Supporting Information

Photochromic upconversion nanoarchitecture: towards activatable bioimaging and dual NIR light-programmed singlet oxygen generation

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Experimental Section

Materials: All the chemicals were used as received without further purification. Rare Earth Oxides, oleylamine (OM, 90%), and 1-octadecene (ODE, 95%) were purchased from Acros. Oleic acid (OA, 90%), and trifluoroacetic acid (99%) were bought from Sigma-Aldrich. 5,10,15,20-Tetrakis (4-carboxyphenyl)porphyrin (H_2TCPP) was purchased from TCI. Diarylethene (DAE) molecule used in this work was synthesized according to our previous reports.[1] The water used throughout the experiments was Millipore water (18.2 MΩ). All the experiments were conducted at room temperature unless mentioned otherwise.

Instrumentations: The UV-vis spectra were obtained from a Hitachi 5300 spectrophotometer (Hitachi Co. Ltd., Japan). Fluorescence spectra were collected using a Hitachi F-4600 fluorimeter (Hitachi Co. Ltd., Japan) with Xe lamp as the excitation source at room temperature. The upconversion luminescence spectra (UCL) were recorded on a Hitachi F-4600 spectrometer (Hitachi Co. Ltd., Japan) with 980 nm NIR laser as the excitation source at room temperature. Fluorescent lifetime was recorded on a Delta flex Ultra Fast lifetime Spectrofluorometer (Horiba Jobinyvon IBH Inc., U.K.) and FLS980 steady-state and time-resolved fluorescence spectrometer (Edinburgh Instruments, U.K.). The singlet oxygen lifetime was measured on a Fluorolog-3-2ultrafast spectrometer (Horiba Scientific, USA) equipped with pulse laser (SpectraLED350) and photomultiplier tube (H10330 NIR) (Solvent: DMSO, Ex: 355 nm, Em: 1270 nm). Transmission electron microscopic (TEM) images were obtained using a Hitachi HT-7700 (Hitachi Co. Ltd., Japan) transmission electron microscope. Confocal microscopic images were obtained using a Zeiss LSM 710 confocal microscope at 63× magnification. Cell viability data were obtained using a Biotech Epoch2 microreader. The in vivo fluorescence images were acquired with IVIS® Spectrum in vivo imaging system (PerkinElmer Inc., Waltham, Massachusetts).

Synthesis of $NaGdF_4$: UCNPs were synthesized using the thermal decomposition method combined with a seed-mediated shell growth strategy.[2-4] Metal-trifluoroacetate precursors including 1 mmol CF_3COONa and 1 mmol $Gd(CF_3COO)_3$ were added to a three-necked flask (100 mL) containing a mixed solution of OA (10 mmol), OM (10 mmol) and ODE (20 mmol). Then the solution was heated to 120 ℃ with vigorous stirring under vacuum and then heated to 310 ℃ and maintained at this temperature for 50 min under N_2 atmosphere. After cooling to room temperature, the products were collected by centrifugation, and then re-dispersed in 10 mL of cyclohexane. A 5 mL of the as-prepared colloidal solution was added into a mixture of OA (20 mmol) and ODE (20 mmol) containing extra metal-trifluoroacetate precursors (0.5 mmol CF_3COONa, 0.5 mmol $Gd(CF_3COO)_3$). The solution was heated to 120 ℃ and kept for 0.5 h under vacuum to remove cyclohexane, water and oxygen. The resulted clear solution was heated to 310 ℃ and maintained for 50 min under N_2 atmosphere. After cooling to room temperature, the $NaGdF_4$ UCNPs were precipitated out by the addition of ethanol, collected by centrifugation, and re-dispersed in 10 mL of cyclohexane.

Synthesis of $NaGdF_4$@$NaGdF_4$:Yb,Er core-shell UCNPs: 2.5 mL of the as-prepared $NaGdF_4$ UCNPs colloidal solution, used as seeds for epitaxial growth, was added into a mixture of OA (20 mmol) and ODE (20 mmol) containing precursors of shell (1 mmol CF_3COONa, 0.78 mmol $Gd(CF_3COO)_3$, 0.2 mmol $Yb(CF_3COO)_3$, and 0.02 mmol $Er(CF_3COO)_3$). The solution was heated to 120 ℃ and kept for 0.5 h under vacuum to remove cyclohexane, water and oxygen. The resulted clear solution was heated to 310 ℃ and kept for 50 min under N_2 atmosphere. After cooling to room temperature, the core-shell UCNPs were precipitated out by the addition of ethanol, collected by centrifugation, and stocked in 10 mL of cyclohexane.

Synthesis of $NaGdF_4$@$NaGdF_4$:Yb,Er@$NaGdF_4$ sandwich-structure UCNPs: 5 mL of the as-prepared $NaGdF_4$@$NaGdF_4$:Yb,Er core-shell UCNPs colloidal solution, used as seeds for epitaxial growth, was added into a mixture of OA (20 mmol) and ODE (20 mmol) containing precursors of shell (0.5 mmol CF_3COONa, 0.5 mmol $Gd(CF_3COO)_3$). The solution was heated to 120 ℃ and kept for 0.5 h under vacuum to remove cyclohexane, water and oxygen. The resulted clear solution was heated to 310 ℃ and kept for 50 min under a N_2 atmosphere. After cooling to room temperature, the core-shell-shell UCNPs were precipitated out by the addition of ethanol, collected by centrifugation, and stocked in 10 mL of cyclohexane.

Synthesis of the TPS: TPS was synthesized according to previous reports.[5] Typically, APTES (15 μmol) and TCPP (3.75 μmol) were dissolved in DMF, EDC (15 μmol) and NHS (15 μmol) were added to this solution. The mixture was stirred for 24 h at room temperature to form TPS precursors via the amidation reaction between the amino group of APTES and the carboxylic acid groups of TCPP.

