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Supporting Information for

CuS and BODIPY loaded nanoscale covalent organic framework for synergetic photodynamic and photothermal therapy

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Content

1. Materials and Instrumentations

1,3,5-Tris(4-aminophenyl)benzene, 2,5-dimethoxyterephthaldehyde and 10-(4-chlorophenyl)-5,5-difluoro-2-formyl-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide (BODIPY) were purchased from Jilin Province Yanshen Technology Co., Ltd. 1,3-Diphenylisobenzofuran (DPBF) were purchased from TCI (Shanghai) Development Co., Ltd. Benzaldehyde were purchased from Aladdin Reagent Co., Ltd.All reactants were used as purchased without further purification. Acetonitrile, acetic acid, copper acetate monohydrate, thioacetamide, ethylenediamine were purchased from Sinopharm Chemical Reagent Co., Ltd. Ultra-pure water was prepared with an Aquapro System (18 MΩ). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Shanghai) Trading Co. Ltd. Singlet Oxygen Sensor Green (SOSG) was purchased from Thermo Fisher Scientific Inc. Phosphate-Buffered Saline (PBS), Dulbecco's Phosphate-Buffered Saline (DPBS), and Fetal bovine serum (FBS) was purchased from VivaCell (Shanghai, P. R. China). Dulbecco's Modified Eagle Medium (DMEM), Penicillin Streptomycin Mixtures (Pen-Strep), and Trypsin-EDTA Solution (0.25%) were purchased from HyClone Laboratories, Inc. Normocin was purchased from Invivogen (San Diego, CA, USA).

520 nm laser (FC-520-2W-MM) and 1064 nm laser (FC-1064-10W-MM) Fourier transform infrared (FT-IR) spectra were obtained in the 400-4000 cm^{-1} range using a Thermo Scientific Nicolet iS50 FT-IR Spectrometer equipped with diamond attenuated total reflection (ATR) module. Each spectrum was the average of 16 scans. Ultraviolet–visible (UV−vis) absorption spectra were recorded on a Shimadzu UV-2700 Double Beam UV−vis Spectrophotometer. Scanning electron microscopy (SEM) micrographs were recorded on a Hitachi SU8010 Scanning Electron Microscope. Transmission electron microscope (TEM) micrographs were recorded on a Hitachi HT7700 120 kV Compact-Digital Transmission Electron Microscope. Powder X-ray diffraction (PXRD) patterns were obtained on a Rigaku SmartLab SE X-Ray Powder Diffractometer with Cu Kα line focused radiation (λ = 1.5405 Å) from 2θ = 2.00° up to 50.00° with 0.01° increment. Nitrogen adsorption isotherms were measured at 77 K with a Micromeritics ASAP2020 HD88 Surface Area and Porosity Analyser. Before measurement, the samples were degassed in vacuum at 120°C for 12 h. The Brunauer–Emmett–Teller (BET) equation was utilized to calculate the specific surface areas. The pore size distribution was derived from the sorption curve by using the non-local density functional theory (NLDFT) model. Hydrodynamic particle size and zeta potential were measured using Malvern Zetasizer Nano ZS90 System. Laser scanning confocal fluorescence images were captured with a Leica TCS SP8 Confocal Laser Scanning Microscopy with an objective lens (×20). Glass bottom dishes were purchased from

Cellvis (Mountain View, CA, USA). Microplate assays were carried out on a Molecular Devices SpectraMax i3x Multi-Mode Microplate Detection System.

2. Cell Culture and Laboratory Animals

The MCF-7 (human breast adenocarcinoma cell line) was provided by Stem Cell Bank, Chinese Academy of Sciences (Shanghai, P. R. China), and cultured in DMEM supplemented with FBS (10%), Normocin (50 μg/mL), penicillin (100 U/mL) and streptomycin (100 μg/mL) in an atmosphere of CO_2 (5 vol%) and air (95 vol%) at 37°C.

Nude mice (BALB/c-nu, femina, aged 4 weeks, 15–20 g) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. Animal experiments were reviewed and approved by the Ethics Committee of Shandong Normal University, Jinan, P. R. China (approval number AEECSDNU 2021009). All the animal experiments complied with relevant guidelines of the Chinese government and regulations for the care and use of experimental animals.

