Electronic Supplementary Information (ESI)
For

BODIPY-containg nanoscale metal-organic frameworks for photodynamic therapy

Weiqi Wang,[a,b] Lei Wang,*[,a] Zhengsheng Li,[a] Zhigang Xie,*[,a]

[a] State Key Laboratory of Polymer Chemistry and Physics,
Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 5625 Renmin Street, Changchun, Jilin 130022, P.R. China.
[b] The University of Chinese Academy of Sciences, Beijing 100049, P. R. China.

E-mail: leiwang@ciac.ac.cn, xiez@ciac.ac.cn

1 Material and Methods

Materials. All starting materials were commercially available and used without further purification. N,N-dimethylformamide (DMF) was stored over activated molecular sieves and was distilled under reduced pressure. Ultrapure water was prepared using a Millipore Simplicity System (Millipore, Bedford,USA). Calcein-AM/ propidium iodide (PI) cell kit was obtained from KeyGen Biotech. Co. Ltd. (Nanjing, China).

Methods. FTIR was measured by Nicolet Impact 410 Fourier transform infrared spectrometer. UV and Fluorescence were recorded on SHIMADZU UV-2450 and Edinburgh Instrument FLS-920 spectrometer, respectively. TEM and SEM images were recorded by JEOL JEM-1011 electron microscope (acceleration voltage of 100 kV) and JEOL JXA-840 (acceleration voltage of 15 kV). The size distribution and zeta potential were measured by Malvern Zeta Sizer-Nano ZS90 instrument. The Solid-state ^{13}C and ^{1}H NMR spectra were recorded at 5K Hz. PXRD was performed

by a Riguku D/MAX2550 diffractometer using CuKα radiation, 40 kV, 200 mA with scanning rate of 0.4 °/min. The thermogravimetric analysis (TGA) was performed using a NetzchSta 449c thermal analyzer system at a rate of 10 °C/min under air atmosphere. Carbon, hydrogen, nitrogen contents was determined by CHN-1000 elemental analyzer. Zirconium and iodine contents were determined by the inductively coupled plasma (ICP) spectra spectrometry (X-series II, Thermoscientific). The nitrogen adsorption isotherm was measured on a Micromeritics ASAP 2010 analyzer.

2 Synthesis

2.1 Synthesis of I2-BDP

3-(2',6'-Diiodo-1',3',5',7'-tetramethyl-4',4'-difluoro-4'-bora-3'a,4'a-diaza-s-indacen-8'-yl) propanoic acid was synthesized according to previous work.[S1]

2.2 Synthesis of UiO-66 nanocrystals

UiO-66 was synthesized by modified solvothermal method according to pioneered.[S2] An amount of 9 mL of DMF containing Zircomiun tetrachloride (116.6 mg, 0.5 mmol), benzene-1,4-dicarboxylic acid (78.7 mg, 0.47 mmol), benzoic acid (0.92 g, 7.6 mmol) and hydrochloric acid (83 μL) were heated at 120 °C for 48 h. After cooling, the resulting product was washed with DMF and methanol for several times, collected through centrifugation (8000 rpm × 10 min) and dried under vacuum. Anal. Calcd (wt %): $\{Zr_6O_4(OH)_4\}(BDC)_4$, C, 28.77 wt%; H, 1.51 wt%; Zr, 40.97 wt%.

Found (wt %): C, 29.51 wt%; H, 3.11 wt%; N, 0.27 wt%; Zr, 38.94 wt%.

2.3 Synthesis of UiO-PDT nanocrystals

UiO-PDT was prepared by solvent-assisted ligands exchange approach. I2-BDP (10 mg) and UiO-66 (10 mg) were dispersed into 2 mL DMF and stirred at 65 ^{o}C overnight. After cooling, the dark purple powders were washed with DMF and methanol for several times, collected by centrifugation (8000 rpm × 10 min) and dried under vacuum. Anal. Calcd (wt %): $\{Zr_6O_4(OH)_4\}(BDC)_{3.5}$(I2-BDP), C, 29.02 wt%; H, 2.00 wt%; N, 2.03 wt%; Zr, 26.45 wt%; I, 13.91 wt%. Found (wt %): C, 35.25 wt%; H, 3.37 wt%; N, 2.09 wt%; Zr, 26.65 wt%; I, 12.84 wt %.

