# Supporting Information

# A Highly Efficient Red Metal-free Organic Phosphor for Time-resolved Luminescence Imaging and Photodynamic Therapy

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## I. Experimental Section

### Calculation Methods

The monomer was extracted from the crystal structure, one model was also built which the monomer was surrounded with one BrH molecule. Their excitation energies and nature transition orbitals for the singlet and triplet states were then evaluated at TD-DFT/M06-2X/6-31G(d) level using Gaussian 09 program.[1] At the same level, the spin-orbit couplings between singlet ant triplet states were also calculated by PySOC code.[2]

### Preparation of phosphorescence nanodots (PNDs)

The DBCz-BT (3 mg) was added into the water solution containing F127 (3 mg/mL) under sonication at room temperature for 10 min (SCIENTZ-II D with 30% output). The homogenous nanoparticles were attained after filtering with a 0.22 μm filter. The particles were kept in water in 4 $^{o}$C storage.

### Cell Culture and Imaging

HeLa cells were cultured in RPMI DMEM, containing 10% fetal bovine albumin and 1% penicillin streptomycin. Cells were maintained at 37 °C in air with 5% $CO_2$. Confocal luminescence imaging was carried out laser scanning confocal microscope (LSCM). A semiconductor laser was served as the excitation of the HeLa cells incubated with PNDs at 405 nm. The emission was collected at 550-650 nm, for the Hela cells incubated with PNDs. PNDs were added to RPMI DMEM to yield 75 μg/mL solution. The HeLa cells were incubated with the PNDs for 3 h at 37 °C.

For the phosphorescence lifetime imaging, The HeLa cells were incubated with PNDs (75 μg/mL) and a commercial nucleus dye, Hoechst 33342 (1 μg/mL) for 3 h at 37 °C. Then the lifetime imaging was conducted through both fluorescence and phosphorescence channels.

## Assay for Cell Viability by MTT

HeLa cells were seeded into a 96-well cell-culture plate at a density of $1\times10^4$ cells/well for 24 h at 37 °C and 5% CO2. PNDs with different concentrations (0, 5, 10, 25, 50, 75, 100, 150, 200 μg/mL) were added into the wells, respectively, and further cultured for

another 3 h. The 475 nm xenon lamp with a dose of 35 mW $cm^{-2}$ for 20 min was used to perform PDT treatment. After irradiation, the cells were cultivated continuously for 24 h. Then MTT (5 mg/mL, 10 μL/well) was added to the wells and the cells were incubated at 37 °C for another 4 h. DMSO (150 μL/well) was added to dissolve the produced formazan after discarding the supernatant. The plates were shaken for 10 min and the absorbance values of the wells were then read with microplate reader at 490 nm. The cell viability rate (VR) was calculated according to the following equation:

$VR = (A_{experimental\ group}/A_{control\ group}) \times 100\%$.

## ROS Measurements in Vitro

After the HeLa cells were incubated with PNDs for 3 h, they were further incubated with 1 μM 2'7'-dichlorodi-hydrofluorescein diacetate (DCFH-DA) for 10 min and irradiated with a 475 nm xenon lamp at a power of 35 mW $cm^{-2}$ for 0, 2, 4, 6, 8 min, and perform the fluorescence detection of DCF with the CLSM, respectively, to detect the level of intracellular ROS generation. The emission was collected at 500-550 nm.

## Confocal Imaging of Photo-induced Cell Death by Calcein-AM and PI

After the HeLa cells were incubated with PNDs for 3 h, irradiated by a 475 nm xenon lamp at a power of 35 mW $cm^{-2}$ for 20 min. After continuous cell cultivation for 3 h, the medium was replaced with 1 mL PBS containing Calcein-AM ($2 \times 10^{-6}$ M), PI ($4 \times 10^{-6}$ M) for 30 min, respectively. Each well was washed to remove the excess dye solution and replaced with fresh PBS. The wells were then observed via a confocal microscopy system. (Calcein AM $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ =500-550 nm， PI $\lambda_{ex}$ = 488 nm, $\lambda_{em}$ =600-650 nm).

## Assay for Cell Viability Population by Flow Cytometry

The HeLa cells were seeded in the six-well plates at a density of $\approx 1 \times 10^5$ for 24 h, 37 °C. Then the cells were washed once with PBS. RPMI DMEM medium with PNDs (75 μg/mL) was added for 3 h. Afterward, PDT treatments (35 mW $cm^{-2}$, 20 min) together with dark and light control were conducted on the cell plates. After continuous cell cultivation for 3 h, the cells were stained with Annexin V–FITC/PI cell apoptosis kit according to the manufacturer's instruction to perform flow cytometric assay using BD

FACSCanto II flow cytometry. Here the FITC (fluorescein isothiocyanate) was a derivative of fluorescein, used to stain the apoptotic cells; and PI was labeled the late apoptotic cells and death cell nucleus.