3. Synthesis of materials

3.1 Synthesis of CuS@COF (2)

TPB–DMTP-COF was prepared according to our previous report.[1] A mixture of TPB-DMTP-COF (17 mg, 0.07 mmol) and copper acetate (10 mg, 0.05 mmol) in aqueous solution (10 mL) was stirred at 80°C for 12 h. The resulting solids were completely washed with water and then redispersed in water (5 mL). After addition of aqueous solution (30 mL) of thioacetamide (207 mg, 2.75 mmol) and ethylenediamine (20 μL), the mixture was stirred at 80°C for additional 12 h. After filtering and completely washing with water, **CuS@COF** (**2**) was obtained as yellow green solids. FT-IR (ATR, cm^{-1}): 3372 (w), 2998 (w), 2946 (w), 2859 (w), 2829 (w), 1682 (m), 1618 (m), 1593 (m), 1505 (m), 1486 (m), 1466 (m), 1409 (s), 1393 (m), 1289 (m), 1211 (s), 1182 (w), 1142 (m), 1039 (m), 1013 (w), 979 (w), 972 (w), 831 (m), 736 (w), 694 (w), 608 (w), 540 (w).

3.2 Synthesis of CuS@COF-BDP (3)

A mixture of 2 (5 mg, 0.02 mmol), BODIPY (7.5 mg, 0.02 mmol) and glacial acetic acid (50 μL, 3 M) in 5 mL ethanol was stirred at 75°C for 24 h. The resulting solids were completely washed with ethanol to generate **CuS@COF-BDP** (**3**) as brick red solids. ICP-OES analysis indicated that the loading amounts of CuS in **CuS@COF-BDP** (**3**) was 3.46 wt%. The ion chromatography analysis indicated that the loading amounts of BODIPY in **CuS@COF-BDP** (**3**) was 15.05 wt%. After immersed in H_2O, PBS, and DMED 72 h, ICP-OES analysis showed the content of CuS in **CuS@COF-BDP** (**3**) were 3.41, 3.34, and 3.40, respectively. FT-IR (ATR, cm^{-1}): 3370 (w), 2949 (w), 2946 (w), 2869 (w), 2834 (w), 1677 (m), 1618 (m), 1592 (m), 1505

(m), 1486 (m), 1466 (m), 1410 (s), 1393 (m), 1374 (m), 1292 (m), 1209 (s), 1182 (w), 1142 (m), 1039 (m), 1013 (w), 979 (w), 972 (w), 831 (m), 736 (w), 696 (w), 608 (w), 538 (w).

3.3 Synthesis of COF-BDP

A mixture of TPB-DMTP-COF (4.9 mg, 0.02 mmol), BODIPY (7.5 mg, 0.02 mmol) and glacial acetic acid (50 μL, 3 M) in 5 mL ethanol was stirred at 75°C for 24 h. The resulting solids were completely washed with ethanol to generate **COF-BDP** as brick red solids. The ion chromatography analysis indicated that the loading amounts of BODIPY in **COF-BDP** was 15.50 wt%. FT-IR (ATR, cm^{-1}): 3365 (w), 2957 (w), 2869 (w), 2834 (w), 1677 (m), 1618 (m), 1594 (m), 1501 (m), 1461 (m), 1410 (s), 1397 (m), 1374 (m), 1294 (m), 1203 (s), 1190 (w), 1142 (m), 1041 (m), 1013 (w), 972 (w), 830 (m), 734 (w), 693 (w), 608 (w), 539 (w).

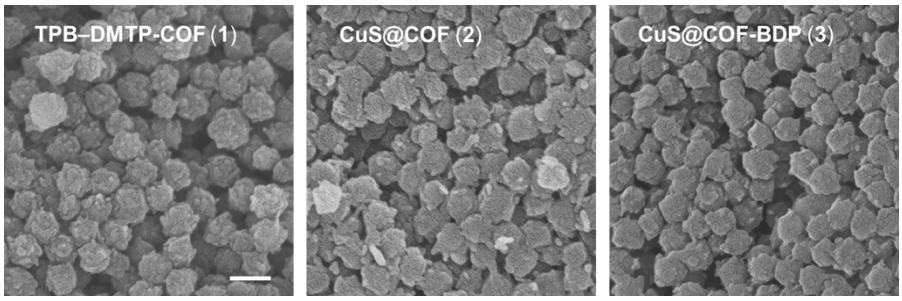

Fig. S1 SEM images of **1**, **2** and **3** (Scale bar, 200 nm).

