Supporting Information

AIE Nanodots Scaffolded by Mini-Ferritin Protein for Cellular Imaging and Photodynamic Therapy

Xuehong Min,[a] Ti Fang,[a] Lingling Li,[a] Chaoqun Li,[a] Zhi-Ping Zhang,[a] Xian-En Zhang,[b] Feng Li*[a]

[a] State Key Laboratory of Virology, Wuhan Institute of Virology, Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan 430071, PR China

[b] National Key Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, PR China

* Corresponding author. Tel.: +86 27 8719 9279; Email: fli@wh.iov.cn

1. Experimental section

1.1 Materials

Malononitrile, magnesium ethoxide, and 3-hydroxy-3-methylbutan-2-one were purchased from Aladdin (Shanghai, China). Anhydrous magnesium sulfate, NaOH, NaCl, sucrose, ethanol, ethyl acetate (EA), petroleum ether (PE), and dichloromethane (DCM) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 4-(1,2,2-Triphenylvinyl)benzaldehyde (TPE-CHO) was purchased from AIEgen Biotech. Co., Limited (Hong Kong, China). Water was purified by a Millipore filtration system.

1.2 Synthesis of RAIE molecules

RAIE molecules were synthesized according to the procedures in the literature.[1] Mass spectrometry of RAIE molecules was conducted on a Tandem Quadrupole (Triple Quadrupole) Mass Spectrometry (ACQUITY UPLC H-class-Xevo TQ MS, Waters). *m/z*: $[M]^+$; calcd. for $C_{38}H_{27}N_3O$: 541.2154, found: 541.1954.

1.3 Preparation of Dps

A single-mutation gene G104C (Gly 104 to Cys) was generated by overlap PCR with pET32a-wtDps as the template and inserted into the pET32a vector at NdeI-XhoI. The resultant pET32a-G104C was transformed into the E. coli BL21 (DE3) strain. When OD_{600} reached 0.6, Dps expression was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 12 h at 25 °C. Extraction and purification of the recombinant Dps protein were performed as previously described.[2] Mass spectrometry analysis was carried out using a MALDI TOF/TOF 5800 mass spectrometer (AB Sciex, USA) with sinapinic acid as the matrix with a set mass range of 12000 to 27000 Da.

1.4 Preparation and purification of Dps-RAIE nanodots

RAIE molecules dissolved in DMSO were added to depolymerized Dps solution, stirred for 30 min, and then dialyzed against the assembled buffer. Next, Dps-RAIE nanodots were purified by sucrose density gradient centrifugation (SDGC), where

nanodots were loaded onto a two-layer sucrose cushion with 5 mL of 25% (w/v) sucrose as the upper layer and 5 mL of 45% (w/v) sucrose as the lower layer, and then centrifuged at 38,000 rpm (SW41 Ti rotor, Beckman) for 4 h at 4°C. The band between the two layers of sucrose was harvested and dialyzed against PBS to eliminate sucrose. The SDGC tube was imaged using a digital camera with excitation by a portable UV lamp at 365 nm. The product was concentrated by ultrafiltration using an Amicon Ultra-15 Centrifugal Filter Unit with a molecular weight cut-off of 100 kDa (Millipore).

1.5 Size and zeta potential measurements

Dynamic light scattering (DLS) measurement of Dps-RAIE nanodots in PBS was carried out using a Zetasizer® Nano ZS (Malvern Instruments, Malvern, UK) at 25 °C with the samples being filtered through 0.1 μm syringe filters. Zeta-potentials of Dps and Dps-RAIE nanodots in PBS were measured using the same instrument.

1.6 Transmission electron microscopy (TEM)

A drop of sample (20 μL) was deposited onto a carbon-coated copper grid. After 4 min of incubation, excess solution was removed with filter paper. The grid was then stained with 20 μL of 2.0% phosphotungstate (PTA) for 3 min. The grid was observed under a Hitachi H7000 TEM equipped with an Olympus MegaView G2 camera. ImageJ software (NIH, USA) was used for TEM image processing and data analysis.

1.7 Fluorescence measurement

Fluorescence detection was performed on a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies). The excitation wavelength (λ_{ex}) was 465 nm, and the emission wavelength (λ_{em}) ranged from 550 nm to 850 nm, with slit widths of 10 nm in both excitation and emission.