3 Characterization

3.1 Singlet-Oxygen Generation Measurements.

In vitro single oxygen generation measurement was carried out by modified method using 1,3-diphenylisobenzofuran (DPBF) as capture agent.[S3, 4] An amount of 2 mL of DMF containing 100 μL of DPBF (1 mM) and 5 μM of photosensitizer samples (free I2-BDP molecule and UiO-PDT nanocrystals) in a quartz cuvette was illuminated under a LED lamp (power density of 20 mW/cm^2) at room temperature for 60 s. The absorbance intensity of DPBF at 410 nm in the mixture was recorded at five-seconds intervals. The rate of singlet oxygen generation was determined from the reduced absorbance intensity over time. For the control experiments, DPBF absorption was also recorded for negative comparison at the same conditions in the absence of

photosensitizer.

3.2 Cell culture

The mouse mammary tumor cell B16F10 was cultured in Dulbecco's Modified Eagle Medium (DMEM), whereas CT26 and C26 was grown in RPMI-1640 Medium containing 10 % FBS and 1% penicillin/streptomycin at 37 °C under 5 % CO_2.

3.3 Confocal laser scanning microscopy (CLSM)

The B16F10 cells were seeded into 6-well plates at an initial cell density of 1×10^5 cells/well in 2 mL of DMEM medium. The cells were treated with samples (free I2-BDP and UiO-PDT nanocrystals) at the same I2-BDP concentration of 1 μg mL^{-1} at 37 °C. After 24 h incubation, the cell culture medium was removed and cells were washed with ice-cold PBS buffer (pH = 7.4) for twice times before fixed with fresh 4.0 % paraformaldehyde (1 mL) for 10 min at room temperature. The cells were counterstained with DAPI for cell nucleus. The fixed cells were again washed with PBS 7.4 for three times before observation by confocal laser scanning microscope (Carl Zeiss LSM 780).

3.4 Cellular uptake by flow cytometry

B16F10 cells were seeded in six-well plates (2×10^5 cells/well) and grown in complete DMEM medium for 24 h. The free I2-BDP and UiO-PDT nanocrystals at an equivalent I2-BDP concentration of 1 μg mL^{-1} in fresh culture medium were added

into each well. After incubation for 24 h at 37 °C, the medium was removed and cells washed three times with cold PBS before trypsin treatment. The collected cells were centrifuged at 1000 rpm for 5 min and washed by PBS twice. The supernatants were discarded and the cell pellets were re-suspended in 0.5 mL PBS 7.4. Flow cytometry analysis was performed by a flow cytometer (Beckman, USA) which collected 1×10^4 gated events for each sample.

3.5 Cytotoxicity assay

The dark cytotoxicity of free I2-BDP, UiO-66 and **UiO-PDT** nanocrystals as well as theirs photocytotoxicity were evaluated by 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT) assay. Cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 10^4 cells/well and incubated overnight. Then different concentrations of samples or free BDP were added into each well and incubated for 4 h. Afer the removal of samples, cells were transferred into fresh media and then irradiated by the visible light at a power density of 80 mW/cm^2 for 10 min. The dark cytotoxicity (without irradiation) was monitored without irradiation at the same time as control. The laser power density was measured by a LPE-1C laser powermeter (Wuke Photoelectric Technique, Beijing, China). The cells were then incubated at 37 ^{o}C for additional 48 h before the MTT assay to determine the cell viabilities. Cells could be stained with the calcein-AM/propidium iodide (PI) to determine their viabilities.

4 Results

4.1 Synthesis of I2-BDP

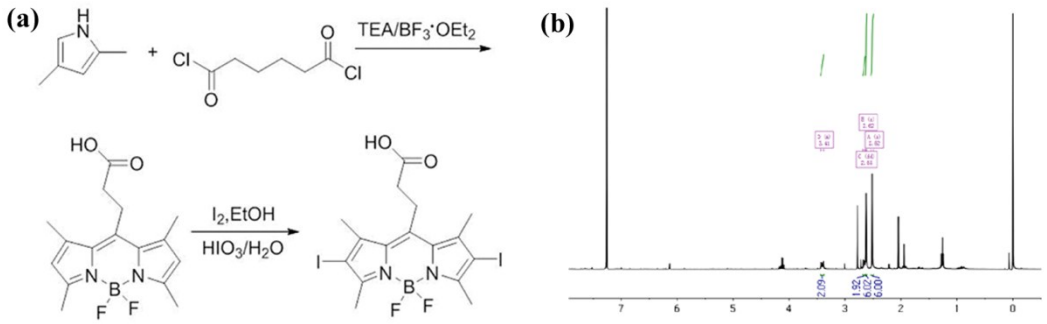


Fig. S1 The synthesis of I2-BDP (a) and its ^{1}H NMR in DMSO-d6.