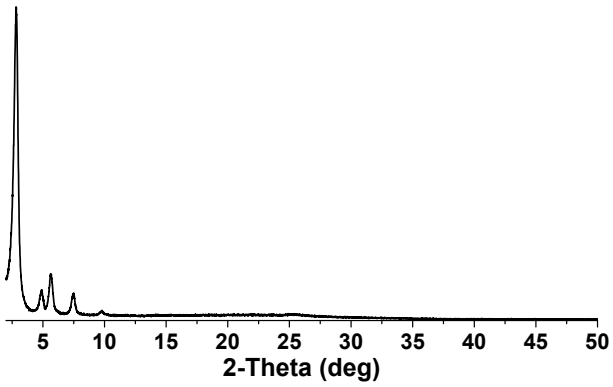

Fig. S2 PXRD of **1.**

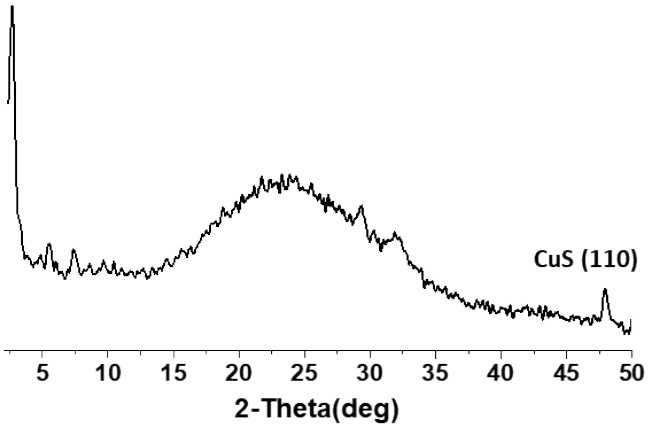

Fig. S3 PXRD of **2.**

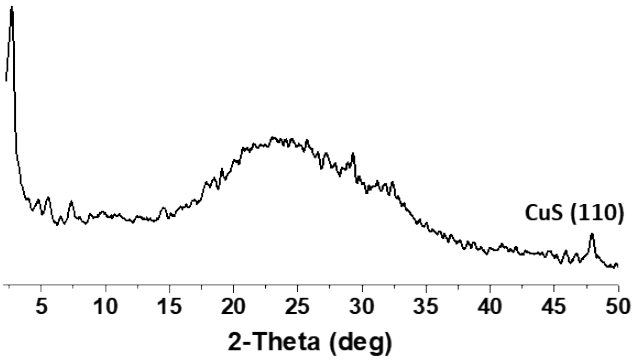

Fig. S4 PXRD of **3.**

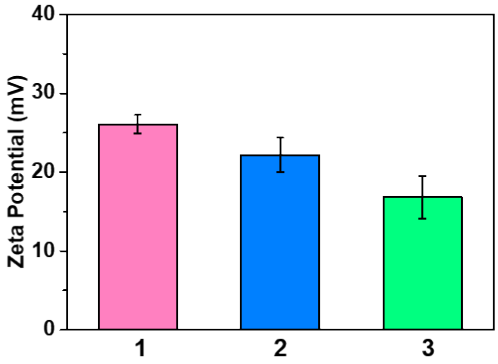

Fig. S5 Zeta potentials of **1**, **2** and **3.**

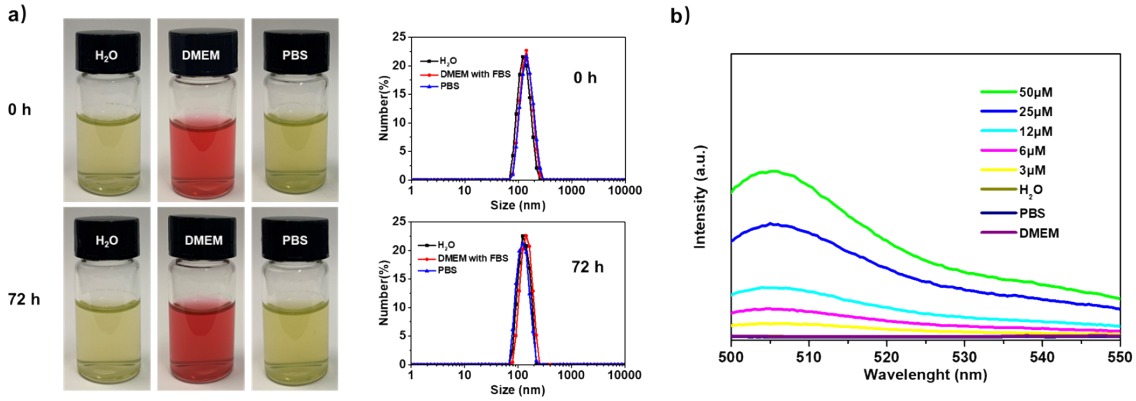

Fig. S6 (a) Stability of **3** under different physiological solutions (H_2O, DMEM with 10% fetal bovine serum, and PBS). (b) Fluorescence spectra of BODIPY with different concentrations and the DMF solution extracted from the supernatant after 72 h.

