Supporting information

For

Self-quenching Synthesis of Coordination Polymer Pre-drug Nanoparticles for Selective Photodynamic Therapy

Yucong Song,[a,b] Yite Li,[a,b] Yuandong Zhang,[c] Lei Wang*,[a,b] and Zhigang Xie*,[a,b]

[a]State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P. R. China. Email: leiwang@ciac.ac.cn. Fax/Tel: +86 431 85262779.

[b]University of Science and Technology of China, Hefei 230026, P. R. China.

[c]Department of Chemistry, Northeast Normal University, 5268 Renmin Street, Changchun, 130024, P. R. China.

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Experimental section

Materials and instruments. Live-Dead Cell Staining Kit was purchased from Nanjing KeyGen Biotech Co., Ltd. MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazoliumbromide) and DCFH-DA ROS Detection Kit was purchased from Beyotime Biotechnology Co., Ltd. IBDP was prepared in our lab. All the other chemical materials and organic solvents used were purchased from commercial suppliers and used without further purification unless otherwise noted.

TEM images were recorded by JEOL JEM-1400 electron microscope (acceleration voltage of 120kV). The hydrodynamic size distribution and zeta potential of the nanoparticles were measured using the Malvern Zetasizer-Nano ZS90 instrument. FTIR was measured by Nicolet Impact 410 Fourier transform infrared spectrometer. UV-Vis absorption spectra were monitored with a Shimadzu UV-2450 PC UV/Vis spectrophotometer. XPS were obtained on a Thermo Scientific ESCALAB 250 Multitechnique Surface Analysis. TGA was performed using a NetzchSta 449c thermal analyzer system at a rate of 10 ℃ min^{-1} under air atmosphere. Inductively coupled plasma mass spectrometry (ICP-MS) was carried out on an Agilent 7700x series ICP-MS instrument.

Synthesis of IBDP.

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 our previous work.[1]

Preparation of Fe-IBDP NPs. 1 mL fresh stock solutions of IBDP dissolved in THF (concentration of IBDP, 500 μg/mL) was rapidly rejected into 10 mL aqueous

solution of ferric chloride (concentration of Fe^{3+}, 2.5 mM) with a syringe under vigorous stirring. After stirring overnight, the excess reactants were finally removed by dialysis using the dialysis bag (molecular weight cutoff: 3.5 kDa), and the purified nanoparticles were obtained by lyophilization.

Stimuli-Response of the Fe-IBDP NPs. The newly prepared nanoparticles were dispersed in aqueous solution, co-incubated with 1mM H_2O_2, 5mM GSH, 1mM HCl (pH = 5.0) and all the three conditions together in a 37°C air bath for 5 hours. DLS, TEM images and UV-vis spectra of the differently treated nanoparticles were determined.

$^1O_2$ generation measurements. To detect the $^1O_2$ generation performance of the Fe-IBDP NPs and IBDP, ABDA as the frequently-used reactive oxygen species trap was employed and the whole process was monitored by the UV-Vis spectroscopy. Free IBDP molecules (2 μg mL^{-1}) blended with ABDA (30 μg mL^{-1}) was dissolved in DMF and then irradiated with a 540 nm LED at a power intensity of 10 mW cm^{-2}. The absorption intensity of the ABDA at the wavelength of 400 nm was detected at different time points. The same experiments were done with free ABDA in DMF as a control group. The ROS generation ability of the Fe-IBDP NPs were also evaluated by the same method (540 nm, 10 mW cm^{-2}). Before light irradiation, Fe-IBDP NPs (concentration of IBDP, 2 μg mL^{-1}), nanoparticles co-incubated with 1mM H_2O_2, 5mM GSH, 1mM HCl (pH = 5.0) or all the three conditions together were blended with ABDA (30 μg mL^{-1}) in H_2O at room temperature in dark for 20 min. the absorption of ABDA at 400 nm of each groups were detected at different time points

with the extension of illumination time. The same experiments were done with free ABDA in H_2O as blank control.

Cell culture. L929 and HeLa cells were propagated to confluence in the Dulbecco modified Eagle medium (DMEM) supplemented with 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 10% FBS, and maintained at 37°C in a humidified atmosphere of 5% CO_2 for further cell experiments.

