Supporting information

Glutathione-responsive nanoscale MOFs for effective intracellular delivery of anticancer drug of 6-mercaptopurine

Ming Gong [a], Jian Yang [a], Yongsheng Li [a] and Jinlou Gu*[,a]

[a]Shanghai Engineering Research Center of Hierarchical Nanomaterials, Key Laboratory for Ultrafine Materials of Ministry of Education, School of Materials Science and Engineering, East China University of Science and Technology, Shanghai 200237, China

Email of Corresponding author: jinlougu@ecust.edu.cn

S1. Experimental section

S1.1 Chemicals and materials

Zirconium chloride ($ZrCl_4$), 6-mercaptopurine (6-MP) and dimethyl sulfoxide (DMSO) and triethylene diamine (DABCO) were bought from Shanghai Macklin Biochemical Co., Ltd. N,N-Dimethylaniline (DMA), N,N-dimethylformamide (DMF), benzoic acid (BA) and ethanol were purchased from Shanghai Titan Scientific Co., Ltd. Potassium hydroxide (KOH) and hydrochloric acid (HCl, 37%) were gained from Shanghai Lingfeng Chemical Reagent Co., Ltd. Iodine, 1,4-benzenedicarboxylate (BDC), 4-morpholineethanesulfonic acid (MES), 2-(4-(2-hydroxyethyl)-1-piperazinyl)ethanesulfonic acid (HEPES) and riboflavin sodium phosphate (FMN) were obtained from Aladdin Industrial Corporation. Glutathione (GSH) and 2,5-dihydroxyterephthalic acid diethyl ester were supplied by Shanghai Dibai Chemical Reagent Co., Ltd. Dimethylthiocarbamoyl chloride was purchased from Shanghai Sa'en Chemical Reagent Co., Ltd. RPMI-1640 medium (RPMI-1640), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and all the other cell detection kits were received from Nanjing KeyGen Biotechnology Co., LTD. All reagents were of analytical grade and used as received without further purification. The NIH/3T3 cell lines and SMMC-7721 cell lines were purchased from the Cell Bank of the Chinese Academy of Sciences.

S1.2 Synthesis of 2,5-bis(dimethylthiocarbamoyloxy)terephthalic acid diethyl ester (1)

2,5-Dihydroxyterephthalic acid diethyl ester (300 mg, 1.17 mmol), DABCO (528 mg, 4.71 mmol) and DMA (3 mL) were mixed under a nitrogen atmosphere and cooled to 0 ºC in an ethanol bath. Dimethylthiocarbamoyl chloride (570 mg, 4.71 mmol) dissolved in DMA (1.5 mL) was added dropwise under nitrogen. The mixture was continuously stirred for 24 h at room temperature. The grey product was dried under vacuum after filtered and washed with large amounts of water.

S1.3 Synthesis of 2,5-bis(dimethylthiocarbamoylsulfanyl)terephthalic acid diethyl ester (2)

Product 1 (130 mg) was heated to 230 ºC and maintained for 1 h under a flowing nitrogen atmosphere. The mixture was cooled down slowly and ethanol was added. The brown product was filtered with ethanol before dried under vacuum.

S1.4 Synthesis of 2,5-dimercaptoterephthalic acid (3) (BDC-$(SH)_2$)

Product 2 (130 mg) and 1.3 M deaerated KOH (in ethanol/H_2O, 1:1, 4 mL) was refluxed under nitrogen for 3 h. The mixture was cooled down to 0 ºC in an ethanol bath, and concentrated HCl (1.5 mL) was dropped, resulting in the bright yellow precipitate. Finally, it was filtered and washed with water and dried at room temperature.

S1.5 Synthesis of UiO-66-$(SH)_2$

$ZrCl_4$ (18.5 mg, 0.08 mmol), BDC-$(SH)_2$ (9.3 mg, 0.04 mmol) were dissolved in 8 mL DMF, and the mixture was sonicated for 20 min. Different amounts of BA (195, 244, 293 and 342 mg, with BA/BDC-$(SH)_2$ ratios range from 40 : 1, 50 : 1, 60 : 1 to 70 : 1) were added into each vial, before another sonication of 20 min. Each vial was put in a 120 ºC-oven for 12 h. Finally, after the centrifugation procedures, HCl and DMF were added to remove the BA coordinated in the frameworks and the mixtures were heated at 100 ºC for 8 h. Ethanol was applied in the washing procedure for three times. MOF powders were obtained by a vacuum drying process at 80 ºC for 24 h.

S1.6 Synthesis of UiO-66

$ZrCl_4$ (466 mg, 2 mmol) and BDC (320 mg, 2 mmol) were dissolved in 45 mL DMF, and the mixture was sonicated for 20 min. BA (2.44 g, 20 mmol) was added into the vial, before another sonication of 20 min. The mixture was put in a 120 ºC-oven for 24 h. Finally, after a centrifugation procedure, HCl and DMF were added to remove the BA coordinated in the framework and the mixture was heated at 100 ºC for 8 h. Ethanol was applied in the washing procedure for three times. UiO-66 was obtained by a vacuum drying process at 80 ºC for 24 h.

S1.7 Drug loading process

200 mg 6-MP was dispersed in 14 mL DMSO under continuous stirring. 3 mL of iodine solution (30 mg mL^{-1} iodine in DMSO) was added to the above solution. The reactants were mixed at room temperature for 24 h. After a degassing progress with N_2, 20 mg UiO-66-$(SH)_2$ (or 20 mg UiO-66 as a control group) was added and the suspension was stirred for another 24 h to form the disulfide bond. The precipitates were centrifuged and washed with DMSO until no redundant 6-MP could be detected by the UV-vis spectrum in the supernatant. The drug-loaded NMOF is named as UiO-66-SS-MP (or UiO-66-MP as a control group).

S1.8 GSH-responsive *in vitro* drug release

3 mg UiO-66-SS-MP was added into the MES buffer solutions (pH = 5.5) with the different GSH concentrations of 0, 1, 3 and 5 mM, and incubated in a 37 ºC environment. At the given time intervals, the supernatant of each sample was collected and measured by UV-vis spectrometer at the wavelength of 324 nm.

S1.9 Influence of pH value on the *in vitro* drug release

3 mg UiO-66-SS-MP was added into the different buffer solutions (MES (pH = 5.5, 6.5) and HEPES (pH = 7.4)) with the GSH concentration of 5 mM, and was incubated at 37 ºC. At the given time intervals, the supernatant of each sample was collected and measured by UV-vis spectrometer at the wavelength of 324 nm.