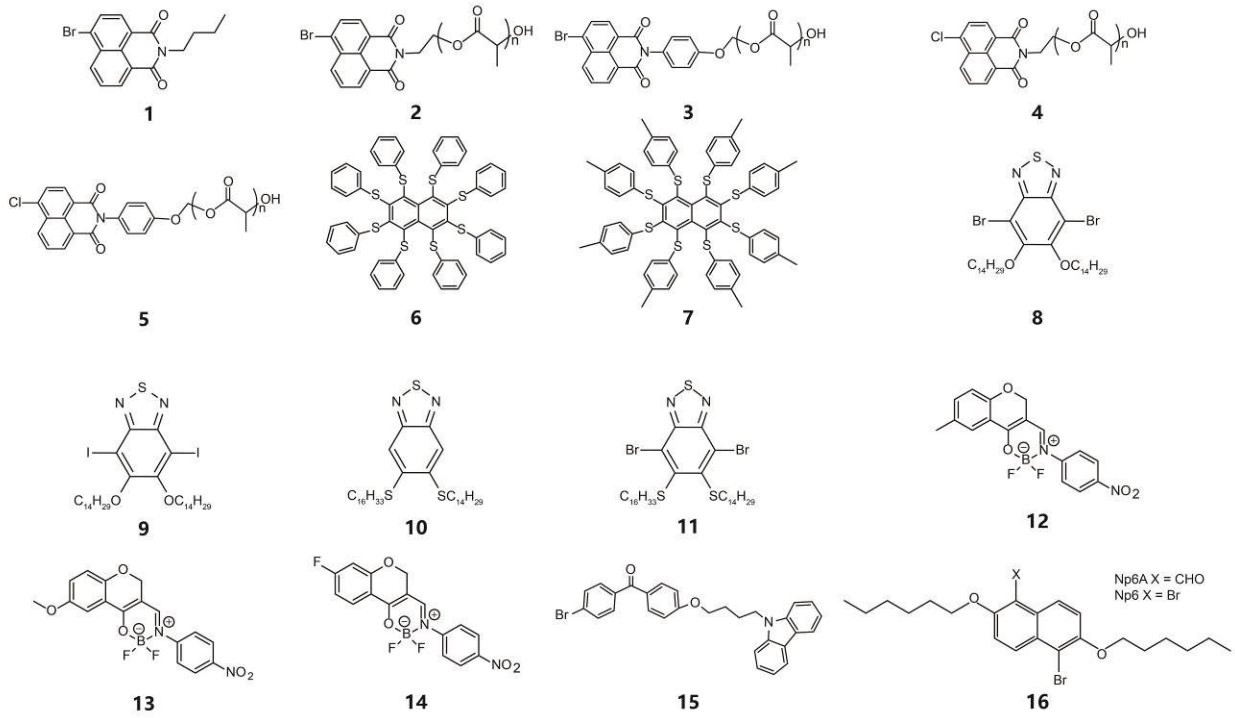
### Zebrafish Imaging

PNDs (150 μg/mL) were injected to the brain of zebra fish by micromanipulator. The lifetime imaging in vivo was performed on a phosphorescence lifetime imaging microscopy (PLIM) excited of 405 nm with a filter of 430 nm.

### Mice Model.

All the animal experiments were performed in compliance with the criterions of the National Regulation of China for Care and Use of Laboratory Animals. Female Balb/c mice with tumor in the flank were purchased from Nanjing Kaiji Biotechnology Development Co., Ltd. When the tumor volumes approached about 150 $mm^3$, imaging and PDT therapy were conducted. The volumes of tumors were measured every 2 days for 15 days and calculated by the following formula: $V = LT^2/2$, where L and T represent the length and width of tumors, respectively. The $V/V_0$ was recorded after the treatments. At the $15^{th}$ day, tumors were excised and fixed in 4% formalin for H&E staining studies.

## II. Additional background information




**Scheme S1.** Reported molecular structures with red phosphorescence.

**Table S1.** Photophysical properties of reported metal-free red phosphors.

| Compound | $\lambda_{em}$ (nm) | $\lambda_{abs/ex}$ (nm) | Φ(%) | $\tau_p$(ms) | Reference |
|---|---|---|---|---|---|
| 1 | 611 | 369 | 0.05[a] | 5.1 | [3] |
| 2 | 600 | 369 | 0.05[a] | 5.6 | [3] |
| 3 | 600 | 369 | 0.0057[a] | 84 | [3] |

| | | | | | |
|---|---|---|---|---|---|
| 4 | 600 | 369 | 0.046[a] | 3.6 | [3] |
| 5 | 600 | 369 | 0.021[a] | 70 | [3] |
| 6 | 650 | 420 | 0.5-1.3[b] | 0.15 | [4] |
| 7 | 660 | 390 | — | 1.5 | [4] |
| 8 | 642 | 325 | 0.55[c] | 2.8 | [5] |
| 9 | 643 | 327 | 0.59[c] | 3.6 | [5] |
| 10 | 624 | 382 | 5.4[c] | 0.7 | [5] |
| 11 | 667 | 347 | 3.4[c] | 0.5 | [5] |
| 12 | 606 | 456 | 40.9[d] | 0.002 | [6] |
| 13 | 653 | 439 | 83.3[d] | 0.002 | [6] |
| 14 | 621 | 426 | 59.5[d] | 0.0013 | [6] |
| 15 | 580 | 365 | 1[e] | 0. 6 | [7] |
| 16 | 602 | 450-550 | 14.6[e] | 0.605 | This work |