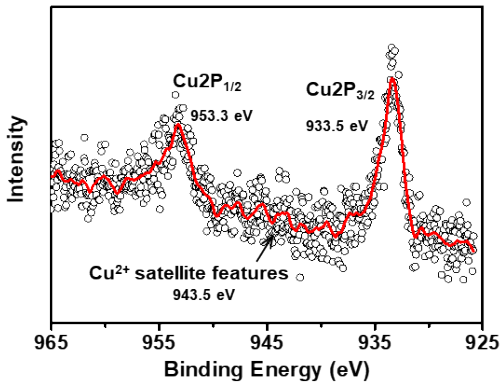

Fig. S7 XPS spectra of Cu in **3.**

3.4 Synthesis of CuS@UiO-68-NH_2-BDP

Nanoscale UiO-68-NH_2 was synthesized according to previous reports.[2] A mixture of UiO-68-NH_2 (17 mg, 0.006 mmol) and copper acetate (10 mg, 0.05 mmol) in aqueous solution (10 mL) was stirred at 80°C for 12 h. The resulting solids were completely washed with water and then redispersed in water (5 mL). After addition of aqueous solution (30 mL) of thioacetamide (207 mg, 2.75 mmol) and ethylenediamine (20 μL), the mixture was stirred at 80°C for additional 12 h. After filtering and completely washing with water, CuS@UiO-68-NH_2 was obtained. FT-IR (ATR, cm^{-1}): 3355 (s), 2968 (w) 2921 (w), 2851 (w), 1668 (w), 1610 (w), 1590 (s), 1550 (m), 1505 (w), 1393 (w), 1153 (w), 641 (w).

A mixture of CuS@UiO-68-NH_2 (5 mg, 0.01 mmol-NH_2 equiv.), BODIPY (7.5 mg, 0.02 mmol) and glacial acetic acid (50 μL, 3 M) in 5 mL ethanol was stirred at 75°C for 24 h. The resulting solids were completely washed with ethanol to generate CuS@UiO-68-NH_2-BDP. ICP-OES analysis indicated that the loading amounts of CuS in CuS@UiO-68-NH_2-BDP was 6.59

wt%. The ion chromatography analysis indicated that the loading amounts of BODIPY in CuS@UiO-68-NH_2-BDP was 11.30 wt%. FT-IR (ATR, cm^{-1}): 3372 (s), 2962 (w), 2924 (m), 2850 (w), 2362 (w), 2316(w), 1648 (m), 1555 (s), 1458 (s), 1412 (s), 1132 (w), 1024 (w), 643 (m), 527 (w).

3.5 Synthesis of CuS@SiO_2-NH_2-BDP

Nanoscale SiO_2-NH_2 was purchased from Macklin Inc. (Product No: M875463). A mixture of SiO_2-NH_2 (4 mg, 0.06 mmol) and copper acetate (10 mg, 0.05 mmol) in aqueous solution (10 mL) was stirred at 80 °C for 12 h. The resulting solids were completely washed with water and then redispersed in water (5 mL). After addition of aqueous solution (30 mL) of thioacetamide (207 mg, 2.75 mmol) and ethylenediamine (20 μL), the mixture was stirred at 80 °C for additional 12 h. After filtering and completely washing with water, CuS@SiO_2-NH_2 was obtained. FT-IR (ATR, cm^{-1}): 3374 (w), 1203 (m), 1094 (s), 953 (m), 800 (m).

A mixture of CuS@SiO_2-NH_2 (58 mg, 0.01 mmol-NH_2 equiv.), BODIPY (7.5 mg, 0.02 mmol) and glacial acetic acid (50 μL, 3 M) in 5 mL ethanol was stirred at 75 °C for 24 h. The resulting solids were completely washed with ethanol to generate CuS@SiO_2-NH_2-BDP. ICP-OES analysis indicated that the loading amounts of CuS in CuS@SiO_2-NH_2-BDP was 5.15 wt%. The ion chromatography analysis indicated that the loading amounts of BODIPY in CuS@SiO_2-NH_2-BDP was 9.36 wt%. FT-IR (ATR, cm^{-1}): 3372 (w), 2927 (m), 1662 (m), 1624 (w), 1309 (m), 1203 (m), 1093 (s), 954 (m), 800 (m),763 (w), 695 (w), 535 (w).