S1.10 Drug release behavior of UiO-66-MP as a control group

GSH-triggering: 3 mg UiO-66-MP was added into the MES buffer solutions (pH = 5.5) with the different GSH concentrations of 0, 1, 3 and 5 mM, and incubated in a 37 ºC environment. At the determined time intervals, the supernatant of each sample was collected and measured by UV-vis spectrometer at the wavelength of 324 nm.

pH-triggering: 3 mg UiO-66-MP was added into the different buffer solutions (MES (pH = 5.5, 6.5) and HEPES (pH = 7.4)) with the GSH concentration of 5 mM, and was incubated at 37

°C. At the given time intervals, the supernatant of each sample was collected and measured by UV-vis spectrometer at the wavelength of 324 nm.

S1.11 Fluorescence labelling process of FMN@UiO-66-SS-MP

5 mg UiO-66-SS-MP and 2 mg FMN were dispersed in 5 mL H_2O and stirred vigorously at room temperature for 12 h in the dark. After centrifugation and washing processes, the FMN labelled FMN@UiO-66-SS-MP was dispersed in 5 mL water without drying.

S1.12 Cell uptake monitored by flow cytometry

A flow cytometry was conducted to assess the endocytosis efficiency of the FMN@UiO-66-SS-MP by cells. The cancer cells SMMC-7721 were diluted into a 6-well plate, with a concentration of 80000 cells per well. After the incubation of 24 h, the suspensions of FMN@UiO-66-SS-MP (25 μg mL^{-1}) in RPMI-1610 media and a control group were added. The cells were co-incubated for 2, 4, 8 and 24 h, respectively. Finally, the cells were digested and washed, and monitored by the flow cytometry.

S1.13 Confocal laser scanning microscopy (CLSM)

The cancer cells SMMC-7721 were set at the concentration of 6000 cells per well in several glass dishes and incubated for at least 24 h. 25 μg mL^{-1} of FMN@UiO-66-SS-MP suspensions were added and co-incubated for 2 and 24 h. The excess materials were washed carefully when the incubation time was over. SMMC-7721 cells were washed with PBS and fixed with formaldehyde before the observation under CLSM.

S1.14 Cytotoxicity

The cytotoxicity studies were performed on both NIH/3T3 normal cell lines and SMMC-7721 cancer cell lines. DMEM for NIH/3T3 and RPMI-1640 for SMMC-7721 with 10% FBS were used as the cell culture media. The cells were conserved in a 5% CO_2 incubator with a humidified atmosphere at 37 °C. Firstly, NIH/3T3 and SMMC-7721 cells were diluted into 96-well plates at 5000 cells per well and cultivated for 24 h at 37 °C in the incubator. Then, the

media were replaced by UiO-66-SS-MP or free 6-MP suspensions with various concentrations as treatment groups, and fresh media were used as the blank control group. After the incubation of 24 h, 10 μL per well CCK-8 (cell counting kit-8) reagent was added, following by another co-incubation for 4 h. A microplate reader was recorded with the absorbance of each well at the wavelength of 450 nm. The cytotoxicity of the UiO-66-SS-MP could be calculated through the comparison of the absorbance ratios of experiment groups to the control group. The biocompatibility of UiO-66-$(SH)_2$ nanocarriers was also assessed in the same way.

S1.15 Instruments and methods

Powder X-ray diffraction (XRD) patterns were conducted on Bruker D8 using Cu Kα radiation. Field emission scanning electron microscopy (FESEM) was conducted on a Hitachi S-4800. Fourier transform infrared (FT-IR) spectroscopy was recorded with a Nicolet 6700 instrument. Ultraviolet-visible (UV-vis) absorption spectrum was worked on a UV-3600 recorded spectrophotometer (Shimadzu). The specific surface area, which was calculated by Brunauer-Emmett-Teller (BET) method, was experimented on a surface area and porosity analyzer (micromeritics, TriStar II) equipped with a sample degas progress (micromeritics, VacPrep 061). The fluorescent spectra were measured with an RF-5301 spectrofluorophotometer (Shimadzu). The dynamic light scattering (DLS) was measured with a ZEN-3700 system.

S2. Characterization

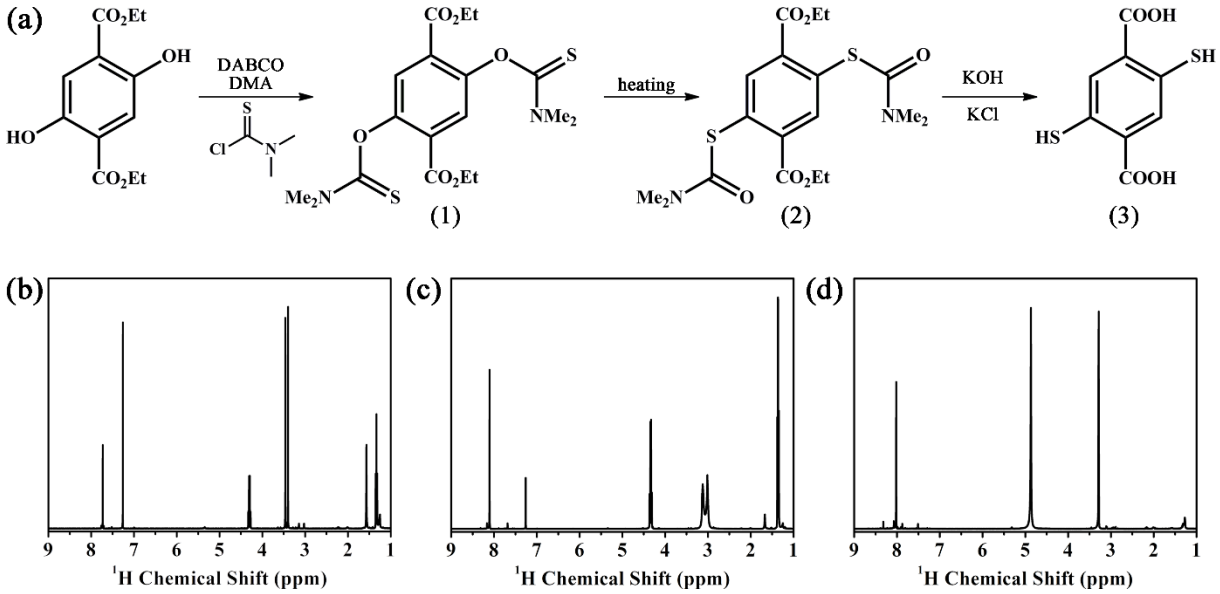

Fig. S1 (a) Synthesis of BDC-$(SH)_2$, the organic ligand of NMOF. ^{1}H NMR spectra of (b) intermediate product 1, (c) intermediate product 2 and (d) final ligand 3.