Endocytosis performance. Endocytosis performance of Fe-IBDP NPs by HeLa cells was monitored by using the inductively coupled plasma mass spectrometry (ICP-MS). Firstly, HeLa cells were employed to be incubated with Fe-IBDP NPs (concentration of IBDP, 1 μg mL^{-1}) at 37°C for 0.5, 2, and 6 h, respectively, and then, the culture medium was removed and the cells were washed three times by PBS solution. The adherent cells were treated with pancreatic digestion and treated with HNO_3, H_2O_2 and H_2O (v/v/v = 1:1:2) at 80°C for 8 h until a clear solution formed. The concentration of Fe was measured by ICP-MS. Endocytosis performance of Fe-IBDP NPs (concentration of IBDP, 1 μg mL^{-1}) for 2 h by HeLa cells at 4 and 37°C were also evaluated by the same method.

Intracellular ROS generation. Intracellular ROS generation was carried out by using the CLSM observation method. HeLa cells were cultured in DMEM at 37°C under a 5% CO_2 atmosphere according to standard cell culture protocols. HeLa cells were seeded at a density of 5×10^4 cells/well in six-well plates and incubated for 24 h. Then, the medium was replaced with 1mL of DMEM containing the Fe-IBDP NPs (concentration of IBDP, 0.5 μg mL^{-1}), futher incubated for 6 h at 37°C. Light

irradiation at 540 nm (10 mW cm^{-2} for 20 min) was employed to two of the plates (with and without NPs pretreated groups) subsequently. Then, DMEM containing DCFH-DA (10×10^{-6} M) solution was added and further incubated for 30 min. The other two plates (with and without NPs pretreated) stayed in dark environment. The fluorescence photos of DCF induced by adding the nanoparticles were observed with CLSM immediately (excitation wavelength, 488 nm; emission band-pass, 500−550 nm).

MTT assay. L929 and HeLa cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 10^5 cells per well and incubated in DMEM for 24 h. The medium was then replaced by 200 μL of DMEM containing predetermined concentrations of the Fe-IBDP NPs, and the cells were incubated for another 6 h, followed by 540nm light irradiation (10 mW cm^{-2}, 30 min). After 24 h incubation, MTT assays were conducted to measure the live cells. 20 μL PBS solution of 5 mg/mL MTT was added into every well, incubating for another 4 h, the culture medium containing MTT was removed, adding 150 μL DMSO to each well to dissolve the formed formazan crystals. Subsequently, cell viabilities were determined by reading the absorbance of the plates at 490 nm with a microplate reader. The same assays were done against L929, HeLa, A549 and HepG2 without light irradiation to evaluate the biocompatibility of the nanoparticles.

Live-Dead cell staining test. L929 and HepG2 cells were co-stained with propidium iodide (PI) and calcein-AM (AM) to distinguish the dead (red) cells from the live (green) cells. L929 and HeLa cells harvested in a logarithmic growth phase were

seeded in 96-well plates at a density of 10^5 cells per well and incubated in DMEM for 24 h. The medium was then replaced by 200 μL of DMEM containing predetermined concentrations of the Fe-IBDP NPs, and the cells were incubated for another 6 h, followed by 540 nm light irradiation (10 mW cm^{-2}, 30 min). The cells incubated with NPs and no light irradiation, treated by light irradiation and no NPs and without any treatment were as three control groups. After 24 h incubation, all the plates were stained with AM and PI for 30 min in dark and washed with PBS, and the obtained various cell samples were imaged with a fluorescence microscope.