3.5 Synthesis of CuS@Polystyrene-NH_2-BDP

Nanoscale polystyrene-NH_2 was purchased from Macklin Inc. (Product No: L815949). A mixture of polystyrene-NH_2 (6 mg, 0.06 mmol) and copper acetate (10 mg, 0.05 mmol) in aqueous solution (10 mL) was stirred at 80°C for 12 h. The resulting solids were completely washed with water and then redispersed in water (5 mL). After addition of aqueous solution (30 mL) of thioacetamide (207 mg, 2.75 mmol) and ethylenediamine (20 μL), the mixture was stirred at 80°C for additional 12 h. After filtering and completely washing with water, CuS@Polystyrene-NH_2 was obtained. FT-IR (ATR, cm^{-1}): 3727 (w), 3370 (w), 3085 (w), 3051 (w), 3025 (w), 2919 (w), 2362 (m), 2332 (m), 1724 (m), 1597 (w), 1443 (m), 1112 (m), 750 (m), 691 (m), 533 (m).

A mixture of CuS@Polystyrene-NH_2 (57 mg, 0.01 mmol-NH_2 equiv.), BODIPY (7.5 mg, 0.02 mmol) and glacial acetic acid (50 μL, 3 M) in 5 mL ethanol was stirred at 75°C for 24 h. The resulting solids were completely washed with ethanol to generate CuS@Polystyrene-NH_2-BDP. ICP-OES analysis indicated that the loading amounts of CuS in CuS@Polystyrene-NH_2-BDP was 2.28 wt%. The ion chromatography analysis indicated that the loading amounts of BODIPY in

CuS@Polystyrene-NH_2-BDP was 8.33 wt%. FT-IR (ATR, cm^{-1}): 3726 (w), 3371 (w), 3084 (w), 3052 (w), 3023 (w), 2973 (w), 2917 (w), 2361 (m), 2333 (m), 1724 (m), 1663 (m), 1624 (w), 1597 (w), 1443 (m), 1310 (m), 1113 (m), 764 (w), 751 (m), 698 (w), 692 (m), 532 (m).

4. Photodynamic Property

The dispersion of **3** (2 mL, 100 μg/mL) and DPBF DMF solution (100 μL, 1 mM) were added in a quartz dish and irradiated with a 520 nm laser (50 mW/cm^2) or/and a 1064 nm laser (1.5 W/cm^2) for 7 min. The absorbance of DPBF at 414 nm in the mixture was recorded at 1 min intervals. The $^1O_2$ generation rate was determined from the reduced the absorbance over time. To characterize the difference in the rate of $^1O_2$ introduced by different lasers, the absorbance of DPBF at 414 nm were calculated. The dispersion of **3** (2 mL, 100 μg/mL) was used as the reference for this UV−vis measurement.

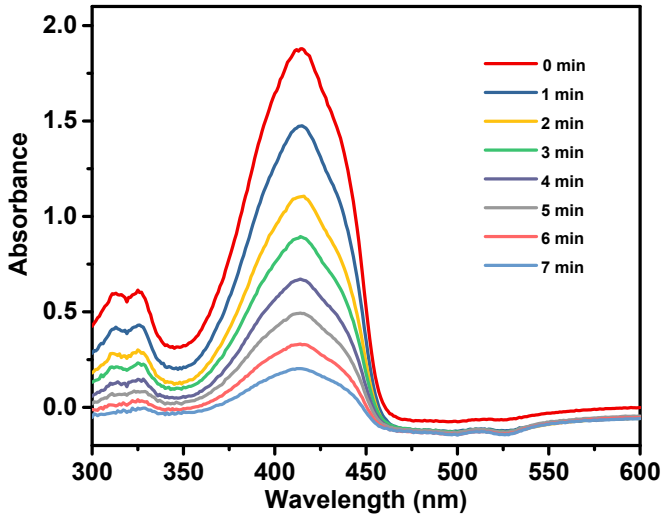

Fig. S8 UV−vis spectra of DPBF induced by **3** under a 520 nm laser.

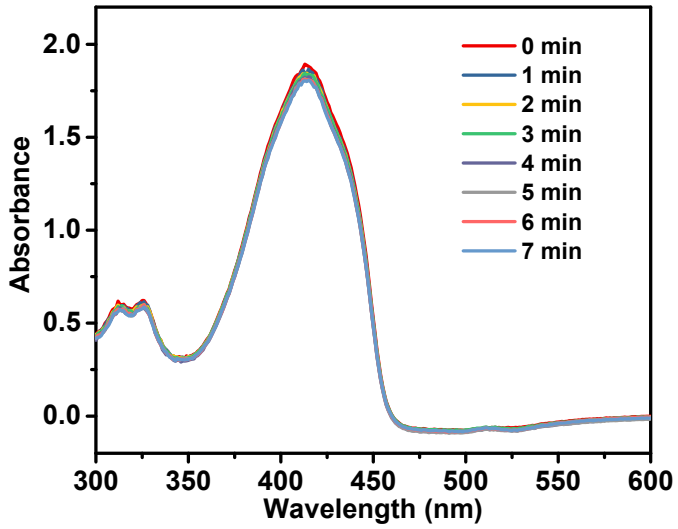

Fig. S9 UV−vis spectra of DPBF induced by **3** under a 1064 nm laser.