Synthesis of UC@PS: Typically, 400 μL cyclohexane solution of UCNPs was mixed with 20 mL water containing 100 mg CTAB. The cyclohexane solvent was evaporated under vigorous stirring at room temperature overnight, leading to a transparent solution of CTAB-coated UCNPs. This solution was mixed with 40 mL water, 6 mL ethanol and 100 μL NaOH solution (2 M). The mixture was heated up to 60 °C under stirring. Then, a mixture of TEOS (80 μL) in 1 mL ethanol and the as-prepared TPS was added dropwise. This mixture was kept at 60 °C for 1.5 h under stirring. The transparent solution became a bit opaque. The resulted purple solid was obtained through centrifugation and washed thoroughly with ethanol. The surfactants CTAB (template) were removed from the nanopores *via* a fast and efficient ion exchange method. Typically, the as-prepared UC@PS (20 mg) were transferred to the 50 mL ethanol solution containing 0.3 g NH_4Cl, which was then stirred vigorously at 60 °C for 2 h. Finally, the resulted UC@PS were collected through centrifugation, washed with ethanol, and stocked in ethanol for further use.

Preparation of UC@PS/C-DAE: UC@PS (4 mg) was added to 500 μL DMF containing C-DAE solid (1 mg). After stirring for 24 h, the UC@PS/C-DAE was collected by centrifugation and gently washed with DMF. During the collection and washing of UC@PS/C-DAE, all the supernatants were collected for calculating the amount of C-DAE that are unencapsulated in the NPs. The C-DAE concentrations were determined by using UV-vis spectrometry at 338 nm through the standard curve (Fig. S5). Then, the loading efficiency (LE) and loading content (LC) of C-DAE were calculated using the following formula: $LE = ((M_i-M_u)/M_i) \times 100\ \%$; $LC = ((M_i-M_u)/(M_i-M_u+M)) \times 100\ \%$, where M_i is the total quantity of C-DAE added initially during the encapsulation, M_u is the total amount of C-DAE unencapsulated in the NPs, and M is the total quantity of UC@PS added during the encapsulation. The LE and LD of C-DAE was calculated to be 35% and 8 wt%, respectively.

SOSG assay: Typically, 10 μL of SOSG stock solution (0.5 mM) was added into 1 mL of UC@PS water solution (100 μg/mL). The solution was kept in the dark or irradiated with 980 NIR lights. For 980 nm treatment, the solution was irradiated with 980 nm laser (1.2 W/cm^2) for various times. For 808 nm + 980 nm treatment, the solution was firstly irradiated with 808

nm laser (0.25 W/cm^2) for 30 s, and then irradiated with 980 nm laser (1.2 W/cm^2) for various times. The fluorescence of SOSG was measured to evaluate $^1O_2$ production.

NIR-activated imaging in live cells by confocal microscopy: Murine breast cancer cells 4T1 (from ATCC) were cultured in RPMI 1640 supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. For confocal imaging, 4T1 cells were seeded in 35 mm glass-bottom confocal dish (2 × 10^5 cells/well) and incubated for 24 h. RPMI 1640 medium was replaced with 500 μL Opti-MEM medium containing UC@PS or UC@PS/C-DAE (100 μg/mL). After 4 h incubation, the cells were washed thoroughly with PBS for 5 times, and 500 μL Opti-MEM medium was replenished. For the photoactivation, the cells were irradiated with 808 nm laser for 3 min for activation (0.5 W/cm^2). The treated cells were collected for fluorescence imaging.

Detection of intracellular $^1O_2$ generation by confocal microscopy: 4T1 cells were seeded and cultured in 35 mm glass-bottom confocal dish (2 × 10^5 cells/well) for 24 h. The medium was replaced with Opti-MEM medium containing UC@PS or UC@PS/C-DAE at an UC@PS dose of 100 μg/mL. After 4 h incubation in the dark, cells were washed to remove noninternalized materials. Fresh culture medium containing DCF-DA (20 μM) was added for another 20 min incubation in the dark. For the photoactivation, the cells were irradiated with 808 nm laser for 3 min for activation (0.5 W/cm^2). For the $^1O_2$ generation, the cells were irradiated with 980 nm laser for 20 min (1.2 W/cm^2, 5 min break after 1.5 min irradiation). The post treatment cells were collected for fluorescence imaging.

Fluorescence quantification with flow cytometry: Cells were seeded in 6-well plate (2 × 10^5 cells/well). After 24 h incubation, cells were treated with different treatments as depicted in the parts of confocal imaging. Then, the cells were trypsinized with 300 μL trypsin for 3 minutes, and neutralized with 600 μL RPMI 1640 medium containing 10% FBS. Cells were centrifuged at 1500 rpm for 3 min, and washed twice with PBS for flow cytometry assay.

Cell viability test: Cells were seeded in 96-well plate (2 × 10^4 cells/well). After 24 h incubation, cells were treated with different concentration (25, 50, 75, 100 μg/mL) of UC@PS for 4 h, and then washed and replaced with fresh culture medium. For each concentration, cells were treated without or with 980 nm irradiation for 20 min (1.2 W/cm^2, 5 min break after 1.5 min irradiation). After these treatments, cells were maintained for another 24 h. Finally, 100 μL fresh culture medium containing 10% CCK-8 was added to each well. After 1 h incubation, the absorbance was measured at 450 nm using the microplate reader. Cells without any treatment was set as negative control.

For validation the 808 nm light activatable PDT, cells were treated with UC@PS or UC@PS/C-DAE at an UC@PS dose of 100 μg/mL for 4 h, and then washed and replaced with fresh culture medium. NIR treatment was performed with 980 nm or 808 nm + 980 nm laser irradiation (808 nm laser for 3 min; 980 nm laser for 20 min, 1.2 W/cm^2, 5 min break after 1.5 min irradiation). After the treatments, cells were maintained for another 24 h. Finally, 100 μL fresh culture medium containing 10% CCK-8 was added to each well. After 1 h incubation, the absorbance was measured at 450 nm using the microplate reader. Cells without any treatment was set as negative control.