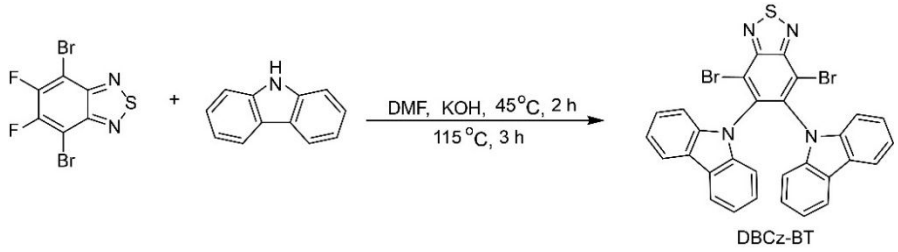
All phosphorescence quantum efficiencies were measured at room temperature. [a] in PMMA under vacuum. [b] in film under air. [c] in solution under air. [d]in solution under $N_2$ atmosphere. [e] in solid under air.

**Table S2.** Crystallographic data for DBCz-BT

| Name | DBCz-BT |
|---|---|
| Formula | $C_{30}H_{16}Br_2N_4S$ |
| Wavelength (Å) | 0.71073 |
| Space Group | C c |
| Cell Lengths (Å) | a 10.171(2)<br>b 13.966(3) |

| | |
|---|---|
| | c 18.345(4) |
| Cell Angles (°) | α 90.00,<br>β 106.09(3),<br>γ 90.00 |
| Cell Volume (Å$^3$) | 2503.8 |
| Z | 26 |
| Density (g/cm$^3$) | 1.656 |
| F (000) | 1240 |
| $h_{max}$, $k_{max}$, $l_{max}$ | 5, 15, 21 |
| CCDC | 1888908 |