4.2 TEM and SEM imagings of UiO-66 and UiO-PDT

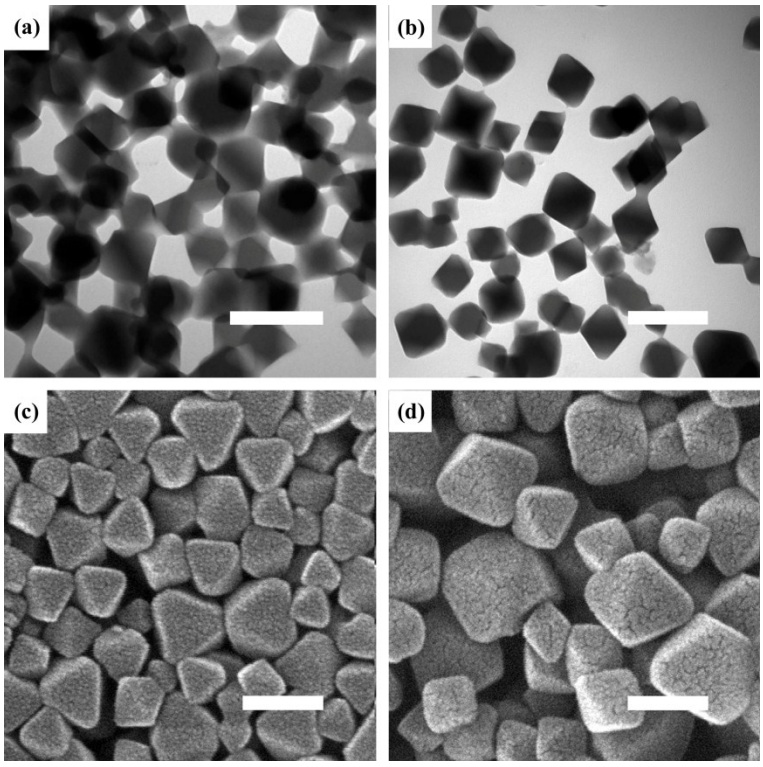


Fig. S2 TEM and SEM imagings of UiO-66 (a,c) and **UiO-PDT** (b,d) before and after solvent-assisted ligands exchange. scale bar: 200 nm.

4.3 DLS of UiO-PDT

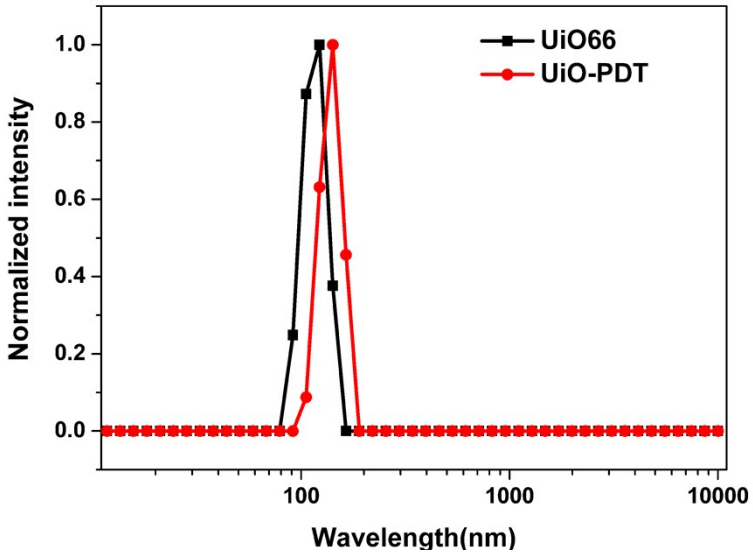


Fig. S3 The DLS of UiO-66 (black) and **UiO-PDT** (red) in H_2O.

4.4 N2 adsorption of UiO-66 and UiO-PDT

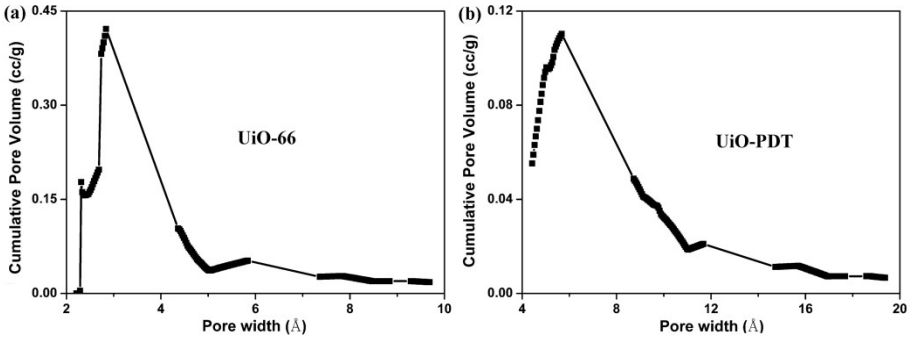


Fig. S4 Pore size distributions (HK method) of UiO-66 (left) and **UiO-PDT** (right) determined from nitrogen uptake measurements at 77 K.