1.8 Measurement of fluorescence quantum yield (Φ_f)

Rhodamine B in EtOH at 20 °C was used as a standard (Φ_f = 0.5).[3] The fluorescence quantum yield of the Dps-RAIE nanodots is related to that of a standard by the following equation:

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2 \quad (1)$$

Where subscripts S and X denote the standard and the tested Dps-RAIE nanodot sample, respectively. A is the absorbance at the excitation wavelength, F is the area across the emission band, and n is the refractive index of solvent.

1.9 Cell culture and imaging

All cells were cultured in Dulbecco's minimum essential media (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin, and 1% amphotericin B. The day before treatment, cells were seeded in 35-mm culture dishes at a confluence of 70-80%. For cell imaging, an aqueous solution of 200 μL Dps-RAIE nanodots was added to the cells and incubated for 2 h. Next, cells were gently washed three times with PBS. Hoechst 33258 was then added (2.5 μM), and the cell plate was kept at room temperature for 10 min in the dark. Finally, cells were gently washed three times with PBS and imaged with a Nikon A1 confocal laser-scanning microscope (Tokyo, Japan). For visualization of Dps-RAIE nanodots, a λ_{ex} of 488 nm was used with the emission was collected in the range of 600-700 nm. For Hoechst 33258, the λ_{ex} was 405 nm, and the λ_{em} ranged from 425 nm to 475 nm.

1.10 Singlet oxygen detection in solution

The probe, 9,10-Anthracenediyl-bis(methylene) dimalonic acid (ABDA), was used to detect singlet oxygen production in solution. The ABDA stock solution in DMSO was mixed with Dps-RAIE nanodots at final concentrations of 50 μM and 10 μM, respectively and exposed to light irradiation for different time intervals at a power density of 0.25 W/cm^2. As a control, a mixture of RAIE molecules (10 μM) and ABDA in DMSO was likewise treated. The decomposition of ABDA was monitored by a decrease in absorbance at 400 nm.

At the same time, another probe named SOSG was also used for singlet oxygen detection in solution. An SOSG stock solution (5 mM) was prepared by dissolving

100 μg of SOSG in methanol. A PBS solution containing Dps-RAIE nanodots (10 μM) and SOSG (2 μM) was irradiated by light (0.25 W/cm^2). The fluorescence was recorded at 525 nm using an excitation wavelength of 488 nm to determine the singlet oxygen concentration at 5-min intervals.

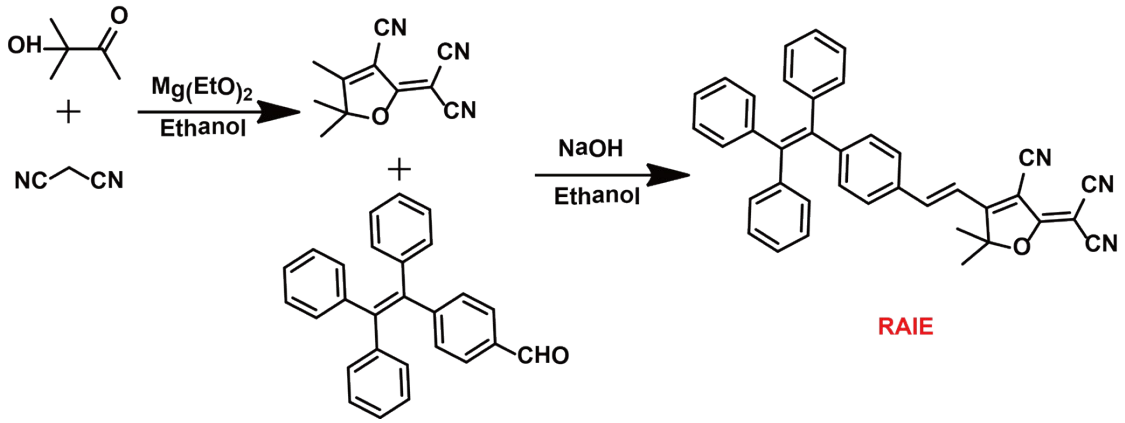
1.11 Intracellular ROS detection

The ROS generation inside cells upon light irradiation was investigated using a cell-permeable indicator 2',7'-dichlorofluorescin diacetate (DCFDA). Hela cells in 8-well chambers (Thermo Scientific) were first incubated with Dps-RAIE nanodots (5 μM) for 2 h in the dark, and then DCFDA (10 μM) was loaded into the cells. After 5 min of incubation, the cells were washed with PBS and exposed to white light irradiation (0.25 W/cm^2, 2 min). After irradiation, the cells were studied by a Nikon A1 confocal laser-scanning microscope (Tokyo, Japan). For DCFDA detection, the λ_{ex} was 488 nm, and the λ_{em} was 505-525 nm.