***In vivo* imaging:** All animal experiments were performed in strict accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of National Center for Nanoscience and Technology (NCNST). All animal protocols were approved by IACUC of NCNST (Beijing, China). The female BALB/c mice (18-22 g) from Beijing Vital River Laboratory Animal Technology Co. Ltd were used to set up tumor xenograft model. 4T1 cancer cells (1×10^6 cells/100 μL in 1:1 (v/v) PBS and Matrigel) were injected subcutaneously into the back of the mouse. The length and width of tumors were measured with calipers. The tumor volume (V) was calculated using the following equation: V = length × width × width/2. When the tumors grew to 200-400 mm^3, the tumor-bearing mice were randomly divided into four groups for the treatment with UC@PS, UC@PS + 808 nm, UC@PS/C-DAE, UC@PS/C-DAE + 808 nm. The mice were intratumoral injected with UC@PS or UC@PS/C-DAE at an UC@PS dose of 40 mg/kg. In light-activation experiments, NIR treatment was performed 30 min post-injection by irradiating the tumor regions with an 808 nm laser for 10 min (1.0 W/cm^2, 5 min break after 1.5 min irradiation). After anesthesia, the whole-body fluorescence imaging was performed with IVIS® Spectrum *in vivo* imaging system at specified time intervals post-treatment. The excitation and emission wavelengths were 640 nm and 700 nm, respectively.

***In vivo* tumor therapy:** 4T1 tumor xenograft female BALB/c mice were used to establish breast cancer mouse model. When the tumors grew to 50-100 mm^3, the mice were randomly divided into nine groups for the treatment with 1: saline, 2: saline + 980 nm, 3: saline + 808 nm + 980 nm, 4: UC@PS, 5: UC@PS + 980 nm, 6: UC@PS + 808 nm + 980 nm, 7: UC@PS/C-DAE, 8: UC@PS@C-DAE + 980 nm, 9: UC@PS/C-DAE + 808 nm + 980 nm (UC@PS dose: 40 mg/kg). The mice were treated with UC@PS or UC@PS/C-DAE at an UC@PS dose of 40 mg/kg through intratumoral injection. NIR treatment was performed 30 min post-injection at the tumor regions with 980 nm or 808 nm + 980 nm laser irradiation

(808 nm laser for 10 min, 1.0 W/cm^2, 5 min break after 1.5 min irradiation; 980 nm laser for 20 min, 1.2 W/cm^2, 5 min break after 1.5 min irradiation). Then tumor volume and body weight were recorded every other day. The tumor volume was calculated using the following equation: tumor volume = length × width × width/2. At the end of the experiment, tumors of mice in contrast treatment groups were fixed and sectioned for H & E and TUNEL staining.

Table S1 Glossary of Terms.

Abbreviation	**Full name or explaination**
UCNP	Upconversion nanoparticle
TPS	Tetra-substituted porphyrin silsesquioxane
DAE	Diarylethene
C-DAE	Closed form of DAE
O-DAE	Open form of DAE
MSN	Mesoporous silica
UC@MSN	Mesoporous silica coated upconversion nanoparticle
UC@PS	Mesoporous silica coated upconversion nanoparticle with porphyrin PSs covalently embedded inside the silica walls
UC@PS/C-DAE	UC@PS with closed form DAE in its mesopores
UC@PS/O-DAE	UC@PS with open form DAE in its mesopores

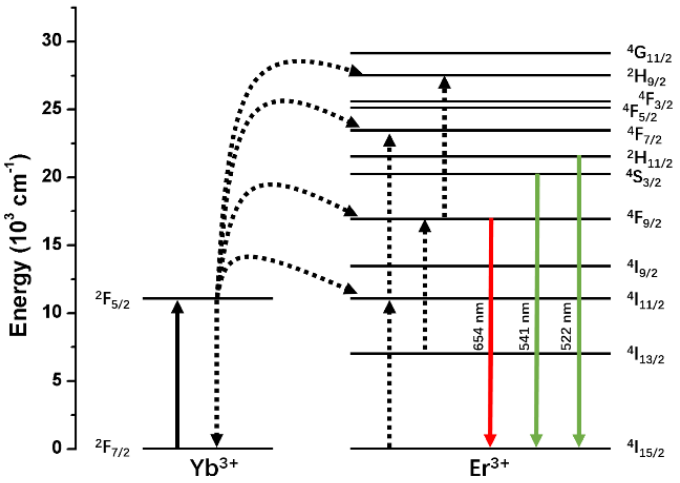

Fig. S1 Energy level diagrams of the Yb^{3+}, Er^{3+} ions and the energy transfer mechanisms of UCNPs. In the UCNPs, Yb/Er ions were co-doped in the NPs to realize visible UCL from Er^{3+} upon 980 nm NIR light excitation.

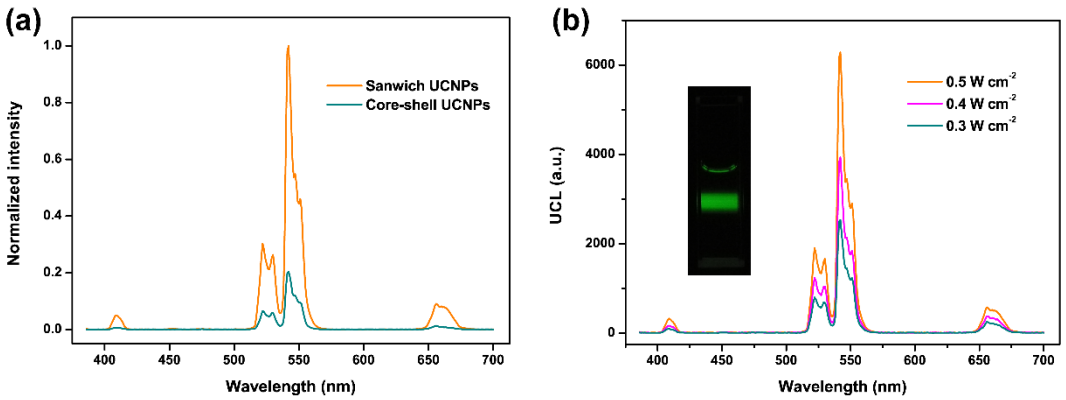

Fig. S2 (a) UCL spectra of the sandwich-structure $NaGdF_4$@$NaGdF_4$:Yb,Er@$NaGdF_4$ UCNPs and the core-shell structure $NaGdF_4$@$NaGdF_4$:Yb,Er UCNPs. (b) UCL spectra of sandwich-structure UCNPs under different power of 980 nm NIR irradiation. From the UCL spectra, it was confirmed that the outer shell of sandwich-structure UCNPs could efficiently avoid environmental quenching effect and thus ensure high upconversion efficiency.