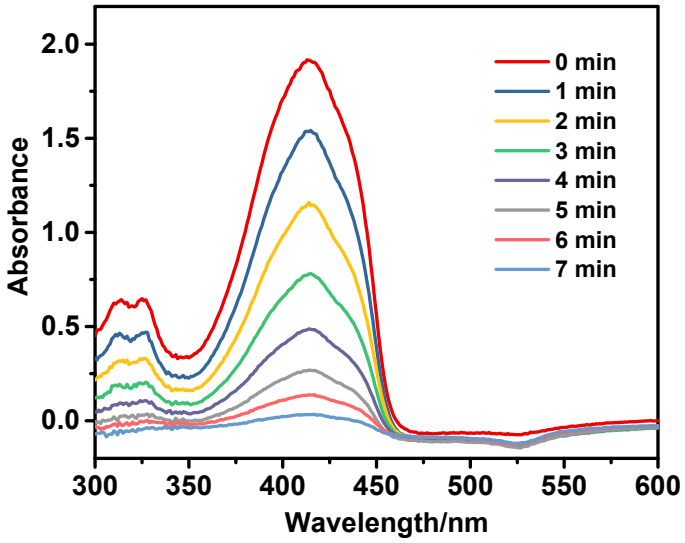

Fig. S10 UV−vis spectra of DPBF induced by **3** under 520 nm and 1064 nm laser.

5. Photothermal Conversion Efficiency

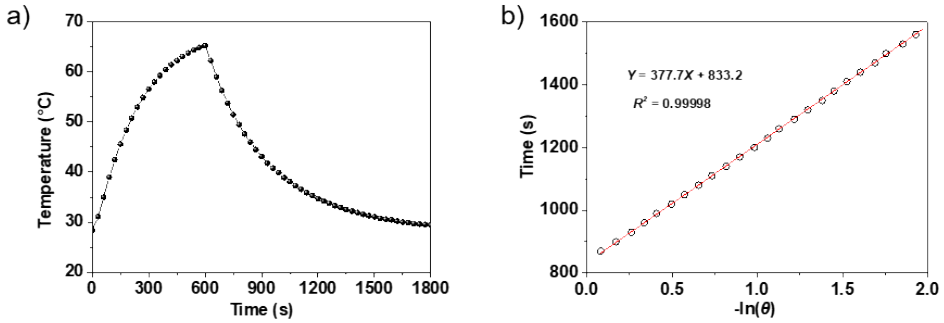

Fig. S11 (a) After 10 min of 1064 nm laser irradiation (1.1 W/cm^2) and natural cooling to room temperature, **3** (600 μg/mL) temperature rise was observed. (B) t – (- ln θ) curve of natural cooling period.

The PBS dispersion of **3** (1 mL, 0–600 μg/mL) was added in a quartz dish and irradiated with an 1064 nm nm laser (0–1.1 W/cm^2) for 10 min. Then, the laser was turned off to allow the dispersion to cool naturally. The temperature of the dispersion was recorded at 30 s intervals. The photothermal conversion efficiency was calculated according to the following formulas:

$$\eta = \frac{Q_s - Q_w}{I\left(1 - 10^{-A1064}\right)}$$

$$Q_s = hS\Delta T_{s,h}$$

$$Q_w = hS\Delta T_{w,h}$$

$$hS = \frac{mc}{\tau}$$

$$\tau = -\frac{dt}{dln\,\theta}$$

$$\theta = \frac{T_{t,c} - T_{min,c}}{\Delta T_{s,c}}$$

η, photothermal conversion efficiency; $A_{1064\ nm}$, the absorption of solution at 1064 nm; I, the power of the laser; $\Delta T_{s,h}$, the changed temperature of solution in the heating curve; $\Delta T_{w,h}$, the changed temperature of water in the heating curve; c, specific heat capacity of water; m, solution mass; τ, slope of t – (-ln θ)graph; t, time in the cooling curves; $\Delta T_{s,c}$, the changed temperature of solution in the cooling curve; $T_{min,\,c}$, the final temperature of solution in the cooling curve; $T_{t,c}$, the temperature of solution at different times in the cooling curve.

