AIE NPs extend into the near-infrared region, and they exhibit a large Stokes shift which can eliminate interference from the background. These factors are favorable

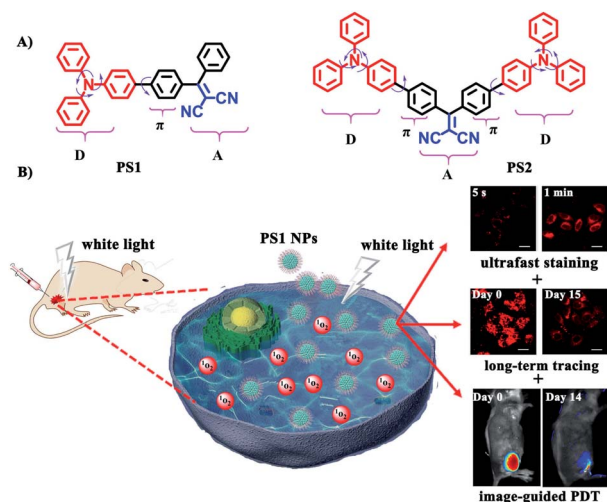


Fig. 1 (A) Structural formulas of **PS1** and **PS2**. (B) Schematic illustration of **PS1 NPs** as PSs for “all-in-one” PDT.







attributed to the brighter red emission of **PS1** NPs than **PS2** NPs. Negligible change in cell morphology was observed from day 1 to day 15 during long-term cell tracing. This indicates that there are no cytotoxicity effects for long-term cell tracing with **PS1** and **PS2** NPs. Under the same conditions, flow cytometry results also demonstrate that both **PS1**NPs and **PS2** NPs have a tendency to accumulate in HeLa cells after 15 days incubation (ESI, Fig. S33<sup>†</sup>). Therefore, the **PS1** NPs could perform as a promising long-term tracing probe.

Motivated by the excellent performance *in vitro*, **PS1** NPs were further evaluated *in vivo* with U14 tumor-bearing mice. In Fig. 4A, obvious fluorescent signals were obtained from the site injected with **PS1** NPs, indicating good *in vivo* imaging. With increasing time, the fluorescence intensity gradually decreased, but still retained 42% of the initial intensity after 14 days (Fig. 4B), indicating the excellent *in vivo* long-term tracing ability of **PS1** NPs. After PDT treatment, faint fluorescence could be observed, suggesting high PDT efficacy of **PS1** NPs. Simultaneously, in Fig. 4C, in the control groups, the relative tumor volumes increased by 10–13 times after 14 days, implying that treatment only by irradiation or by **PS1** NPs, has negligible influence on tumor growth. As expected, tumor volume in the experimental group showed a significant reduction, confirming the good anticancer effect of **PS1** NPs under white-light irradiation. Negligible changes of body weight were observed after various treatments (ESI, Fig. S34D<sup>†</sup>) indicating the low systemic

toxicity of **PS1** NPs. The biosafety of **PS1** NPs was further evaluated by hematoxylin and eosin (H&E) staining of major organs. No pathological changes were found in the major organs of the four groups. These results reveal that **PS1** NPs are excellent candidates for *in vivo* long-term tracing and image-guided PDT.

## Conclusions

In summary, we have successfully constructed for the first time a single PS to simultaneously achieve image-guided PDT, ultrafast staining and long-term tracing. Two red-emitting AIE-active D–A organic molecules and their NPs, were rationally designed and synthesized. The small  $\Delta E_{ST}$  increases the inter-system crossing (ISC) process, leading to effective  $^1O_2$  generation for the two PSs. The construction of nanoparticles further improves the fluorescence intensity and the  $^1O_2$  production ability. Especially the **PS1** NPs are ideal for biological applications owing to the following advantages: bright red emission, large Stokes shift, high fluorescence quantum yield (40%), appropriate  $^1O_2$  generation ability, good biocompatibility, and excellent image-guided PDT activity. More importantly, **PS1** NPs can stain cells in only 5 s at room temperature, and they display excellent long-term imaging for more than 14 days *in vitro* and *in vivo*. This work successfully achieves a smart AIE PS with image-guided PDT, ultrafast staining and long-term tracing functions. This prototype example of “all-in-one” PS NPs should stimulate the design and development of new multifunctional PSs for potential clinical applications.

## Ethical statement

All animal studies were performed in strict accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23 Rev. 1985) and were approved by the guidelines of the Committee on Animal Use and Care of the Chinese Academy of Sciences.

## Conflicts of interest

There are no conflicts to declare.

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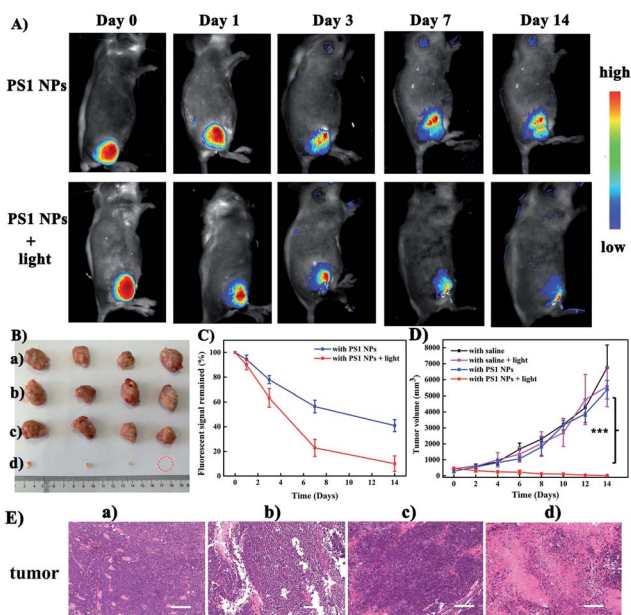


Fig. 4 (A) Time-dependent *in vivo* fluorescence images of U14 tumor-bearing mice after intratumoral injection with **PS1** NPs ( $100 \mu\text{g mL}^{-1}$ ,  $100 \mu\text{L}$ ) or with **PS1** NPs + white light ( $200 \text{ mW cm}^{-2}$ , 20 min). (B) Harvested tumors from various groups treated. (C) Fluorescence intensity of U14 tumor-bearing mice in different mice groups. (D) Tumor volume measurement for different treatments of mice ( $***P < 0.001$ ,  $n = 4$  per group, PDT vs. other groups). (E) H&E staining images of tumor slices from each groups. Scale bars:  $100 \mu\text{m}$ . (a) With saline, (b) with saline and light, (c) with **PS1** NPs, (d) with **PS1** NPs and light ( $100 \mu\text{g mL}^{-1}$ ,  $100 \mu\text{L}$ ), white light ( $200 \text{ mW cm}^{-2}$ , 20 min).



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