1.12 Cytotoxicity study

The cell viability of Hela, A549, and HepG2 cells was determined after incubation with Dps-RAIE nanodots under white light irradiation (0.25 W/cm^2, 2 min) using a WST-8 cell counting kit (CCK-8, Dojindo Laboratories in Japan). Briefly, cells were seeded in 96-well plates at a density of 2,000 cells per well. After 24 h of incubation, cells were incubated with Dps-RAIE nanodots in the dark for the indicated time. After incubation, the cells were washed with PBS and exposed to white light irradiation (0.25 W/cm^2, 2 min). The cells were further incubated in fresh medium for 24 h and washed with PBS. Finally, the CCK-8 reagent was added, and optical density at 450 nm was measured to evaluate cell viability.

2. Supplementary Scheme and Figures



Scheme S1. Synthetic route to the target compound RAIE molecule.

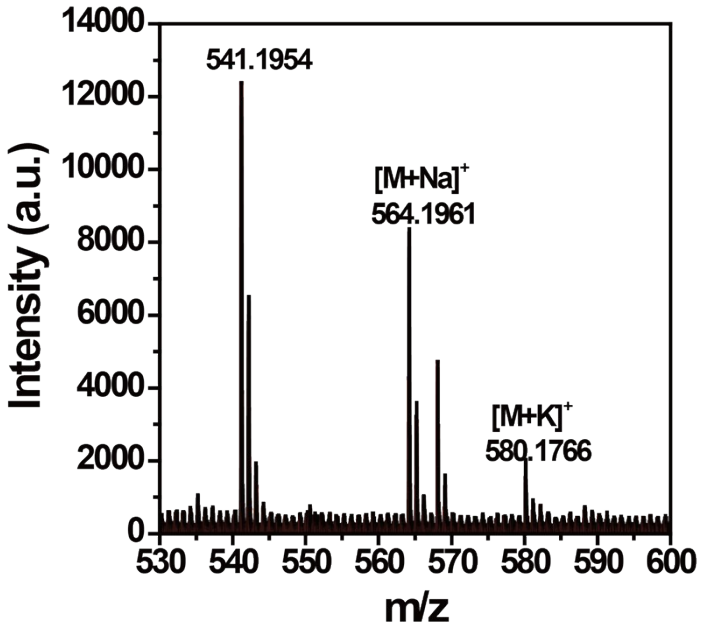


Figure S1. Mass spectrometry of the RAIE molecule.

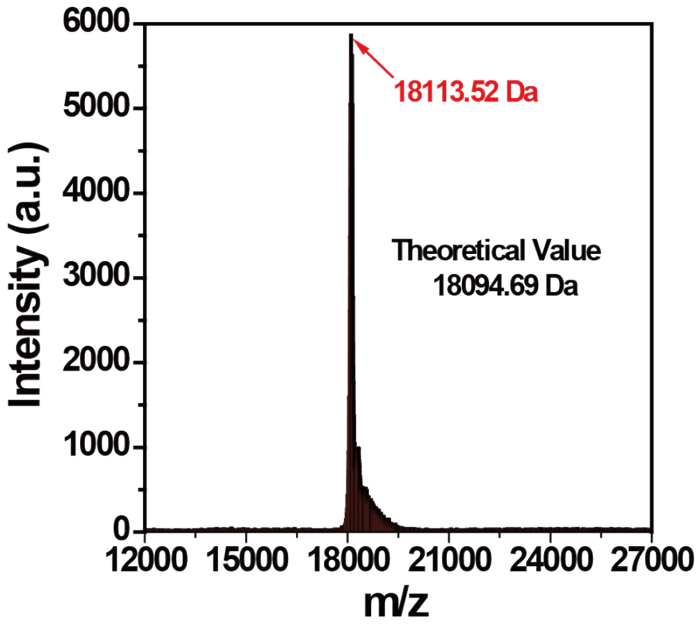


Figure S2. MALDI-TOF mass spectrum of Dps.