## Ⅲ. Synthesis and characterization of organic phosphorescence compounds




**Scheme S2.** Synthetic route of target molecule DBCz-BT.

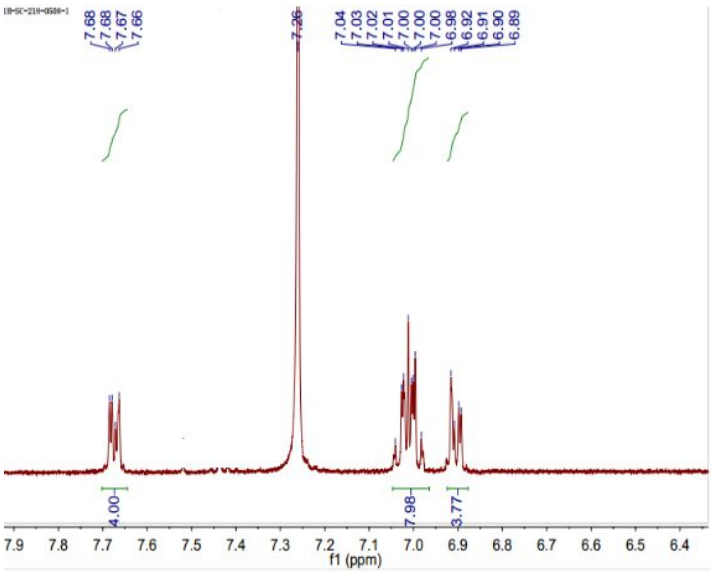



**Figure S1.** $^{1}$H-NMR spectrum of DBCz-BT in $CDCl_3$.

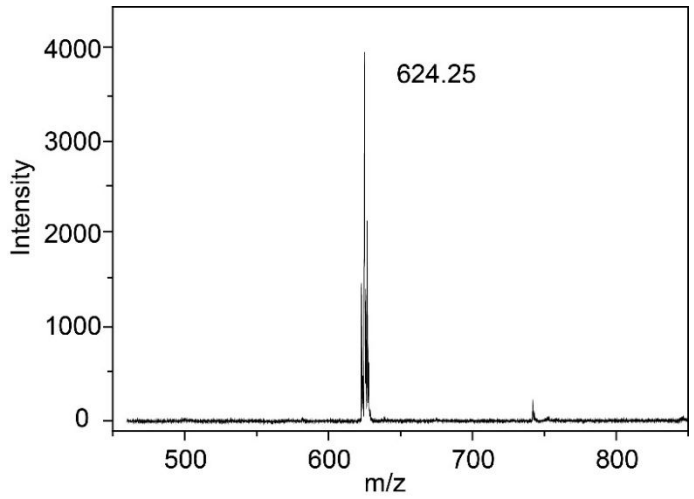



**Figure S2.** MALDI-TOF MS spectrum of DBCz-BT in dichloromethane ($CH_2Cl_2$) at 298 K.

## Ⅳ. Characteristics of DBCz-BT

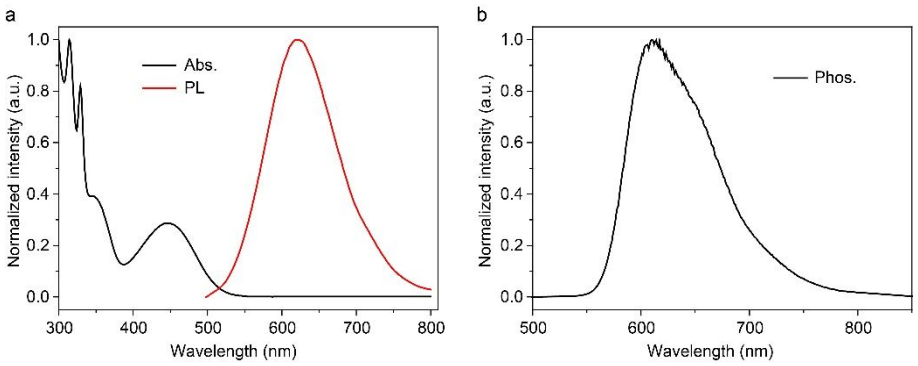



**Figure S3.** a) Absorption and PL spectra of DBCz-BT in $CHCl_3$ dilute solution at 298 K. b) Phosphorescence spectrum of DBCz-BT in $CHCl_3$ dilute solution at 77 K

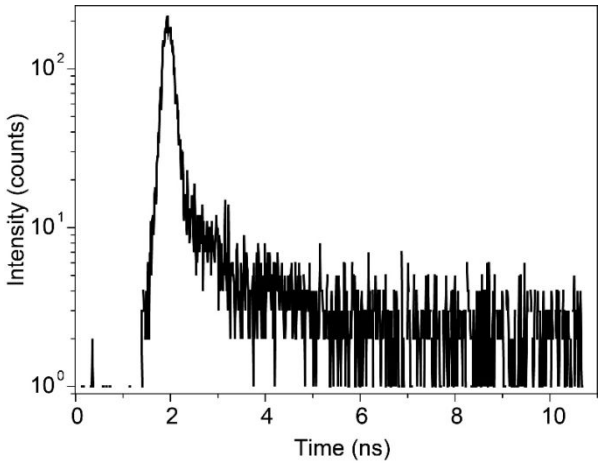



**Figure S4.** Lifetime decay profile of emission band at 605 nm for DBCz-BT in trichloromethane solution at 298 K

**Table S3.** The corresponding lifetime (τ) and its proportion (A) of DBCz-BT at different states[a]

| Compound | State | Wavelength (nm) | Lifetime | | | |
|---|---|---|---|---|---|---|
| | | | $\tau_1$ (μs) | $A_1$ (%) | $\tau_2$ (μs) | $A_2$ (%) |
| DBCz-BT | powder | 602 | 227.7 | 58.40 | 504.6 | 41.60 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | nanoparticles | 602 | 53.5 | 62.23 | 203.1 | 37.77 |

[a] Determined from the fitting function of $I(t) = A_1 e^{-t/\tau 1} + A_2 e^{-t/\tau 2}$ according to the luminescence decay curves.

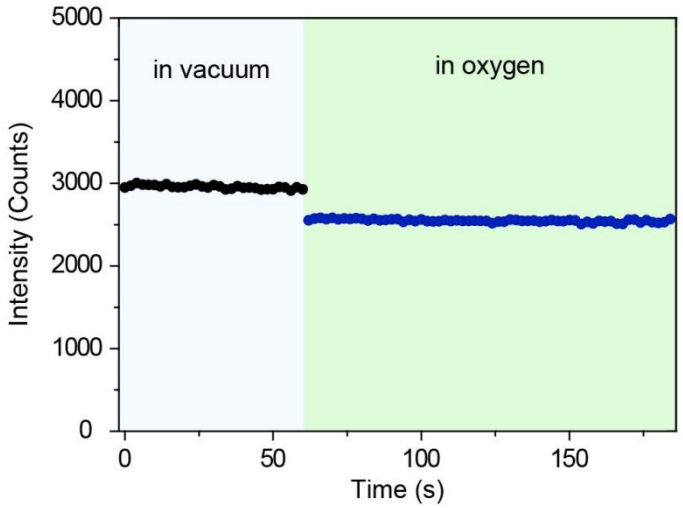



**Figure S5**. The PL intensity tendency of DBCz-BT at 602 nm in solid state under vacuum and oxygen atmospheres with time prolonged.