4.5 FTIR spectra of UiO-66, I2-BDP and UiO-PDT

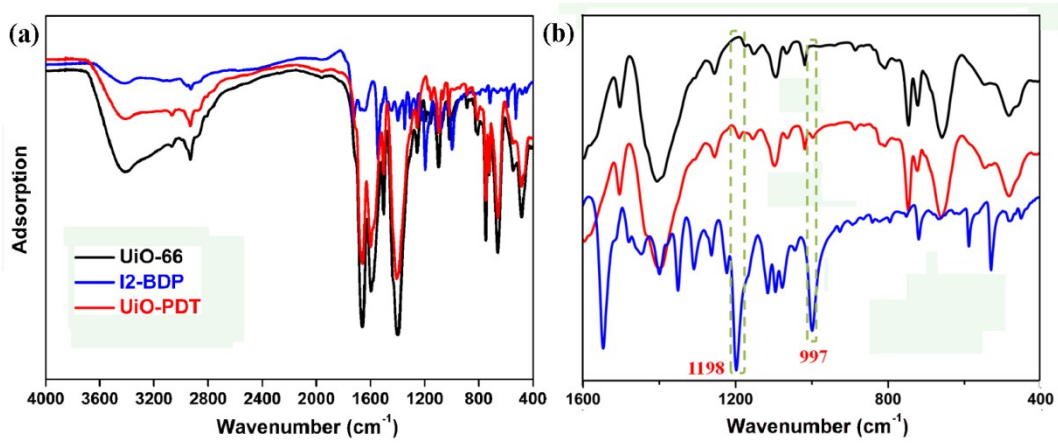


Fig. S5 FTIR spectra of UiO-66 (black), **UiO-PDT** (red) and I2-BDP (blue) in KBr mode.

4.6 The solid ^{1}H NMR of UiO-PDT

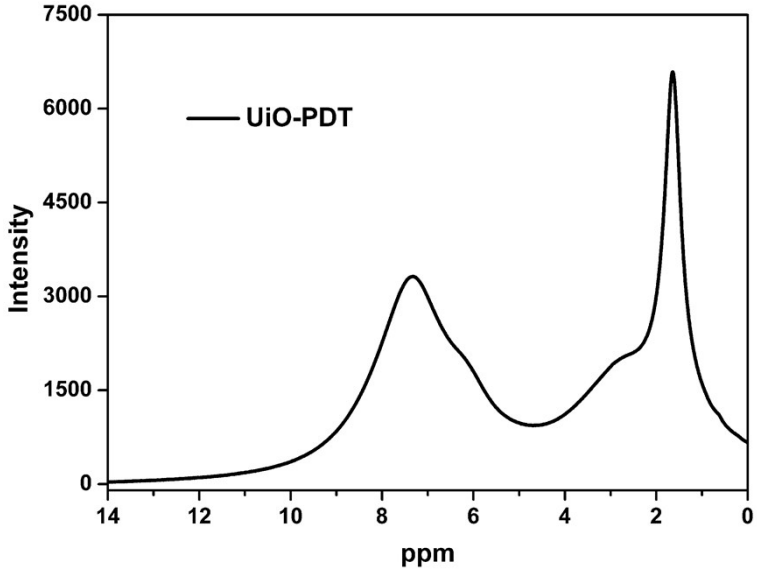


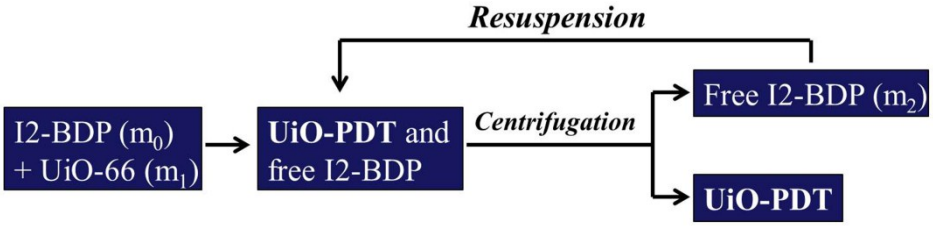
Fig. S6 The solid ^{13}H NMR spectra of **UiO-PDT**.