Table S1 Detail information for ^{1}H NMR analyses of the intermediate products and ligand.

Samples	Solvents	Sample peaks	Solvent & Water peaks
Intermediate product 1	$CDCl_3$	7.73 (2H, CHAr), 4.30 (4H, CH_2), 3.46 (6H, CH_3), 3.40 (6H, CH_3), 1.33 (6H, CH_3)	7.26 & 1.57
Intermediate product 2	$CDCl_3$	8.10 (2H, CHAr), 4.34 (4H, CH_2), 3.13 (6H, CH_3), 3.01 (6H, CH_3), 1.36 (6H, CH_3)	7.26 & /
Final ligand 3	CD_3OD	8.02 (2H, CHAr)	4.87 & 3.29

Table S2 Details of the peak intensity and FWHM at 2θ = 7.3°, as well as the particle size measured from SEM images.

BA/BDC-$(SH)_2$	Peak intensity	FWHM (°)	Diameter from SEM (nm)
40 : 1	101	0.590	/
50 : 1	520	0.317	40
60 : 1	964	0.229	80-120
70 : 1	1796	0.198	110-180

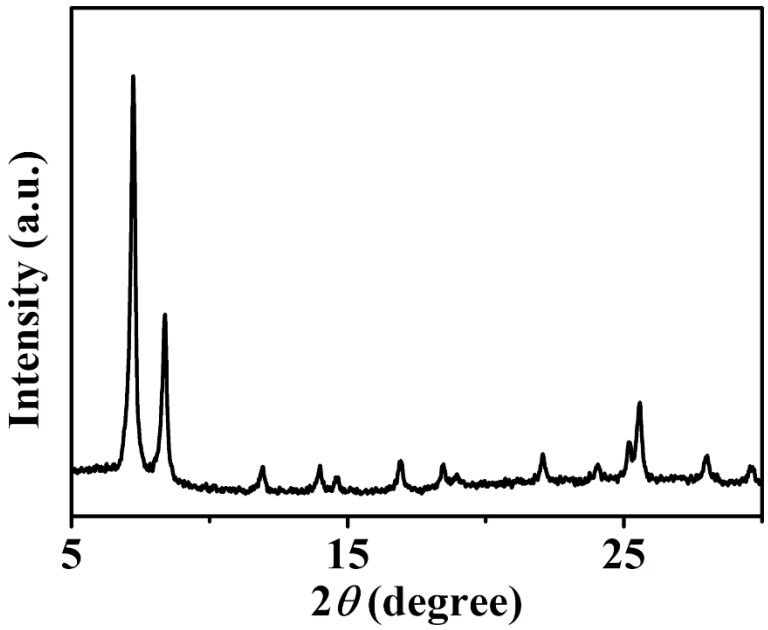

Fig. S2 Powder XRD pattern of the as-synthesized UiO-66.

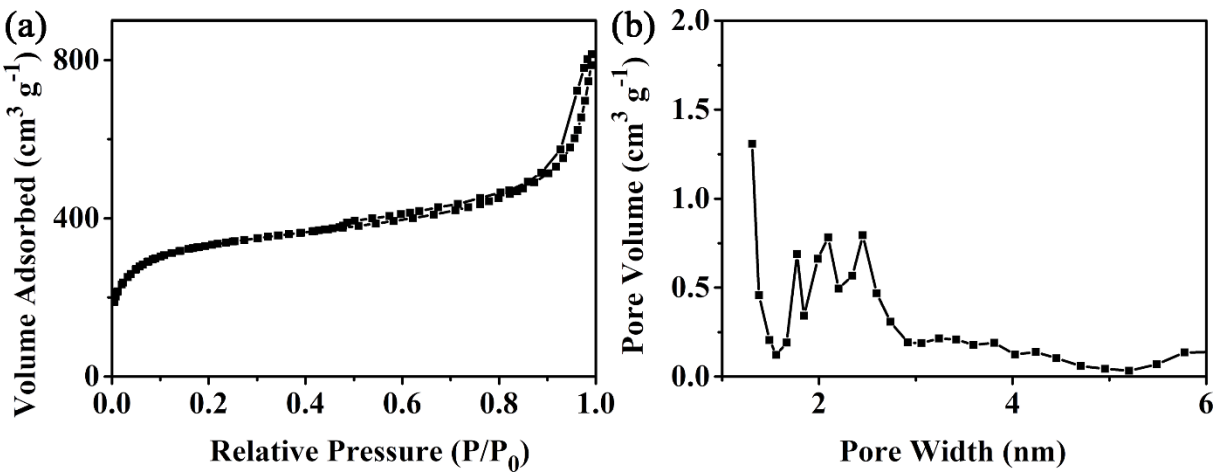

Fig. S3 (a) Nitrogen sorption isotherm and (b) the corresponding DFT pore-size distribution profiles of the as-synthesized UiO-66.

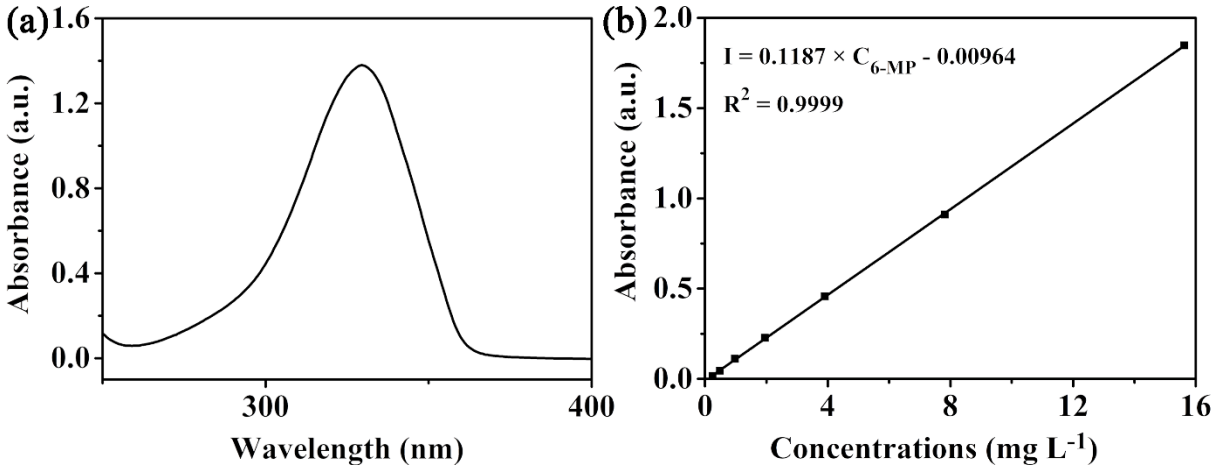

Fig. S4 (a) UV-vis spectrum of 6-MP in DMSO. (b) Linear fitting of 6-MP molecule absorbance at 324 nm in DMSO.