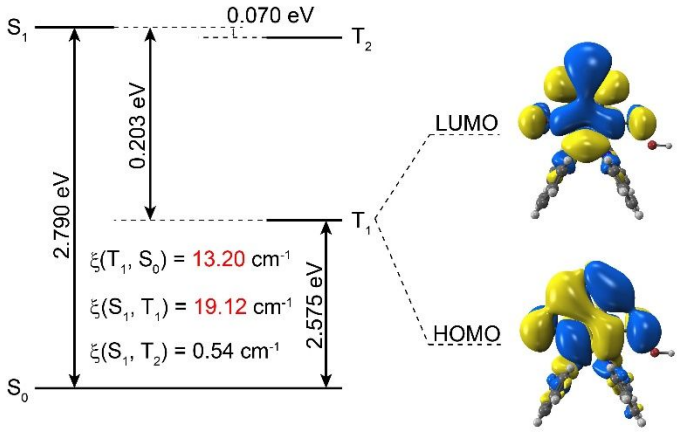



**Figure S6.** Theoretical calculated spin-orbit coupling constant, energy diagram and spin-orbital coupling (ξ) of DBCz-BT model with external heavy atom effect.

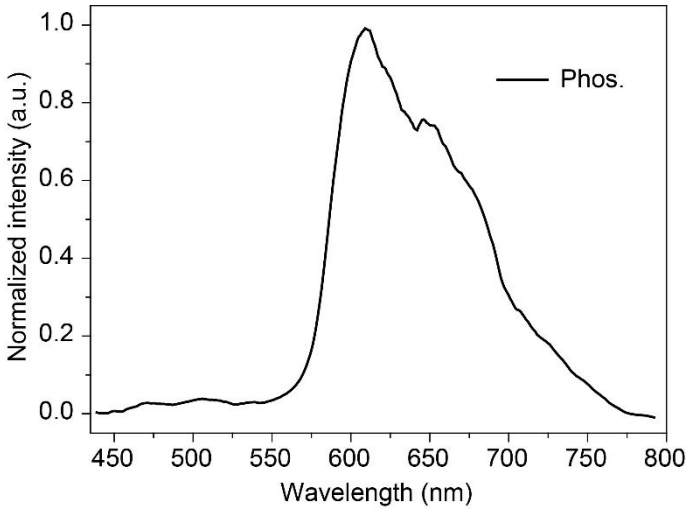



**Figure S7.** Phosphorescence spectrum of the raw material DBF-BT in dilute $CHCl_3$ solution at 77 K (10.0 μM).

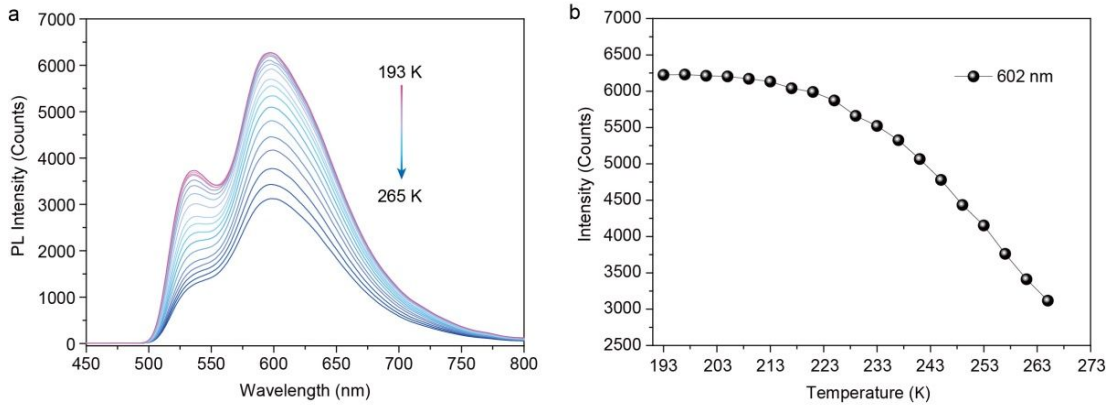



**Figure S8.** (a) Photoluminescence (PL) spectra of DBF-BT phosphorescent nanodots (PNDs) at different temperature. (b) The PL intensity of the emission band at 602 nm with temperature variation.

## Ⅴ. Data of biological application

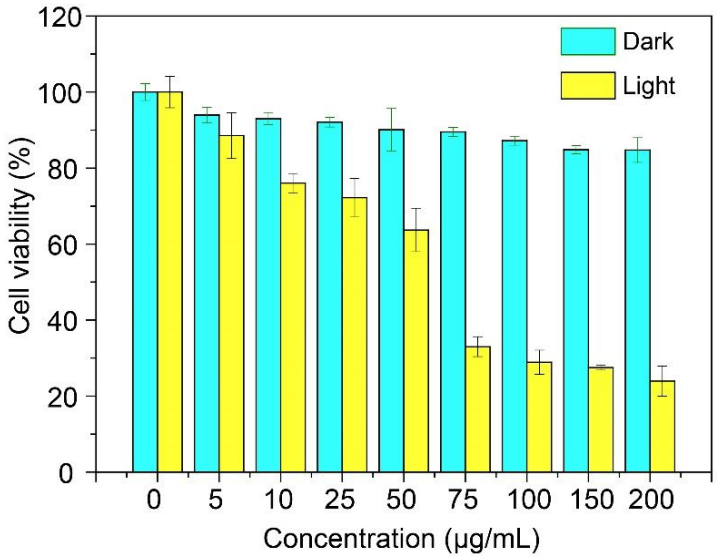



**Figure S9.** MTT cell viability values (%) of HeLa cells incubated with PNDs at various concentrations in dark or under irradiation at 475 nm (Xe lamp: 35 mW $cm^{-2}$) for 20 min.