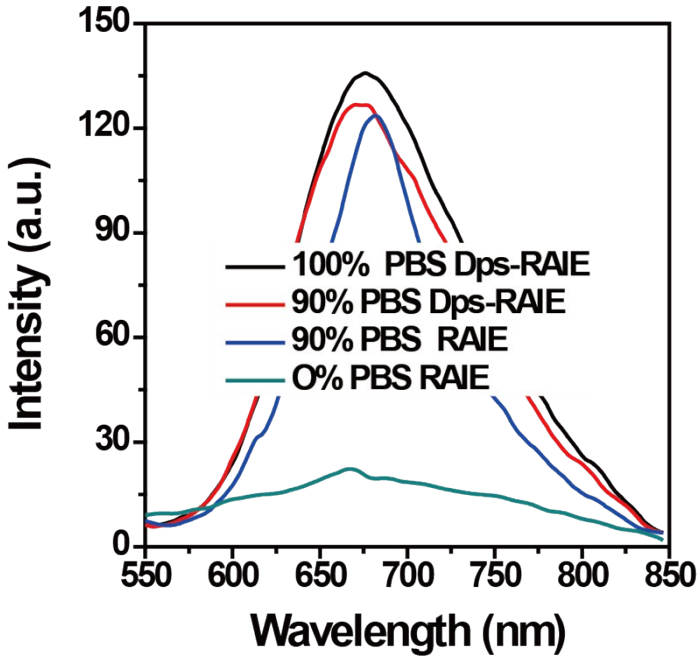


Figure S3. Fluorescence spectra of Dps-RAIE nanodots.

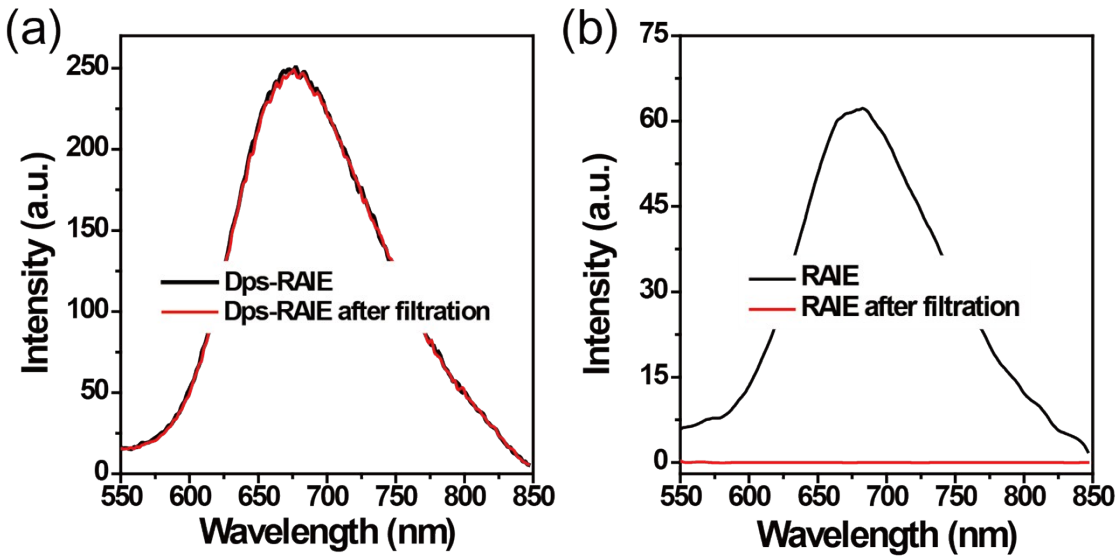


Figure S4. Fluorescence spectra of Dps-RAIE (a) and RAIE (b) samples in PBS before and after filtration using 0.22 μm membranes.