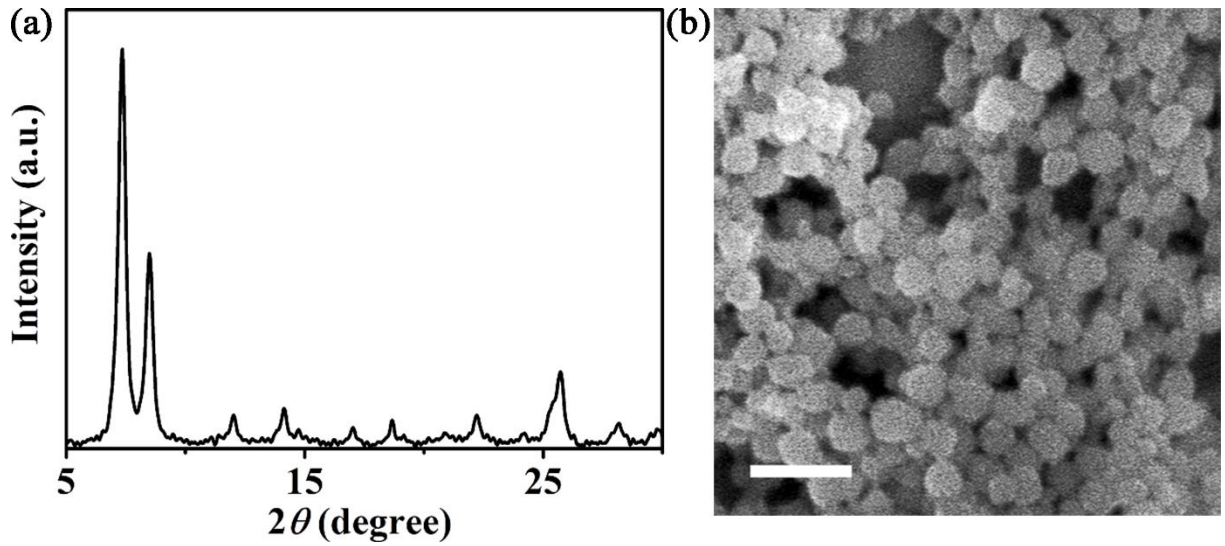

Fig. S5 (a) Powder XRD pattern and (b) SEM image of drug loaded UiO-66-SS-MP. Scale bar: 100 nm.

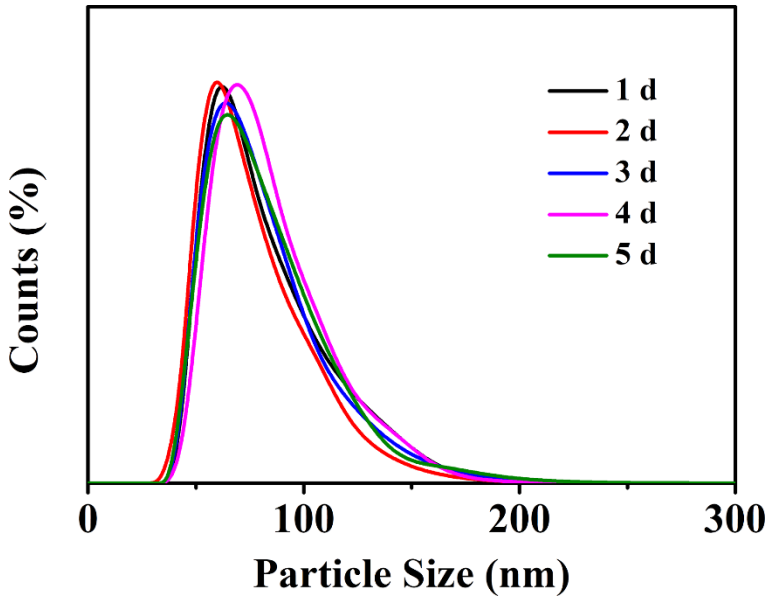

Fig. S6 Long-term dispersities of drug loaded UiO-66-SS-MP suspended in HEPES (pH = 7.4).

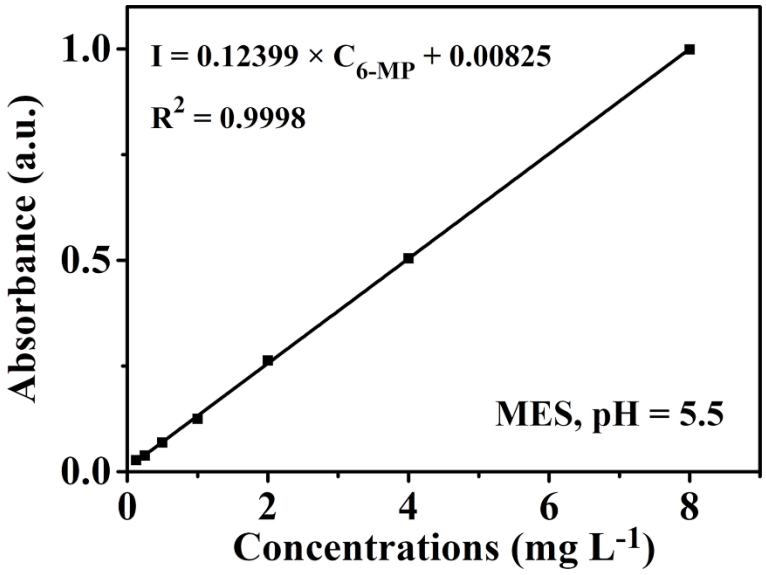

Fig. S7 Linear fitting of 6-MP absorbance at 324 nm in MES buffer solution (pH = 5.5).