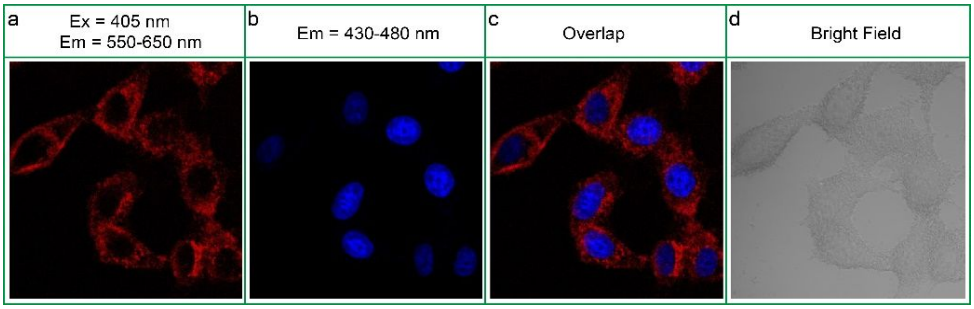


**Figure S10.** Confocal images of HeLa cells collected at a) 550-650 nm, b) 430-480 nm, c) overlap of a) and b) and d) bright field. HeLa cells were incubated with PNDs (75 μg/mL) and Hoechst 33342 (1.0 μg/mL) at 37 °C for 3 h. The excitation wavelength was 405 nm.

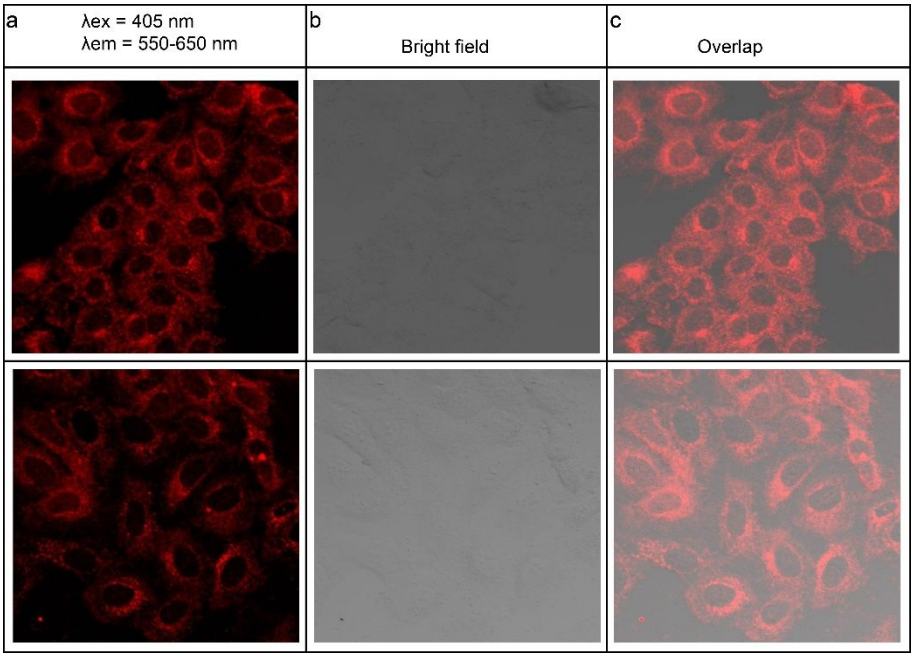



**Figure S11.** Confocal images of HeLa cells collected at a) 550-650 nm, b) bright field and c) overlap. HeLa cells were incubated with PNDs (75 μg/mL) at 37 °C for 3 h. The excitation wavelength was 405 nm.