6. The effect of PDT and PTT in 3 and other nanomaterials

For PTT: **3** (1 mL, 600 μg/mL, 0.22 μmol CuS equiv.), or CuS@UiO-68-NH_2-BDP (1 mL, 315 μg/mL, 0.22 μmol CuS equiv.), or CuS@SiO_2-NH_2-BDP (1 mL, 403 μg/mL, 0.22 μmol CuS equiv.) or CuS@Polystyrene-NH_2-BDP (1 mL, 911 μg/mL, 0.22 μmol CuS equiv.), in PBS was

added in a quartz dish and irradiated with a 1064 nm laser (1.1 W/cm^2) for 15 min. The temperature of the dispersion was recorded at 30 s intervals.

For PDT: **3** (2 mL, 100 μg/mL, 0.078 μmol BODIPY equiv.), or CuS@UiO-68-NH_2-BDP (2 mL, 133 μg/mL, 0.078 μmol BODIPY equiv.), or CuS@SiO_2-NH_2-BDP (2 mL, 161 μg/mL, 0.078 μmol BODIPY equiv.) or CuS@Polystyrene-NH_2-BDP (2 mL, 180 μg/mL, 0.078 μmol BODIPY equiv.) in DMF solution of DPBF (100 μL, 1 mM) was added in a quartz dish and irradiated with a 520 nm laser (50 mW/cm^2) for 7 min. The absorbance of DPBF at 414 nm in the mixture was recorded at 1 min intervals. The $^1O_2$ generation rate was determined from the reduced the absorbance over time.

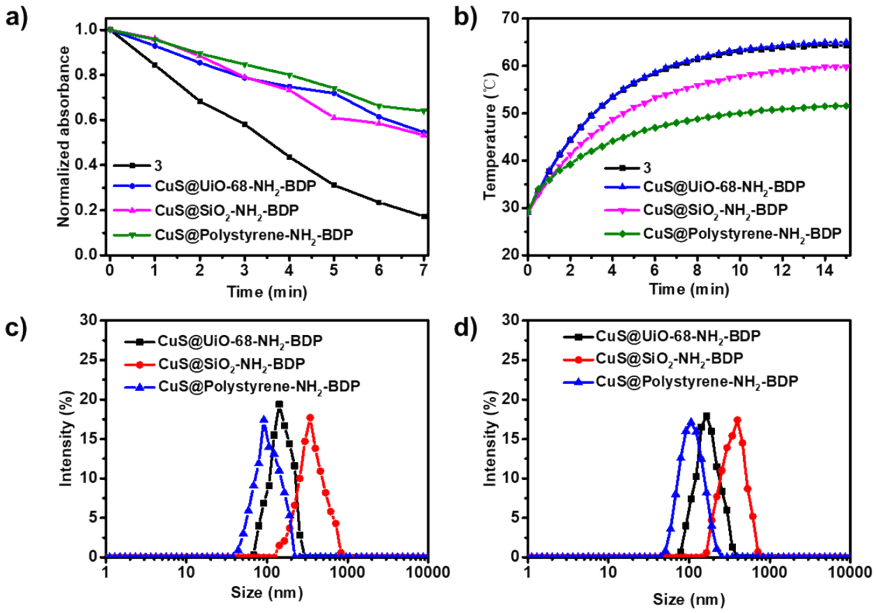

Fig. S12. PDT (a) and PTT (b) effects of **3** and other nanomaterials. The stability of CuS@UiO-68-NH_2-BDP, CuS@SiO_2-NH_2-BDP, and CuS@Polystyrene-NH_2-BDP in PBS (c) and H_2O (d).

7. Cell uptake and subcellular localization

Cells were seeded into glass bottom dishes and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DPBS dispersion of **3** (200 μL, 20 μg/mL) for 2 h in a CO_2 incubator, and washed with DPBS twice carefully. After additional 4 h incubation, cells were incubated with Hoechst 33258 (200 μL, 10 μM) for an additional 10 min, and washed with DPBS twice. Finally, the laser scanning confocal fluorescence images were captured. The green images of **3** were excited by 488 nm light, and the emission wavelength range was collected at 525±20 nm. The red images of nucleus were excited by 405 nm light, and the emission wavelength range was collected at 460±20 nm. Controls were

conducted to make sure images were free of crosstalk. Colocalization was analyzed by ImageJ software.