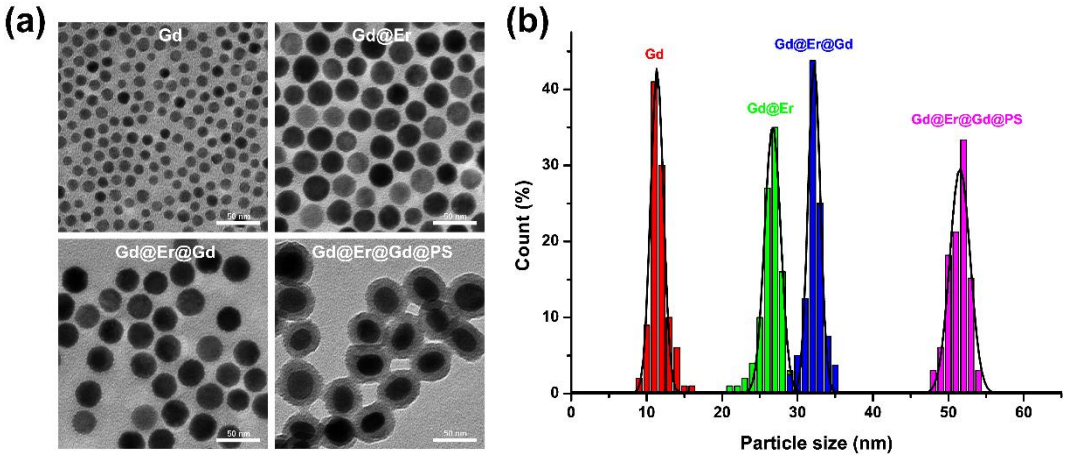

Fig. S3 (a) TEM images and (b) corresponding size distribution of the $NaGdF_4$ (Gd), $NaGdF_4$@$NaGdF_4$:Yb,Er (Gd@Er), $NaGdF_4$@$NaGdF_4$:Yb,Er@$NaGdF_4$ (Gd@Er@Gd) UCNPs, and UC@PS.

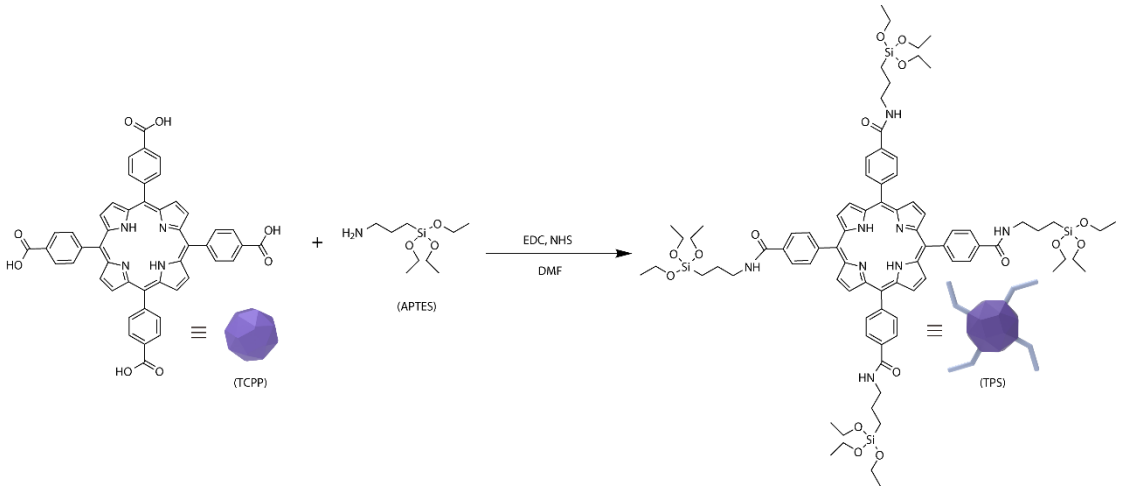

Fig. S4 The synthesis and chemical structure of tetra-substituted porphyrin silsesquioxane (TPS).

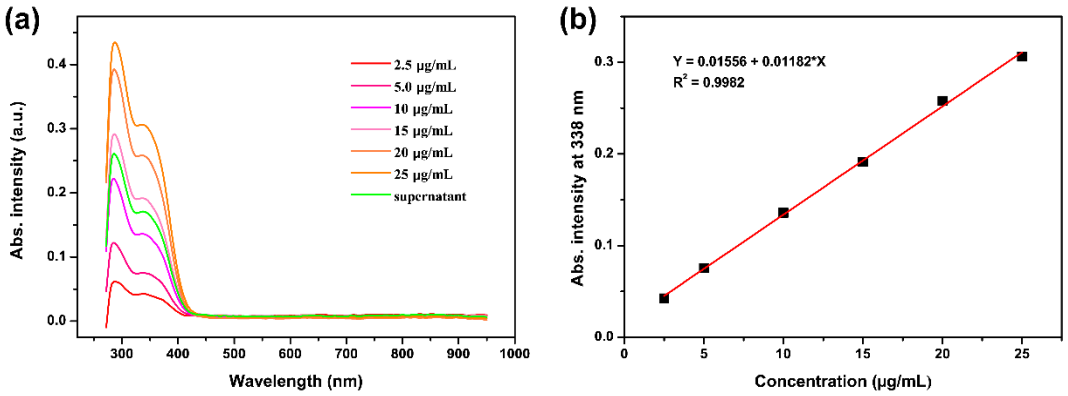

Fig. S5 (a) Absorption spectra of O-DAE at different concentration (DAE stock solution pre-irradiated with 808 nm light until the absorption peak at 712 nm completely disappeared). (b) Absorption intensity of O-DAE at 338 nm as a function of [O-DAE] in a range of 2.5-25 μg/mL in DMF. The DAE loading content was calculated with this standard curve.