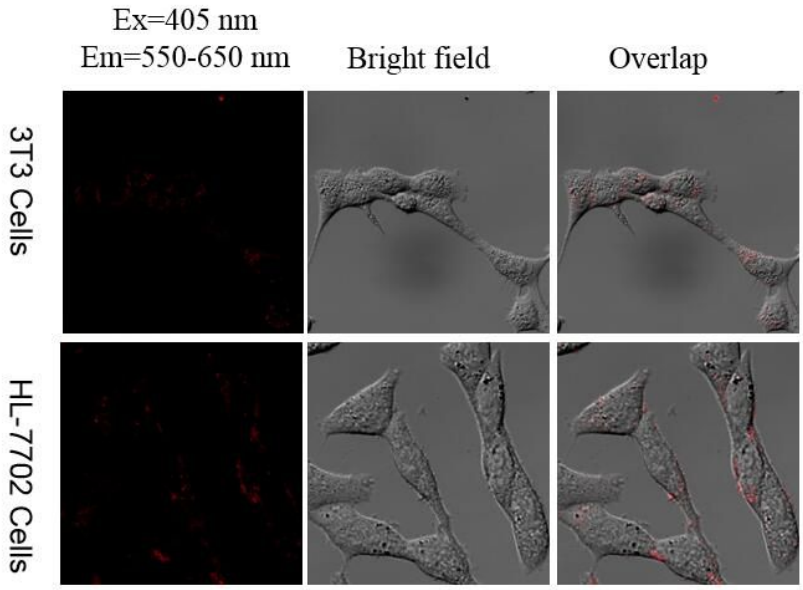



**Figure S12.** Confocal images of two normal cells (3T3 and HL-7702 cells) collected at a) 550-650 nm, b) bright field and c) overlap. Cells were incubated with PNDs (75 μg/mL) at 37 °C for 3 h. The excitation wavelength was 405 nm.

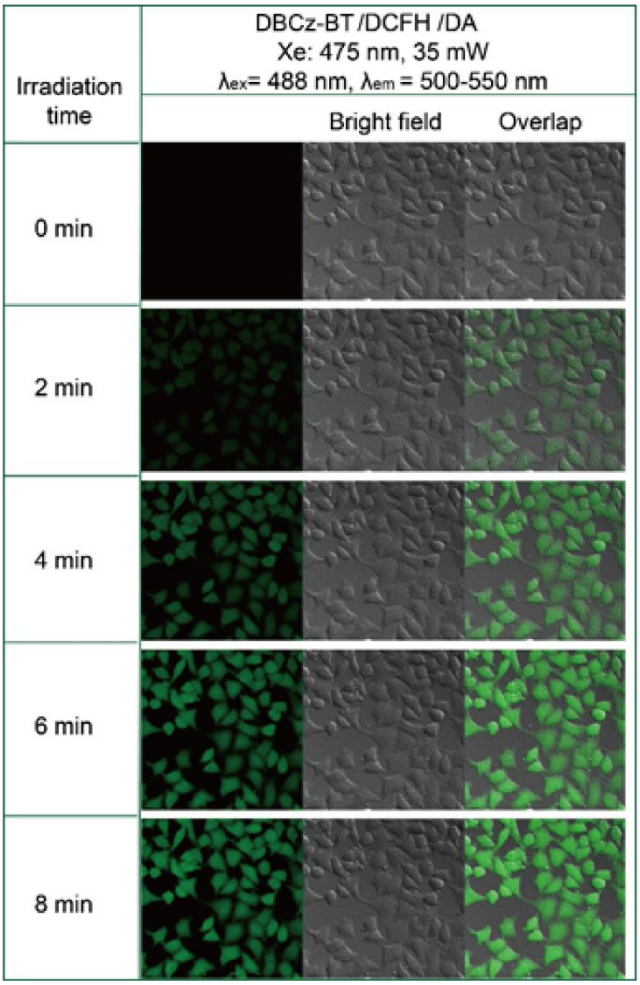



**Figure S13.** Confocal images of HeLa cells collected at 500-550 nm, bright field and overlap under different irradiation time. HeLa cells were incubated with PNDs (75 μg/mL) and DCFH-DA (ROS tracker) at 37 °C for 3 h. The excitation wavelength was 488 nm.