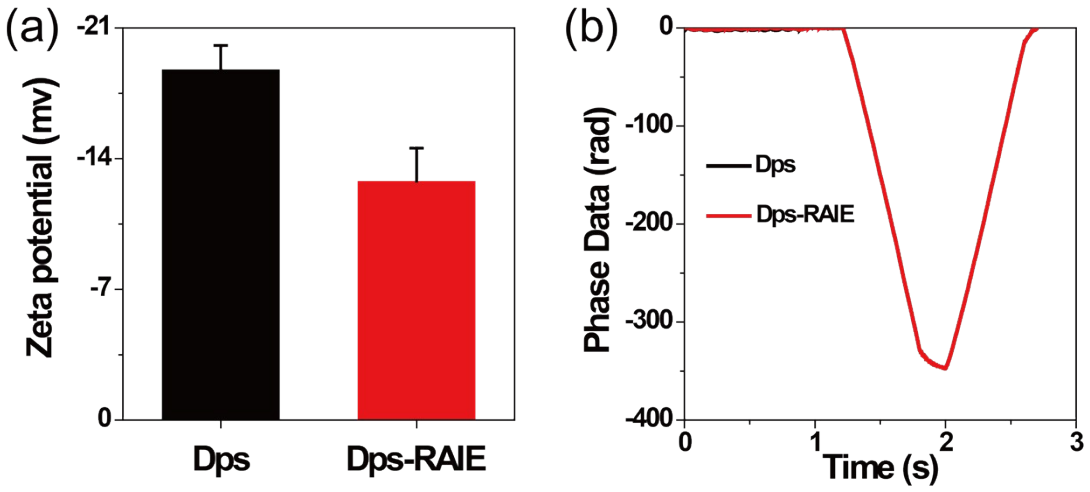


Figure S5. Zeta potential measurement. (a) Zeta potential value of the Dps-RAIE nanodots in comparison with Dps. Error bars represent the standard deviation obtained from three independent measurements. (b) Phase plots of Dps and Dps-RAIE nanodots.

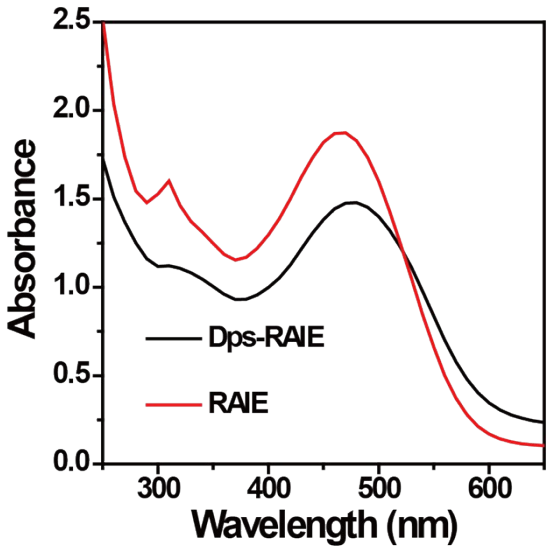


Figure S6. UV-Vis absorption spectra of Dps-RAIE nanodots in PBS and RAIE molecules in DMSO.

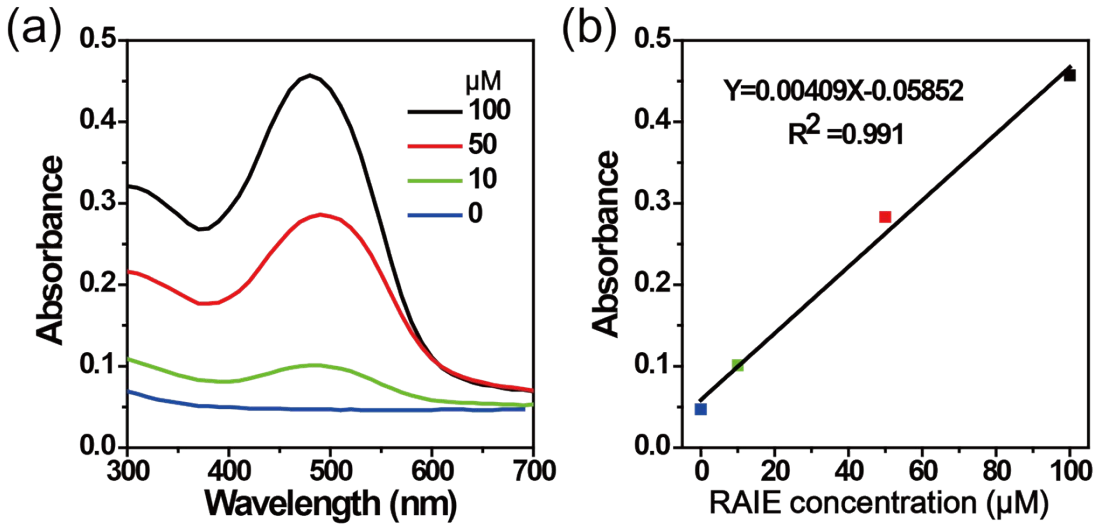


Figure S7. Correlation between absorbance and concentration of RAIE molecules. (a) UV-Vis absorption spectra of RAIE molecules at different concentrations. (b) A linear relationship between absorbance and concentration of RAIE molecules.