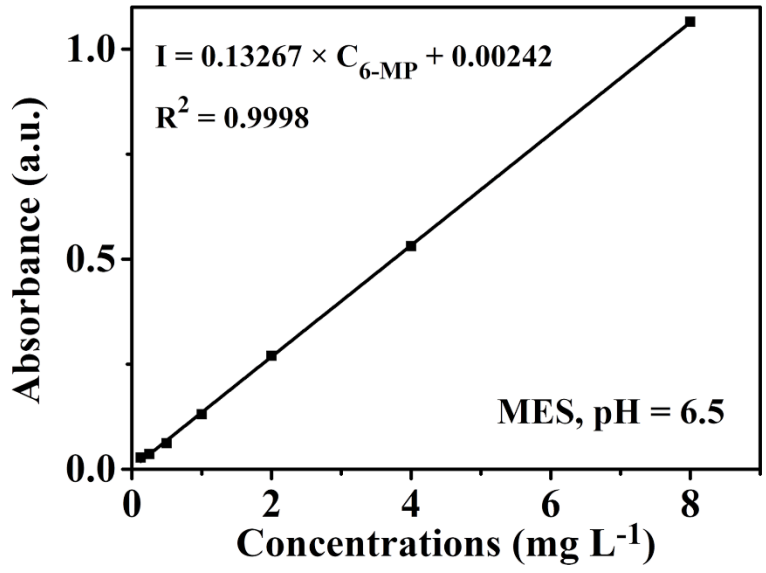

Fig. S8 Linear fitting of 6-MP absorbance at 324 nm in MES buffer solution (pH = 6.5).

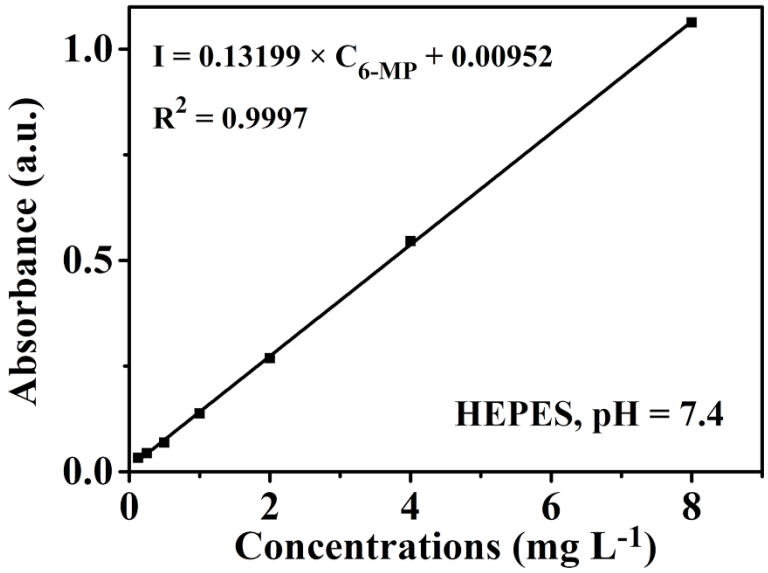

Fig. S9 Linear fitting of 6-MP absorbance at 324 nm in HEPES buffer solution (pH = 7.4).

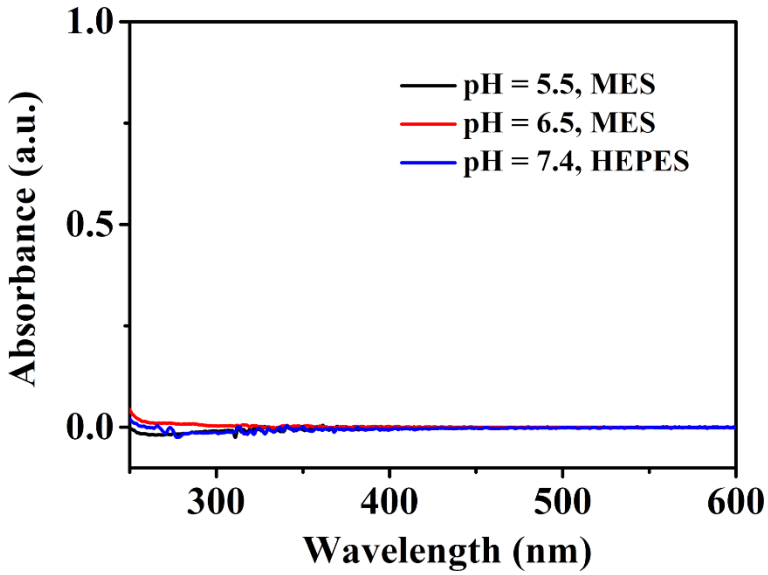

Fig. S10 UV-vis spectra of GSH molecule in different buffer solutions.