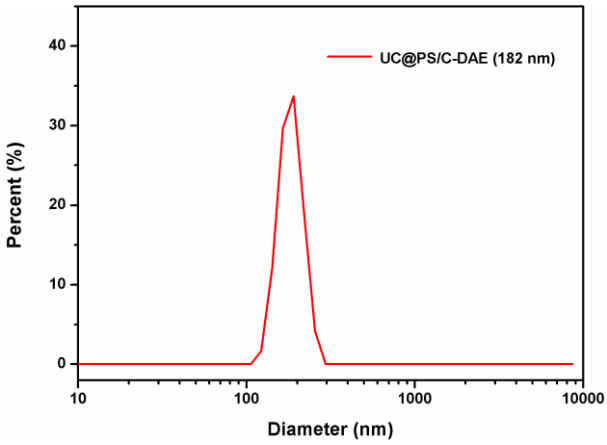

Fig. S6 The hydrodynamic size distribution of UC@PS/C-DAE nanoconstruct, measured by dynamic light scattering (DLS).

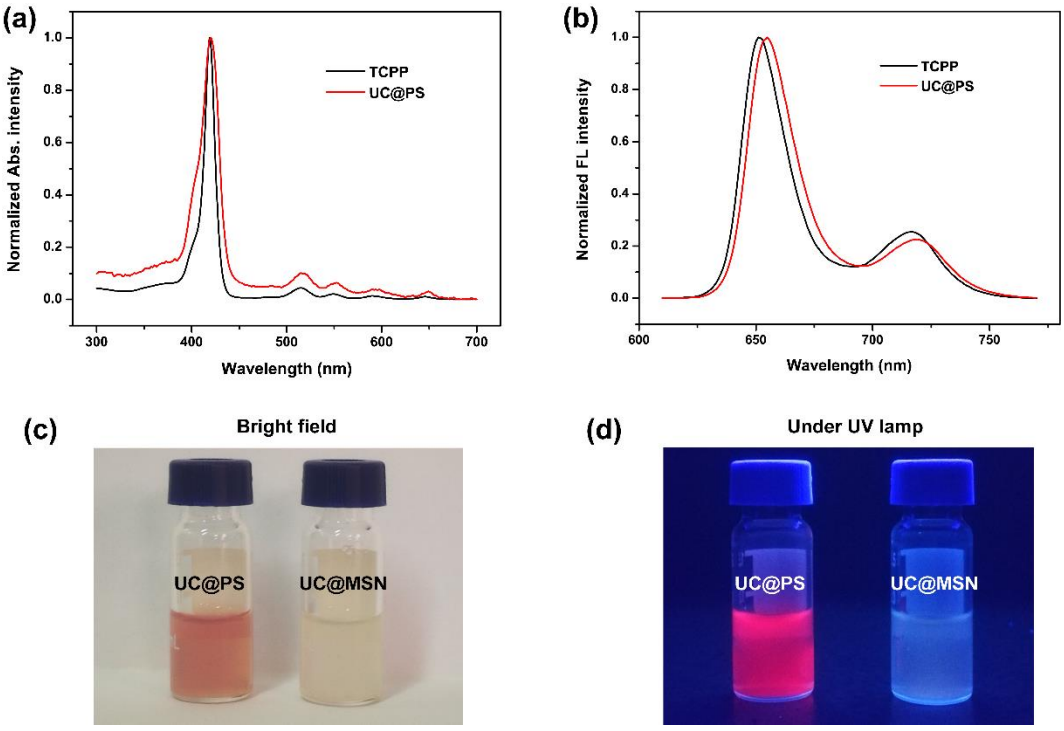

Fig. S7 (a) UV-vis absorption (Abs.) spectra and (b) fluorescence (FL) spectra of TCPP (PSs) and UC@PS in DMF. λ_{ex} = 420 nm. Photographic images of UC@PS and UC@MSN (c) in bright field and (d) under UV lamp.

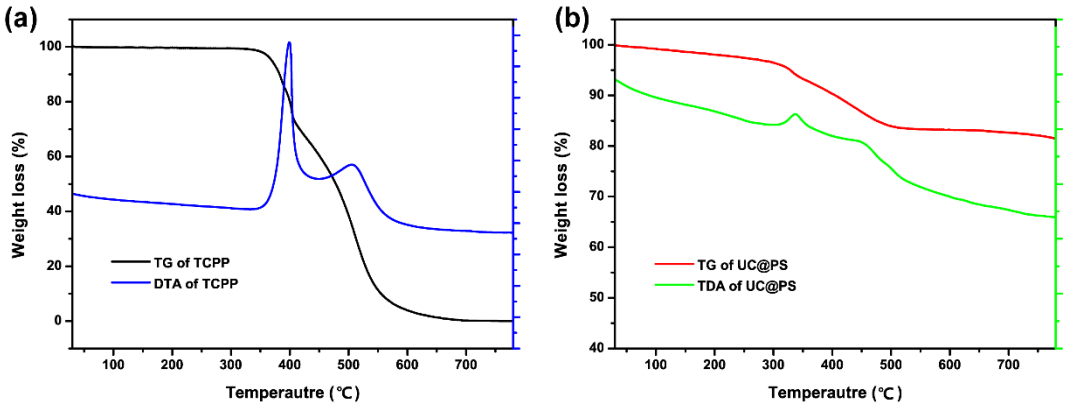

Fig. S8 TG-DTA curves of (a) free TCPP and (b) UC@PS. The TCPP content in the NPs was calculated by the TG-DTA analysis.[5] As shown in Fig. S8a, the free TCPP decomposed through a two-step process from 350 °C to 600 °C. The weight loss of UC@PS in this temperature range could be assigned to loss of TCPP unit (Fig. S8b), allowing calculation of the content of TCPP in the NPs. The content of TCPP unit in the UCNP@PS was calculated to be 18 wt% based on this approach.