4.7 The standard curve of I2-BDP

The content of I2-BDP in as-synthesized UiO-PDT was determined by UV absorption at 524 nm. The detail process for determination the I2-BDP in **UiO-PDT** has been shown in Scheme S1. After ligands exchange in DMF at 65 °C, all washing supernatant were collected to quantify the free I2-BDP content by standard curve of I2-BDP in DMF. The I2-BDP content in final **UiO-PDT** nanocrystals was calculated by Eq. (**1**):

$$\text{I2-BDP content in } \mathbf{UiO\text{-}PDT}\ (\%) = (m_0 - m_2) / (m_1 + m_0 - m_2) \times 100\% \quad (\mathbf{1})$$

m_0: the initial amount of I2-BDP; m_1: the weight of UiO-66, m_2: the cumulative amount of free I2-BDP (obtained from the standard curve).



Scheme S1. Schematic presentation of determination I2-BDP content in **UiO-PDT**.

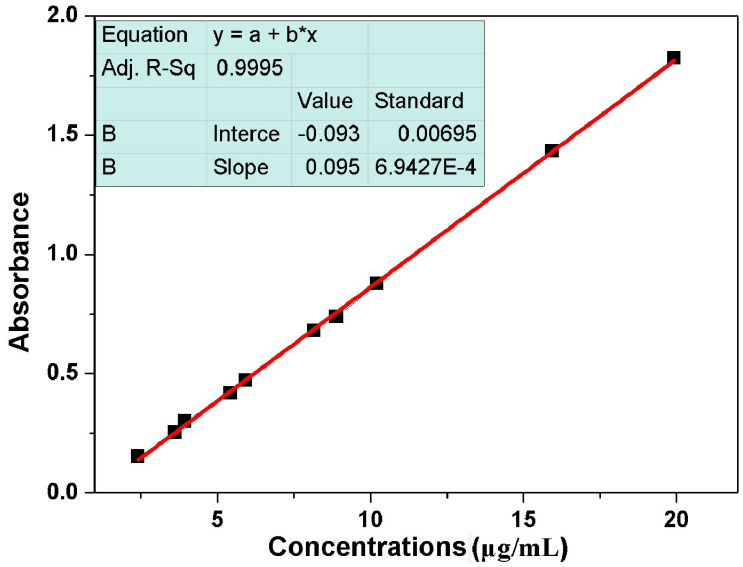


Fig. S7 The standard curve of I2-BDP in H_2O.

4.8 Thermogravimetric analysis of UiO-66 and UiO-PDT

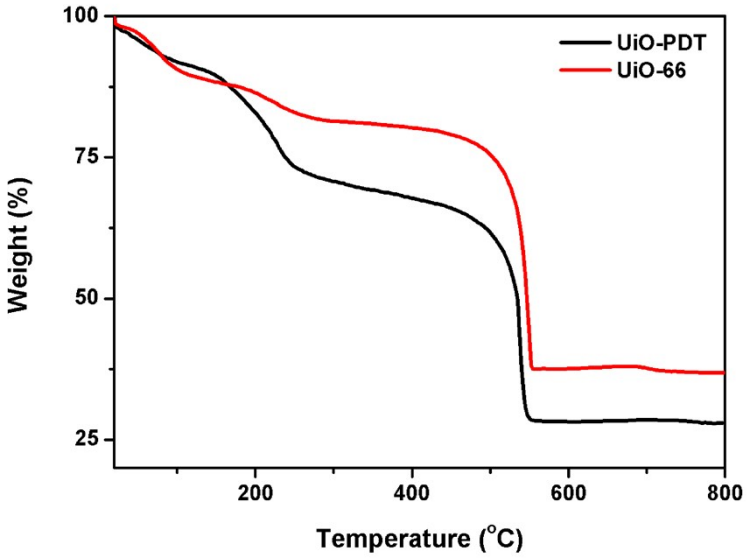


Fig. S8 The thermogravimetric curves of UiO-66 (red) and **UiO-PDT** (black) under air atmosphere.