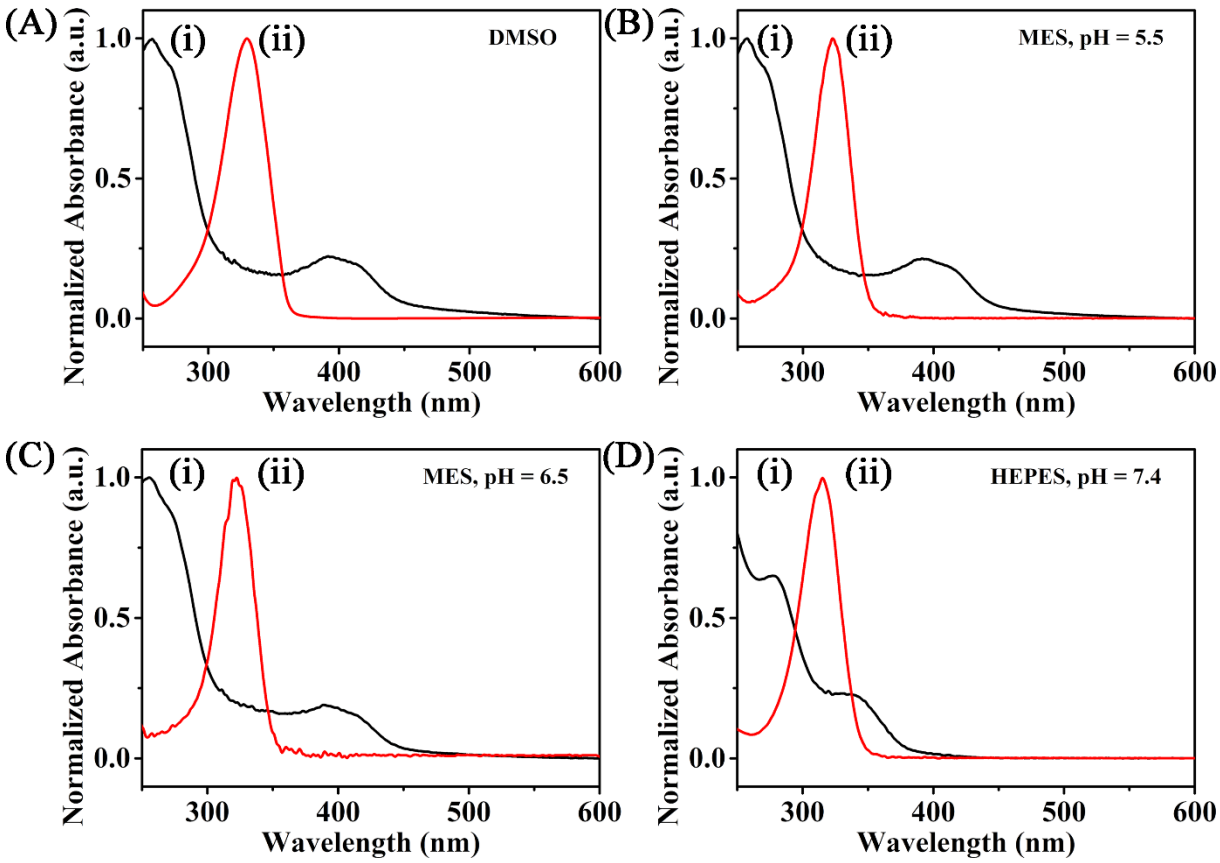

Fig. S11 UV-vis spectra of (i) BDC-$(SH)_2$ and (ii) 6-MP in different solutions: (A) DMSO, (B) pH =5.5 MES, (C) pH = 6.5 MES and (D) pH = 7.4 HEPES.

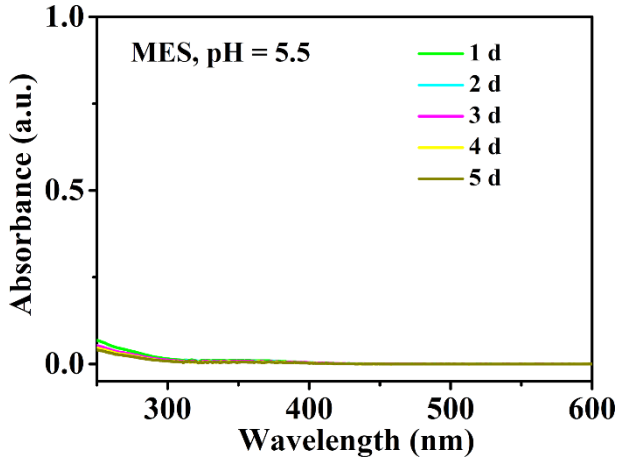

Fig. S12 UV-vis spectra of the supernatants of UiO-66-$(SH)_2$ after 5d-soaking in pH = 5.5 MES with 5 mM GSH.

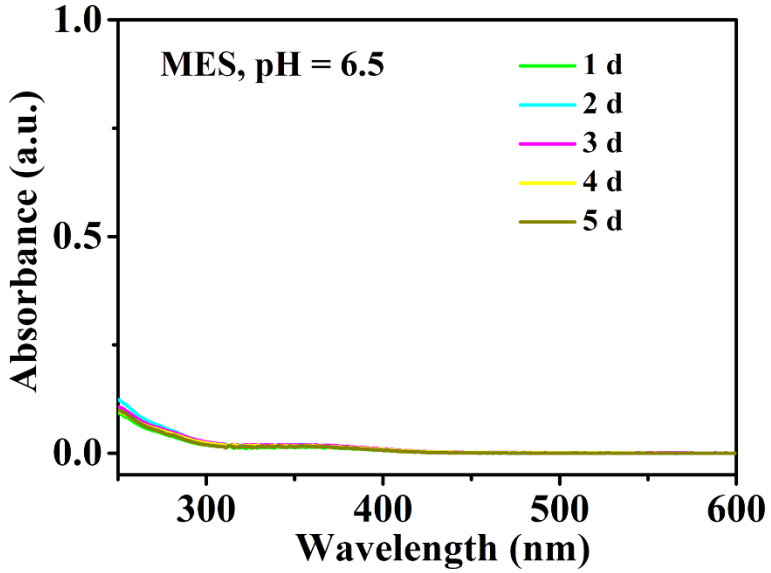

Fig. S13 UV-vis spectra of the supernatants of UiO-66-$(SH)_2$ after 5d-soaking in pH = 6.5 MES with 5 mM GSH.

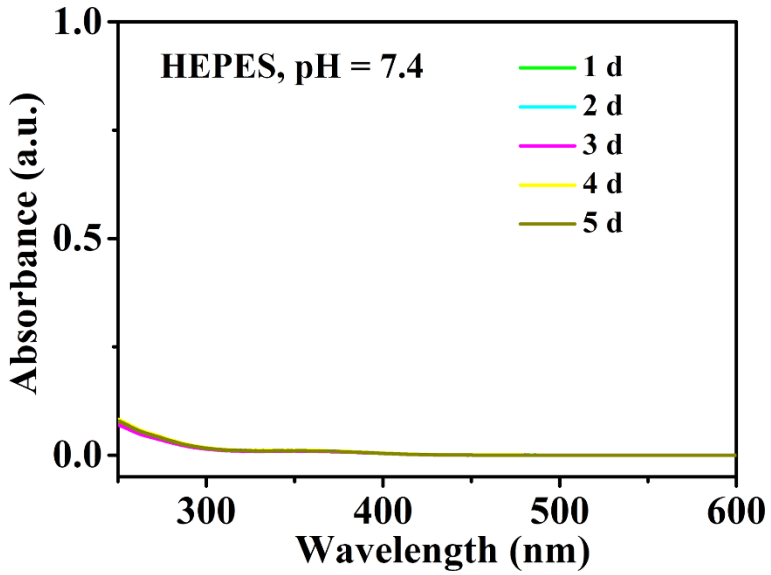

Fig. S14 UV-vis spectra of the supernatants of UiO-66-$(SH)_2$ after 5d-soaking in pH = 7.4 HEPES with 5 mM GSH.