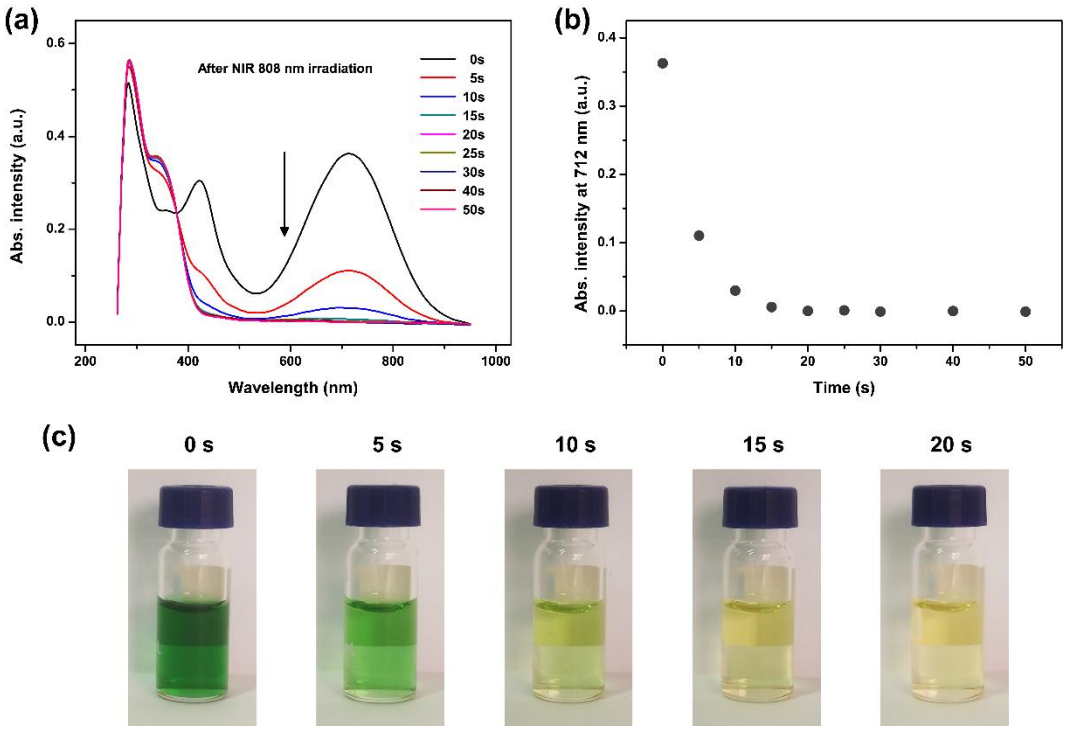

Fig. S9 Changes of the (a) UV-vis-NIR absorption spectrum and (b) absorbance intensity at 712 nm of C-DAE upon NIR 808 nm irradiation of different time. (c) Photographic images of C-DAE solution after 808 nm irradiation of different time.

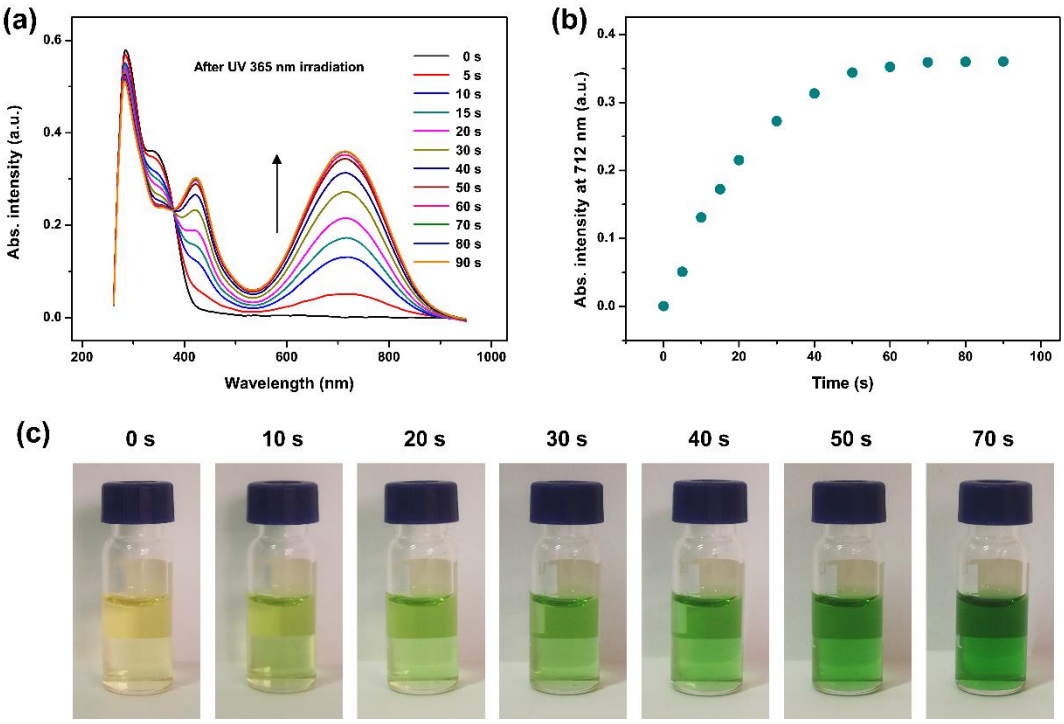

Fig. S10 Changes of the (a) UV-vis-NIR absorption spectrum and (b) absorbance intensity at 712 nm of O-DAE upon 365 nm irradiation of different time. (c) Photographic images of O-DAE solution after 365 nm irradiation of different time.

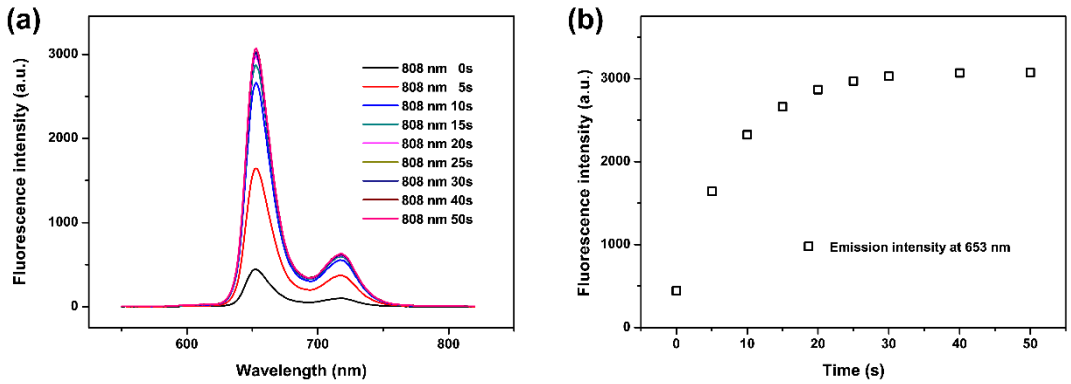

Fig. S11 (a) Fluorescence spectra and (b) fluorescence intensity at 653 nm of UC@PS/C-DAE upon NIR 808 nm irradiation of different time (λ_{ex} = 420 nm).