4.9 Luminescence spectra of UiO-PDT and I2-BDP

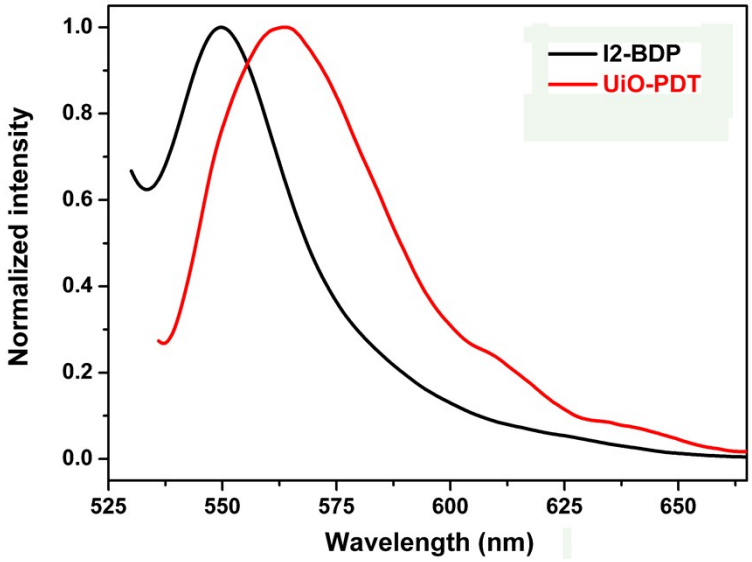


Fig. S9 The luminescence spectra of **UiO-PDT** (red) and I2-BDP (black) dispersed into DMF, λ_{ex}: 524 nm.

4.10 Zeta potential of UiO-66 and UiO-PDT

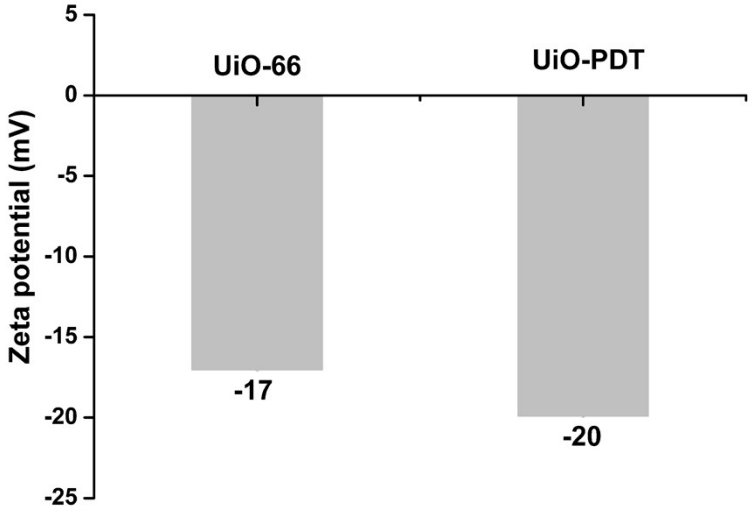


Fig. S10 The zeta potential of UiO-66 and **UiO-PDT** in H_2O before and after ligands exchange monitored by Malvern Zeta Sizer-Nano ZS90 instrument.

4.11 *In vitro* dark cytotoxicity of UiO-PDT

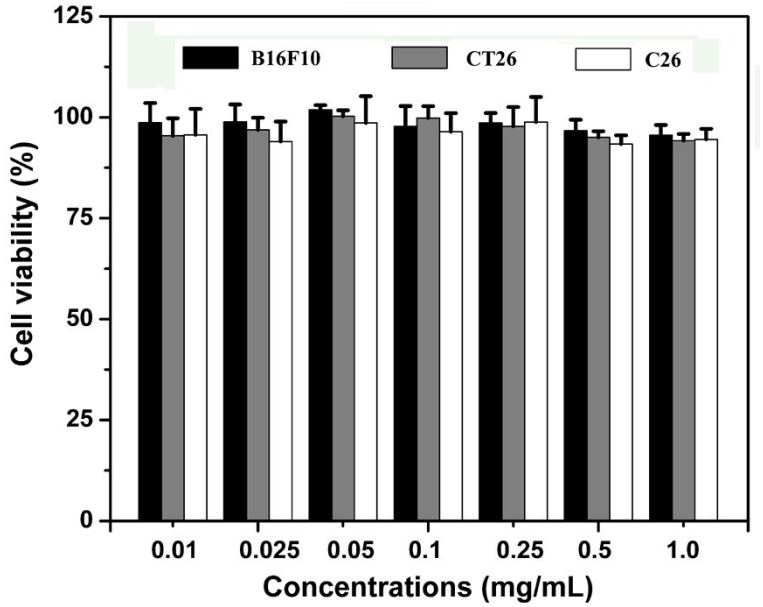


Fig. S11 *In vitro* cytotoxicities against B16F10, CT26 and C26 cell lines of **UiO-PDT** nanocrystals in various concentration after incubation with cells for 48 h.