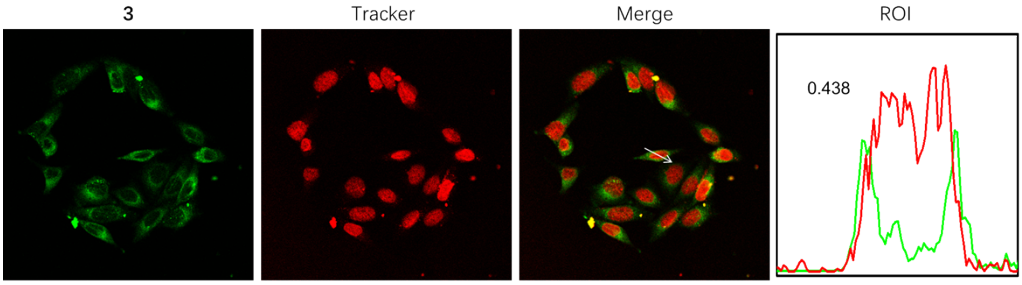

Fig. S13 Cell uptake and nuclei subcellular localization of **3** in MCF-7 cells.

8. Intracellular Singlet Oxygen Measurement

The cells were seeded in a glass bottom culture dish and cultured overnight in a carbon dioxide incubator. After removing the culture medium, **3** (200 μL, 10 μg/mL) dispersion in PBS was used to culture in CO_2 incubator for 2 h, and washed twice with PBS. Then, the cells were incubated with SOSG (200 μL, 5 μM) for 15 minutes and washed twice with PBS. For PDT, cells were exposed to 520 nm laser (50 mW / cm^2, 0, 2 min and 5 min) for different time, and the yield of singlet oxygen was observed. Finally, the laser scanning confocal fluorescence image is obtained. The green image is excited by 488 nm light, and the emission wavelength range is collected at 525 ± 20 nm. Control to ensure that the image is free of crosstalk.

9. In Vitro Antitumor Therapy

Cells were seeded into 96-well plates with a cell number of ~5k cells/well and incubated overnight in a CO_2 incubator. After removal of the culture medium, the cells were incubated with DPBS dispersion of **2** (100 μL, 0–200 μg/mL), or **3** (100 μL, 0–200 μg/mL) or **COF-BDP** (100 μL, 0–200 μg/mL) for 2 h in a CO_2 incubator. For PTT, the cells were exposed to 1064 nm laser (1.5 W/cm^2, 5 min). For PDT, the cells were exposed to 520 nm laser (50 mW/cm^2, 5 min). After additional 24 h incubation, MTT (10 μL, 5 mg/mL) was added to each well and incubated for additional 4 h in a CO_2 incubator. Finally, the supernatants were removed and DMSO (100 μL) was added into each well, followed by recording the absorbance at 490 nm.

10. In Vivo Antitumor Therapy

MCF-7 cancer cells (10^6 cells) suspended in DPBS (100 μL) were subcutaneously injected into the flanks of each mice to establish MCF-7 xenograft model. Length (L) and width (W) of the tumor were determined by digital calipers. The tumor volume (V) was calculated by the formula $V = 1/2 \times L \times W^2$. When the tumor size reached ~150 mm^3, the nude mice bearing MCF-7 tumors (n = 30) were randomly distributed into 6 groups (Table S1). After intratumoral injection, the nude mice were feeding for 4 h, and for the treatment group, light treatment was performed on

the tumor site. The mice continued to be fed for 15 days. The tumor volume and nude mouse body weight were recorded daily during the experimental period.

Table S1. Treatment plan of PDT and PTT experiments *in vivo*.

	Group	Injection	Concentration	Volume	520 nm Laser	1064 nm Laser
i	Control	DPBS	—	100 μL	—	—
ii	Laser	DPBS	—	100 μL	10 min, 50 mW/cm^2	10 min, 1.1 W/cm^2
iii	Dark	DPBS dispersion of material	600 μg/mL	100 μL	—	—
iv	PDT	DPBS dispersion of material	600 μg/mL	100 μL	10 min, 50 mW/cm^2	—
v	PTT	DPBS dispersion of material	600 μg/mL	100 μL	—	10 min,1.1 W/cm^2
vi	PDT+PTT	DPBS dispersion of material	600 μg/mL	100 μL	10 min, 50 mW/cm^2	10 min, 1.1 W/cm^2

11. References

[1] Q. Guan, L.-L. Zhou, Y.-A. Li, W.-Y. Li, S. Wang, C. Song and Y.-B. Dong, *ACS Nano*, 2019, **13**, 13304–13316.

[2] W.-H. Chen, G.-F. Luo, Y. S. Sohn, R. Nechushtai and I. Willner, *Adv. Funct. Mater.*, 2019, **29**, 1805341.