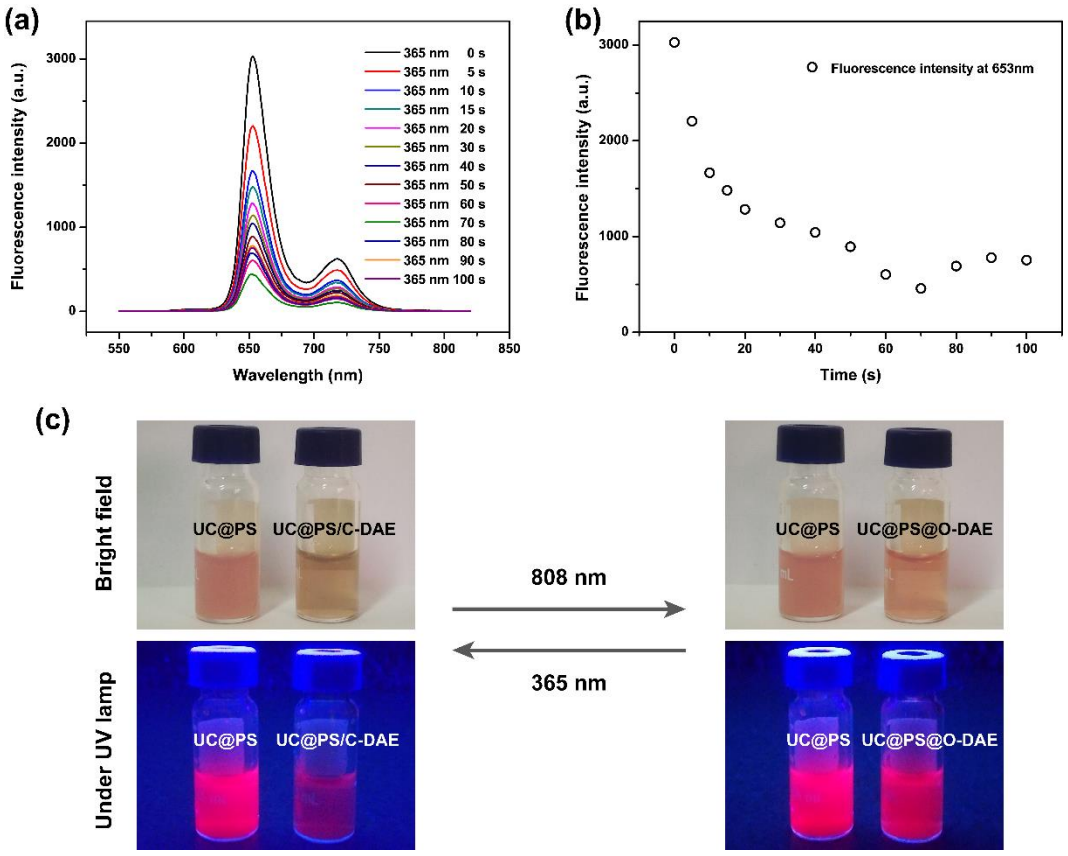

Fig. S12 (a) Fluorescence spectra and (b) fluorescence intensity at 653 nm of UC@PS/O-DAE upon 365 nm irradiation of different time (λ_{ex} = 420 nm). (c) Photographic images of UC@PS and UC@PS/DAE in bright field or under UV lamp upon alternant irradiation with 808 nm and 365 nm lights.

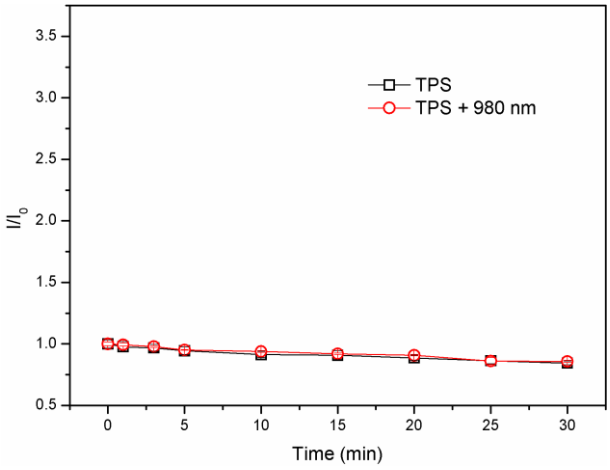

Fig. S13 The $^1O_2$ generation of TPS molecules upon 980 nm light irradiation. The results show that the TPS molecules can not generate $^1O_2$ under this condition.

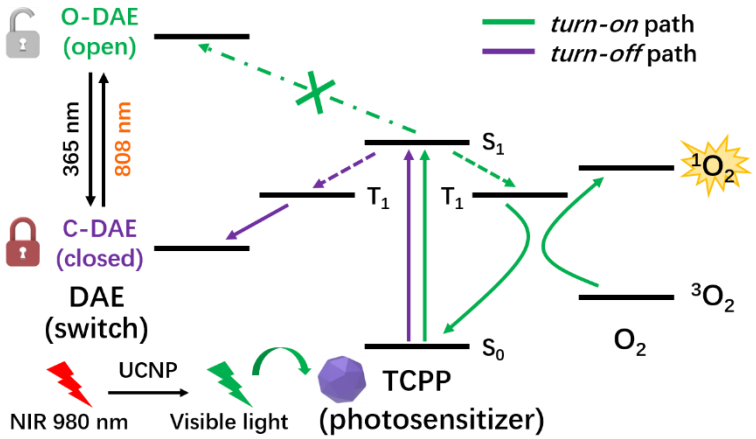

Fig. S14 Schematic showing the energy transfer path and mechanism of turn-on/off singlet oxygen generation by the TCPP-DAE energy transfer pair.