4.12 *In vitro* dark cytotoxicity and phototoxicity of UiO-66

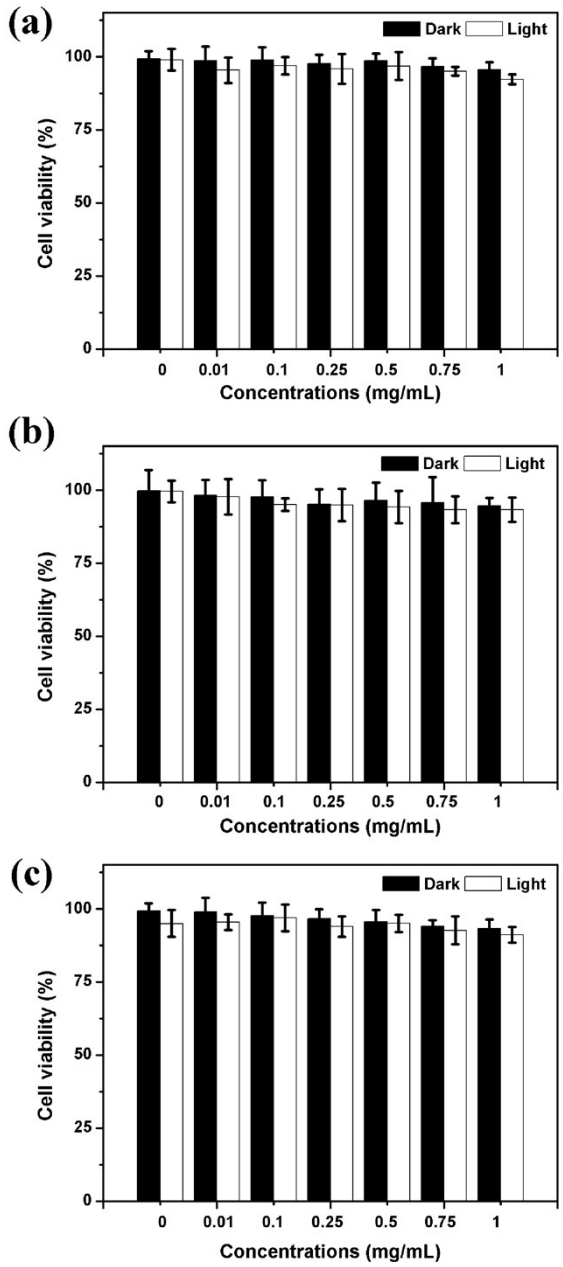


Fig. S12 *In vitro* dark cytotoxicity and phototoxicity of UiO-66 against B16F10 (a), CT26 (b) and C26 (c) in various concentrations after incubation with cells for 48 h.

4.13 Singlet oxygen generation of DPBF blank and freee I2-BDP

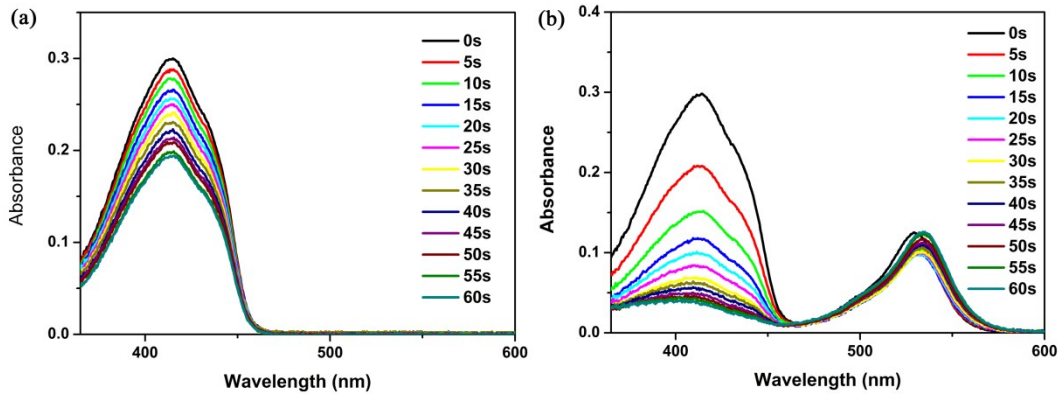


Fig. S13 Singlet oxygen generation of DPBF blank (a) and freee I2-BDP (b) in DMF monitored by disappearance UV absorbance of DPBF at 410 nm with LED light power density of 20 mW cm^{-2} for 60 seconds.