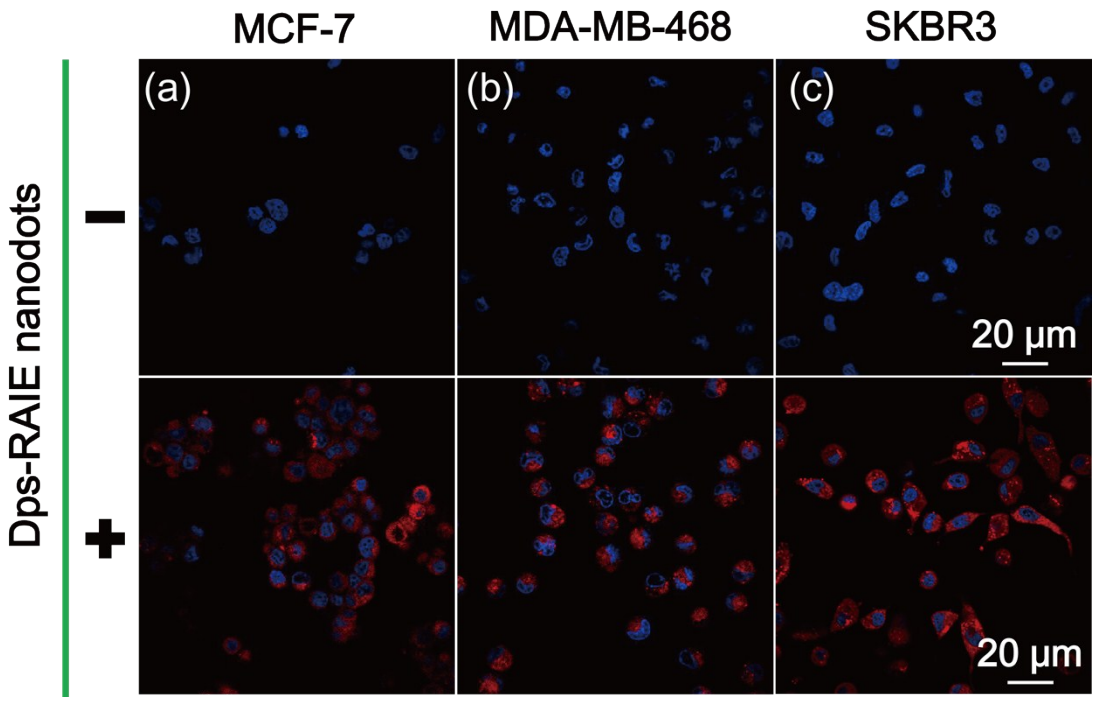


Figure S8. Confocal fluorescence images of MCF-7, MDA-MB-468, and SKBR3 cells incubated for 2 h without (-) and with (+) 5 μM Dps-RAIE nanodots (red). Nuclei were stained with Hoechst 33258 (blue).

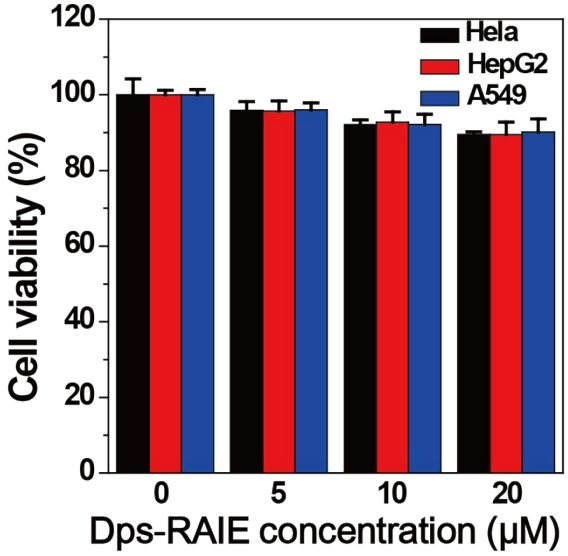


Figure S9. Viability of Hela, HepG2, and A549 cells incubated with different concentrations of Dps-RAIE nanodots for 24 h in the dark.