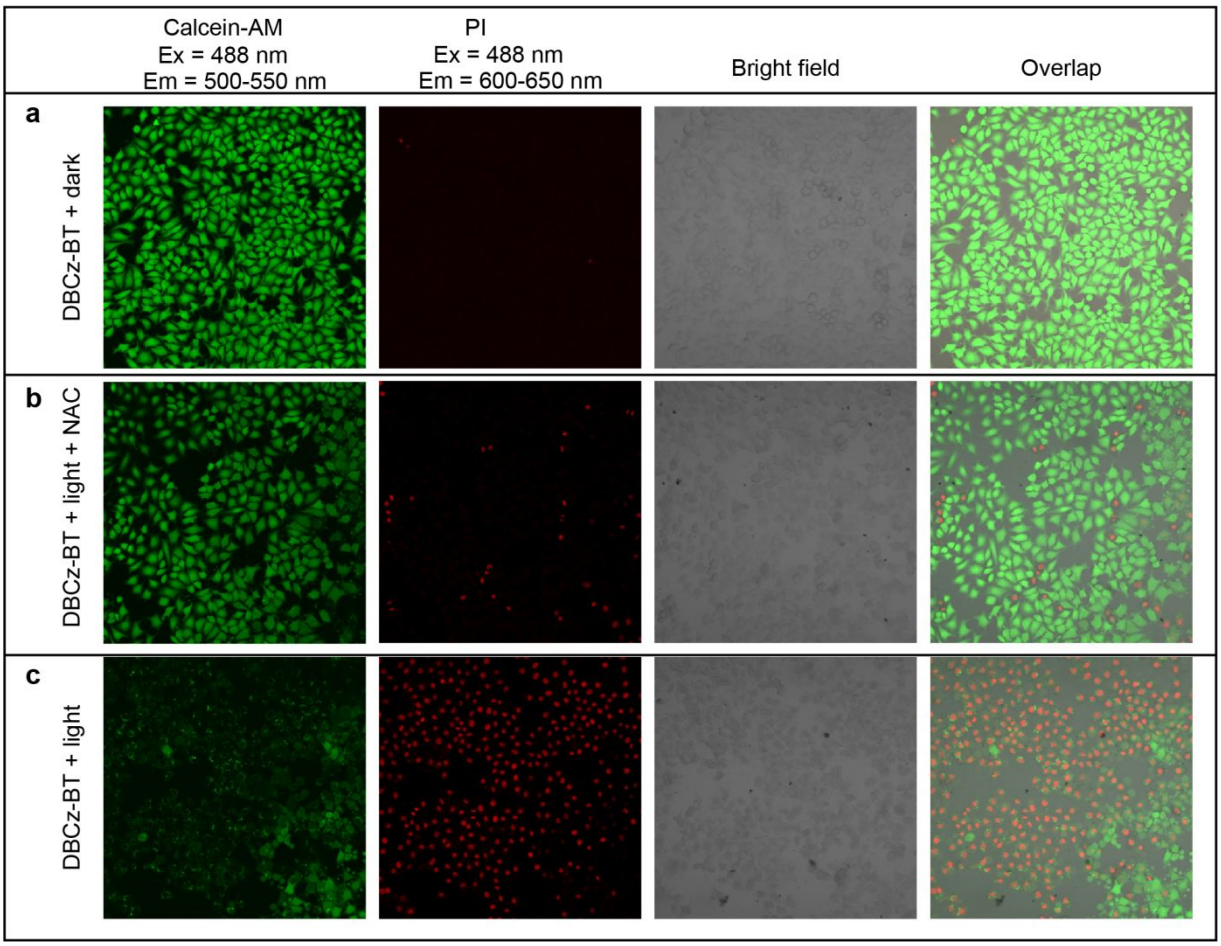



**Figure S14.** Confocal microscopy images of Calcein-AM- and PI-labelled HeLa cells (a) in dark control, (b) incubated with PNDs (75 μg/mL) and NAC under irradiation at 475 nm for 20 min. (c) incubated with PNDs (75 μg/mL) under irradiation at 475 nm for 20 min. Cells were viewed in the green channel for Calcein-AM ($\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 500-550 nm) and red channel for PI ($\lambda_{ex}$ =488 nm, $\lambda_{em}$ = 600-650 nm), respectively.

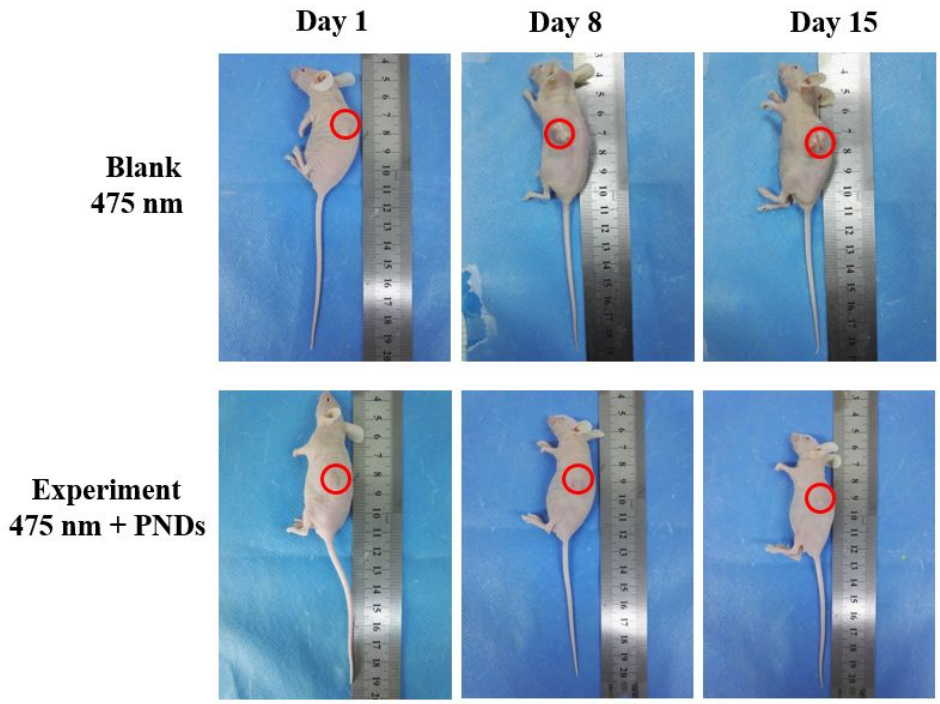



**Figure S15.** Photodynamic anti-cancer effect of the PNDs (150 μg/mL) in vivo.

## VI. References

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