4.14 *In vitro* phototoxicity of free I2-BDP and UiO-PDT nanocrystals

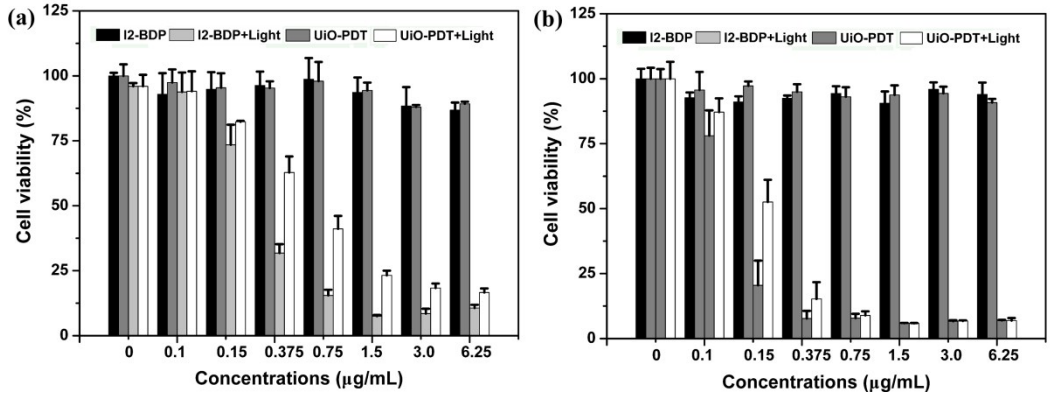


Fig. S14 *In vitro* cytotoxicities of free I2-BDP and **UiO-PDT** nanocrystals against CT26 cells (a) and C26 cells (b) before and after irradiated by visible light at a power density of 80 mW cm^{-2} for 10 minutes.

4.15 live/dead staining

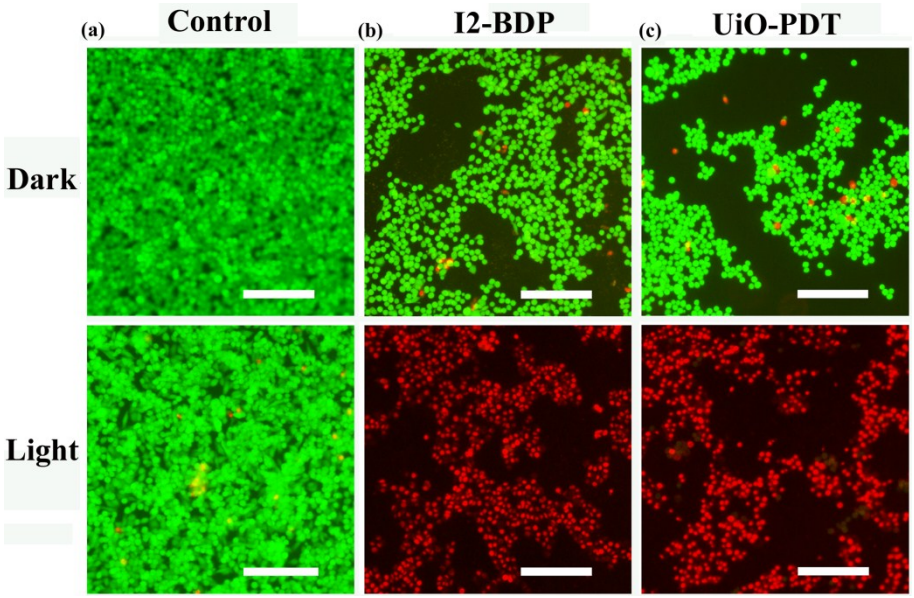


Fig. S15 live/dead staining of blank (a), free I2-BDP (b) and **UiO-PDT** nanocrystals (c) with and without irradiation. Images of calcein-AM (green, live cells) and propidium iodide (red, dead cells) co-stained B16F10 cells. The concentration of I2-BDP is 1 μg/mL in both I2-BDP and **UiO-PDT** and both of them wereirradiated by the visible light at a power density of 80 mW/cm^2 for 10 min.

References

S1 T. Yogo, Y. Urano, Y. Ishitsuka, F. Maniwa and T. Nagano, *J. Am. Chem. Soc.*, 2005, **127**, 12162-12163.

S2 J. H. Cavka, S. Jakobsen, U. Olsbye, N. Guillou, C. Lamberti, S. Bordiga and K. P. Lillerud, *J. Am. Chem. Soc.*, 2008, 130, 13850-13851.

S3 H. Ding, B. D. Sumer, C. W. Kessinger, Y. Dong, G. Huang, D. A. Boothman and J. Gao, *J. Control. Release.*, 2011, 151, 271-277.

S4 X. Ding and B.-H. Han, *Chem. commun.*, 2015, 51, 12783-12786.