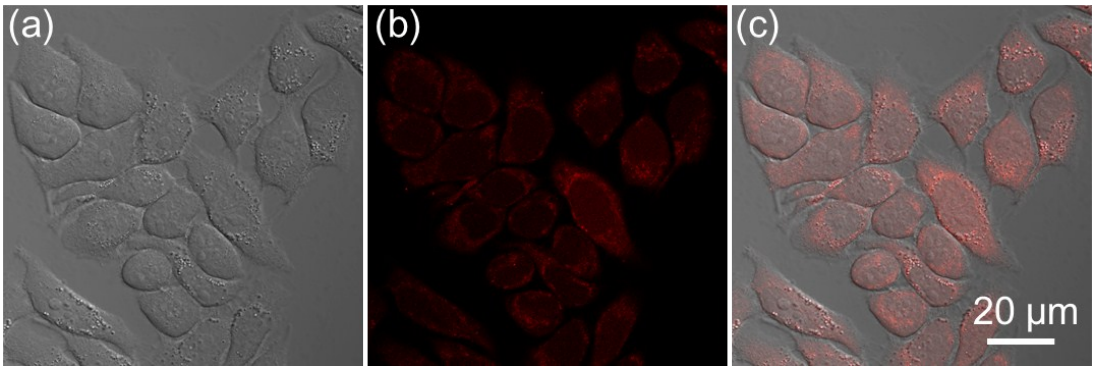


Figure S10. Confocal fluorescence images of Hela cells incubated with 5 μM Dps-RAIE nanodots (red) for 2 h.

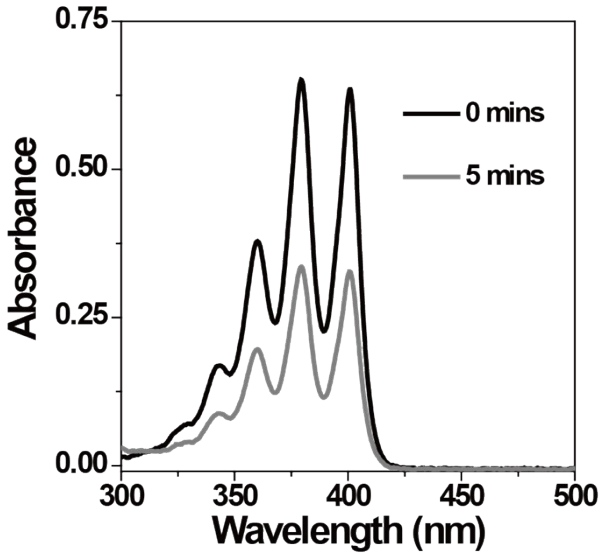


Figure S11. Absorption spectra of ABDA in the presence of Dps-RAIE nanodots without irradiation and after 5 min of irradiation.

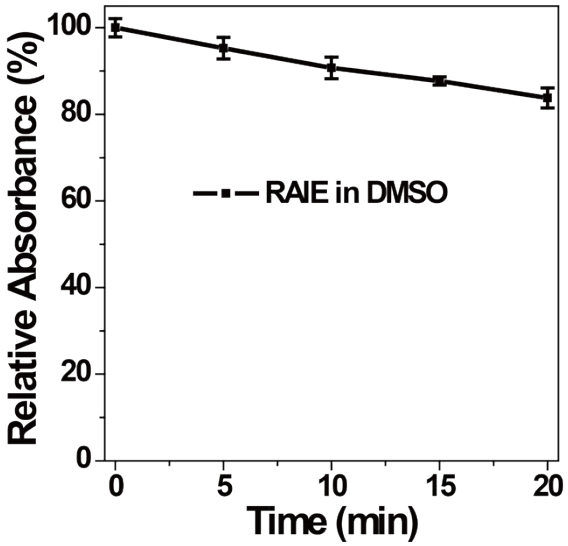


Figure S12. Time-dependent absorption (400 nm) of the singlet oxygen indicator ABDA (50 μM) in the presence of RAIE in DMSO at a power density of 0.25 W/cm^2 for 20 min at 5-minute intervals. Error bars represent the standard deviation of three repetitive experiments.