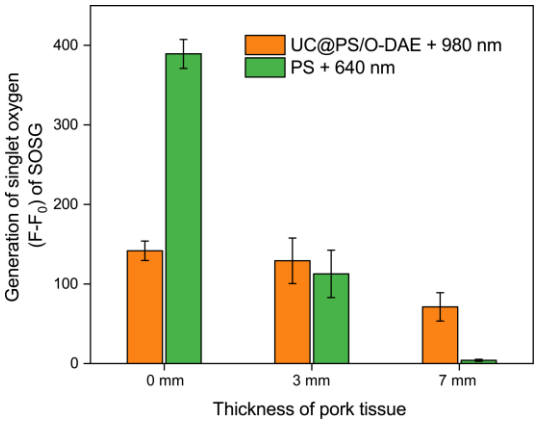

Fig. S15 The $^1O_2$ generation of UC@PS/O-DAE and free PS (TCPP) upon 980 nm and 640 nm light irradiation, respectively, which were blocked by tissue of different thickness.

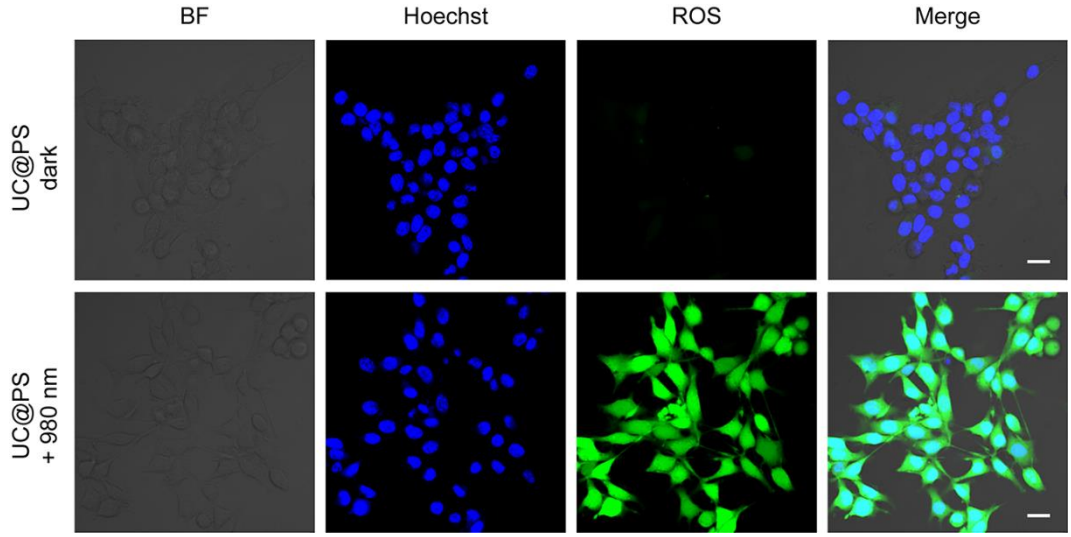

Fig. S16 Confocal fluorescence images of 4T1 cells treated with UC@PS and UC@PS + 980 nm, DCF-DA was used as an indicator of $^1O_2$ in cells. Nuclei stained with Hoechst 33342. Scale bar: 20 μm.

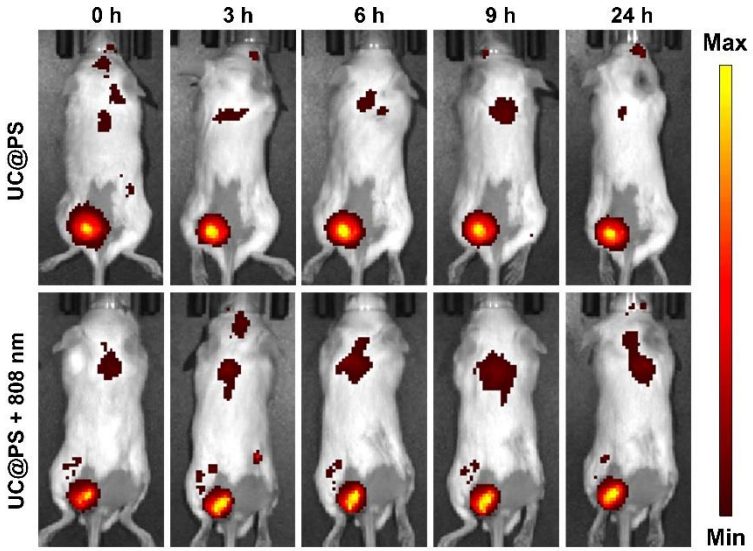

Fig. S17 Representative time-dependent whole-body fluorescence images of 4T1 tumor-bearing mice after treatment with UC@PS without or with 808 nm irradiation.

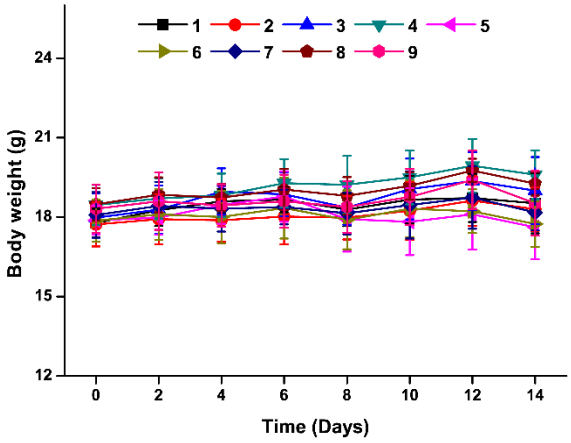

Fig. S18 Body weight changes with time of Balb/c mice under different treatments. (1: saline, 2: 980 nm, 3: 808 nm + 980 nm, 4: UC@PS, 5: UC@PS + 980 nm, 6: UC@PS + 808 nm + 980 nm, 7: UC@PS/C-DAE, 8: UC@PS@C-DAE + 980 nm, 9: UC@PS/C-DAE + 808 nm + 980 nm). Data are means ± SD; N = 5.

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