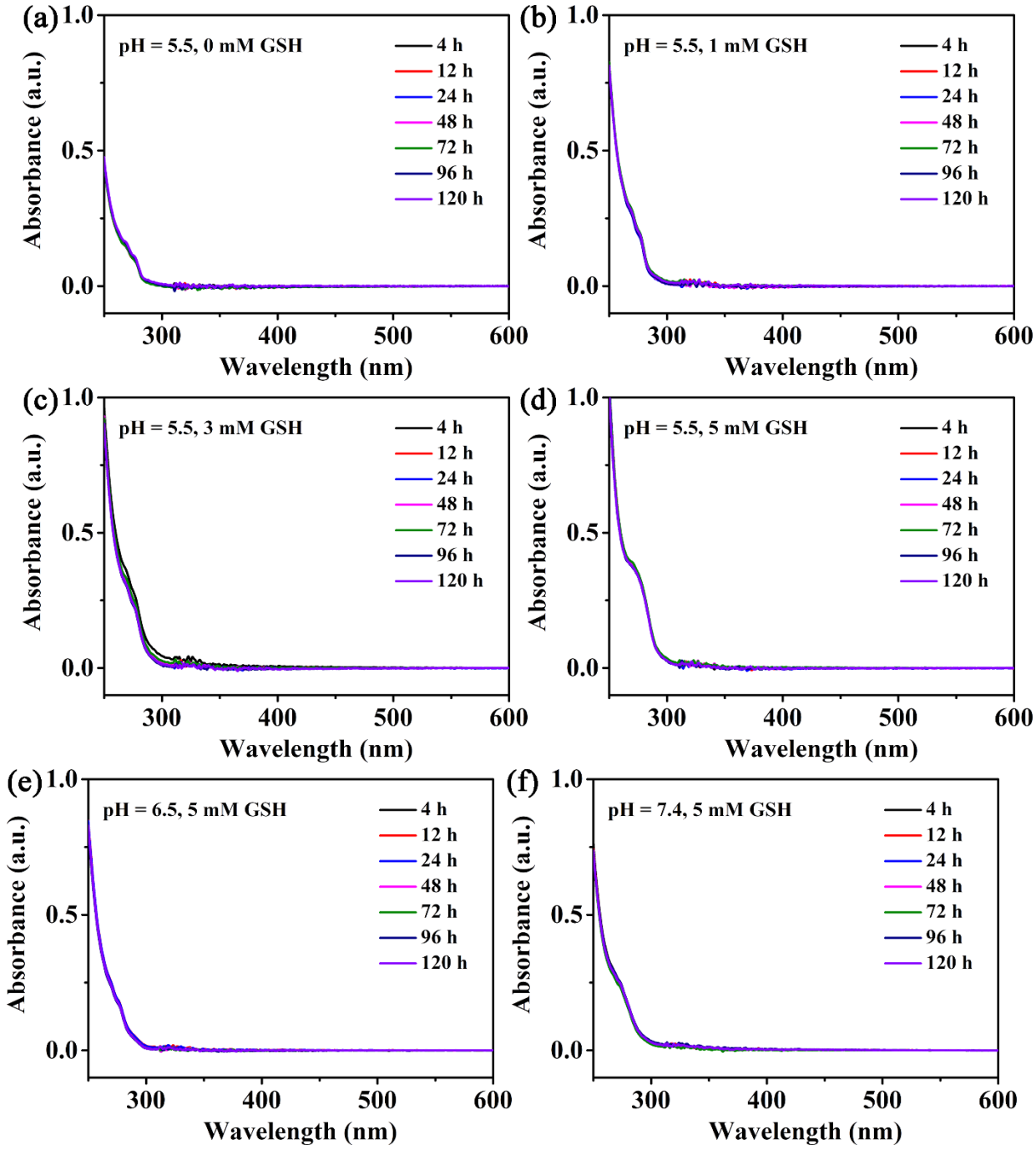

Fig. S15 UV-vis spectra of the supernatants of UiO-66-MP in different releasing environments: (a) pH = 5.5 MES with 0 mM GSH, (b) pH = 5.5 MES with 1 mM GSH, (c) pH = 5.5 MES with 3 mM GSH, (d) pH = 5.5 MES with 5 mM GSH, (e) pH = 6.5 MES with 5 mM GSH and (f) pH = 7.4 HEPES with 5 mM GSH.

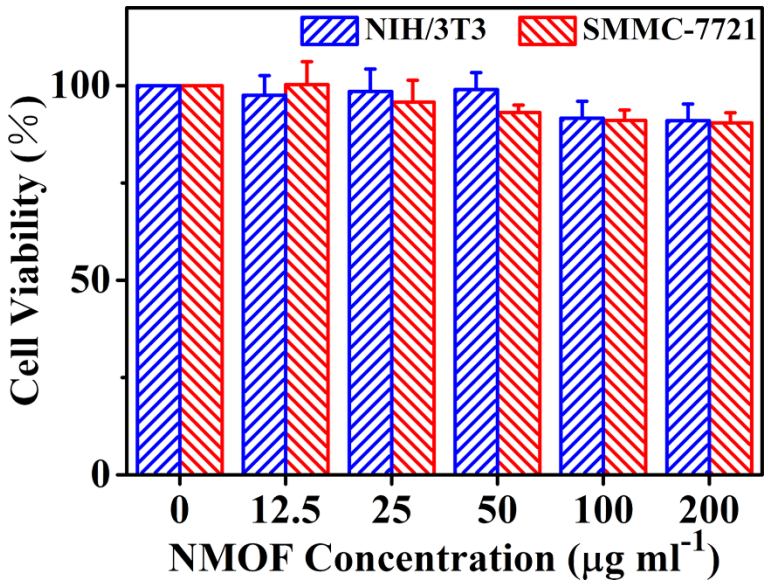

Fig. S16 Cell viabilities of UiO-66-$(SH)_2$ against NIH/3T3 normal cells and SMMC-7721 cancer cells at different concentrations (0-200 μg mL^{-1}) with the incubation time of 24 h.

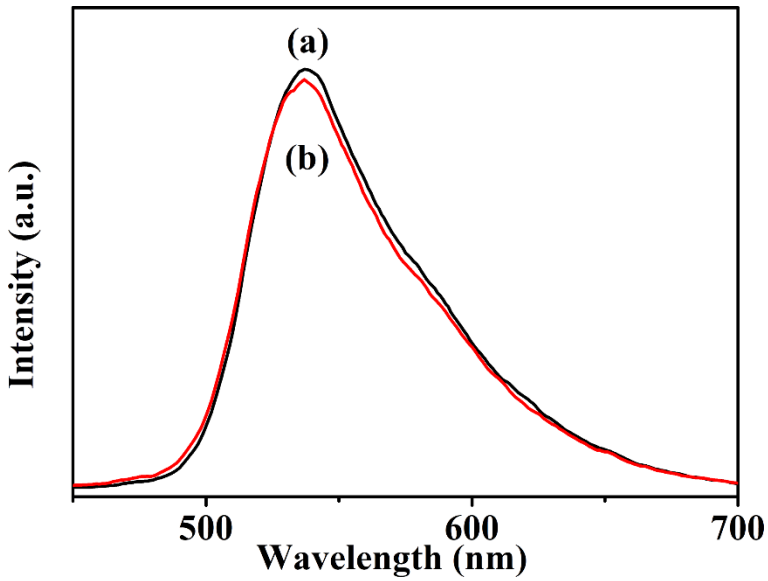

Fig. S17 Fluorescent spectra of FMN@UiO-66-SS-MP (a) before and (b) after the soaking in water for 24 h.