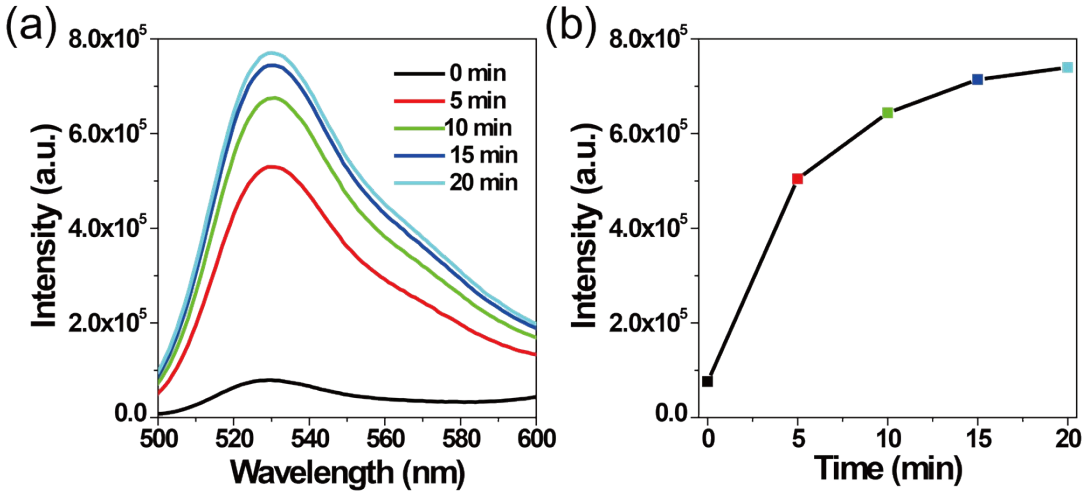


Figure S13. Detection of singlet oxygen using the SOSG probe. (a) Time-dependent fluorescence spectra of SOSG (2 μM) in the presence of the Dps-RAIE nanodots (10 μM) in PBS at a power density of 0.25 W/cm^2 for 20 min. (b) Fluorescence intensity of SOSG at 525 nm at different times.

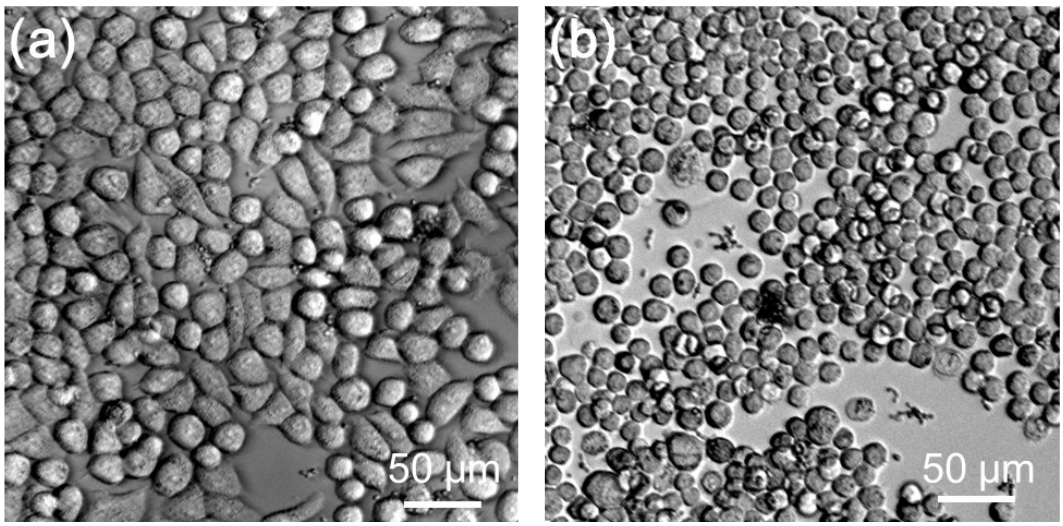


Figure S14. PDT of Hela cells with Dps-RAIE nanodots. Hela cells were incubated with 5 μM Dps-RAIE nanodots for 2 h, treated without (a) or with (b) white light irradiation (0.25 W/cm^2, 2 min) and cultured for another 24 h. Then bright-field images were taken.

3. References

(1). Y. J. Wang, Y. Shi, Z. Y. Wang, Z. F. Zhu, X. Y. Zhao, H. Nie, J. Qian, A. J. Qin, J. Z. Sun and B. Z. Tang, *Chem. Eur. J.* 2016, **22,** 9784.

(2). L. Z. Ma, F. Li, T. Fang, J. T. Zhang and Q. B. Wang, *ACS Appl. Mater. Interfaces*, 2015, **7**, 11024.

(3). J. H. Wang and Y.Q. Shen, *Adv. Mater. Opt. Electron.* 1999, **9**, 129.