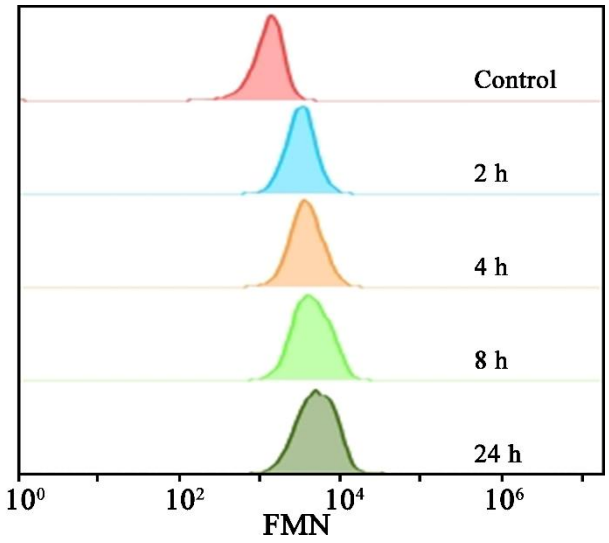

Fig. S18 Flow cytometric analysis of SMMC-7721 cancer cells after their incubation with FMN@UiO-66-SS-MP at the concentration of 25 μg mL^{-1} for 2, 4, 8 and 24 h.

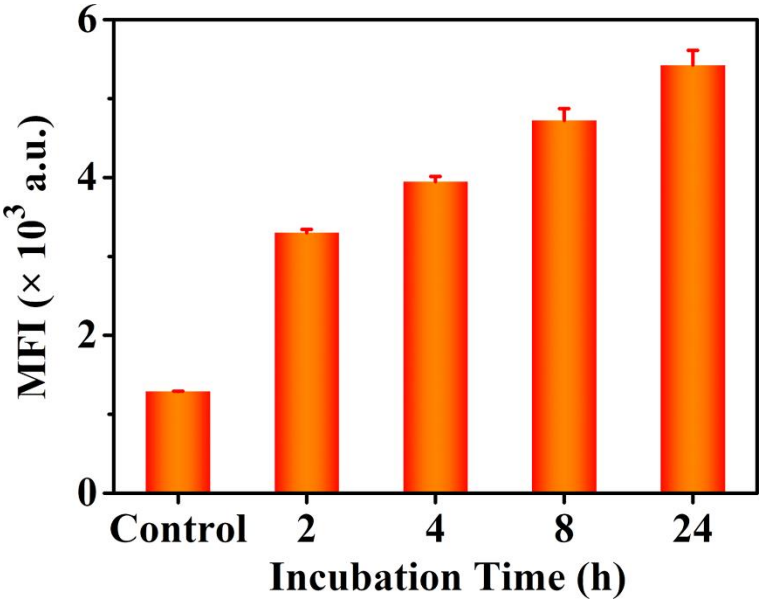

Fig. S19 The mean fluorescent intensity (MFI) of FL2-A channel in SMMC-7721 cancer cells, after incubation with FMN@UiO-66-SS-MP under different times of 2, 4, 8 and 24 h.

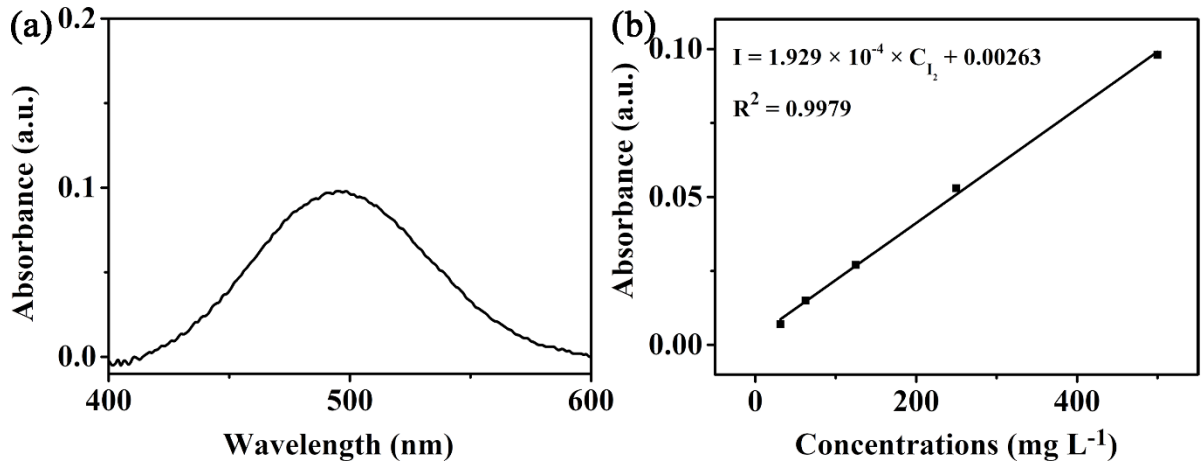

Fig. S20 There might be I^- in the final products, which could be oxidized to I_2 by concentrated sulfuric acid (H_2SO_4). (a) UV-vis spectrum of I_2 in H_2SO_4 (conc.). (b) Linear fitting of I_2 absorbance at 495 nm in H_2SO_4 (conc.).

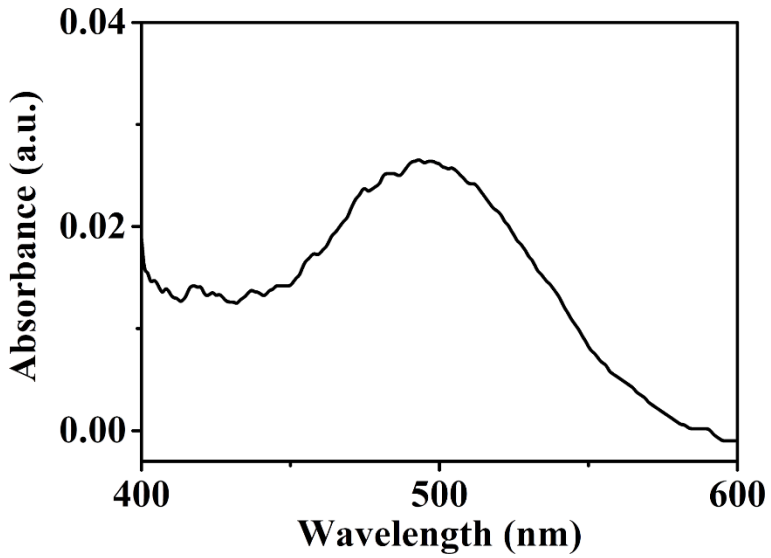

Fig. S21 UV-vis spectrum of H_2SO_4 (conc.)-treated UiO-66-MP. The concentration of I in the MOF is calculated to be 72 mg g^{-1}.