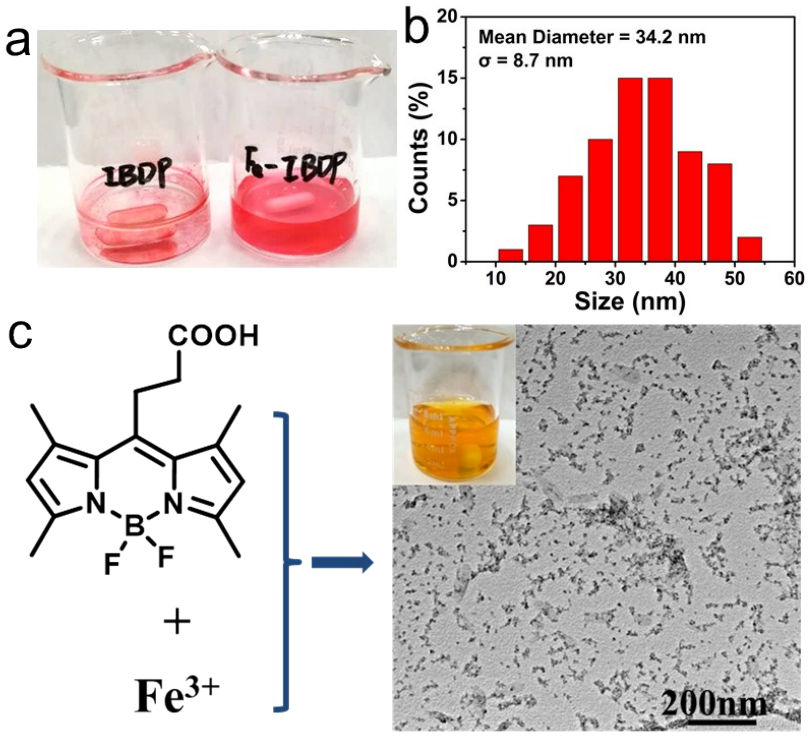

Fig. S1 (a) The photograph of IBDP in a THF solution rejected into water (left) or ferric aqueous solution (right). (b) Size distribution of the obtained Fe-IBDP NPs. (c) Schematic Illustration of the preparation of Fe-BDP NPs and its TEM image with the picture of the sample shown in the inset.

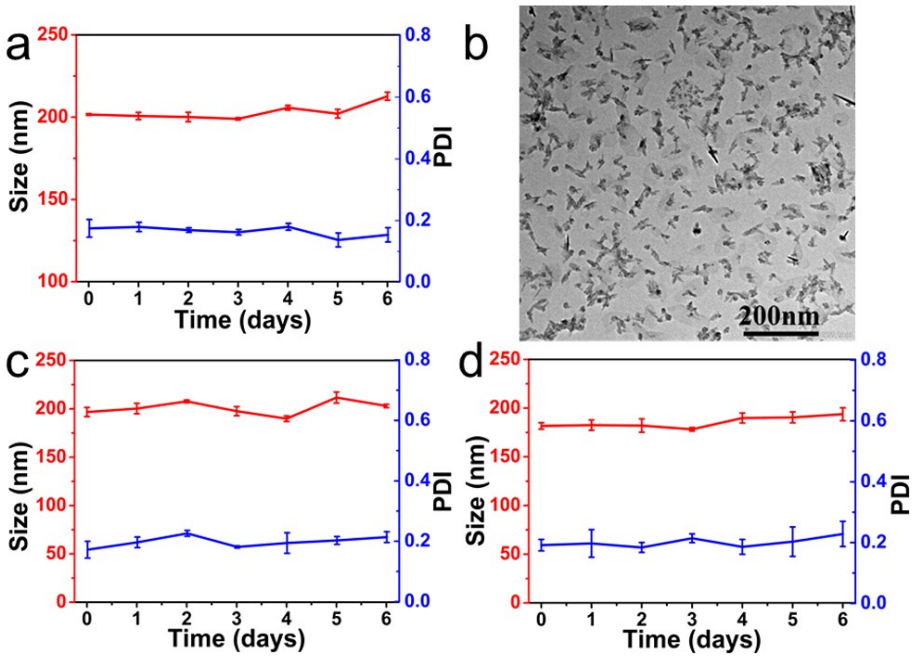

Fig. S2 (a) Changes in hydrodynamic sizes and PDI of Fe-IBDP NPs stored in water for seven days. (b) TEM image of the Fe-IBDP NPs after being stored in water at 4°C for two months. (c) Changes in hydrodynamic sizes and PDI of Fe-IBDP NPs during incubation in a PBS buffer (pH 7.4) at 37°C for seven days. (d) Changes in hydrodynamic size and PDI of Fe-IBDP NPs during incubation in a PBS buffer (pH 7.4) supplemented with 10% (v/v) FBS at 37°C for seven days.

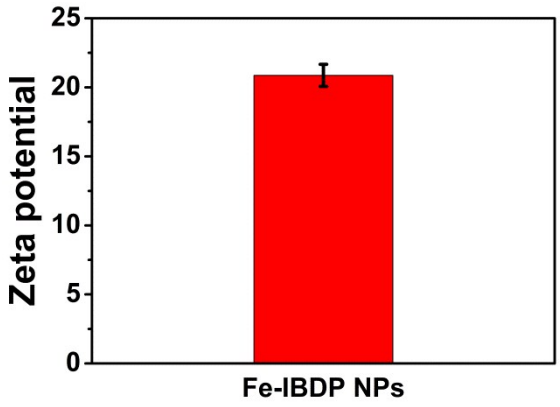

Fig. S3 Zeta potential of Fe-IBDP NPs dispersed in water.

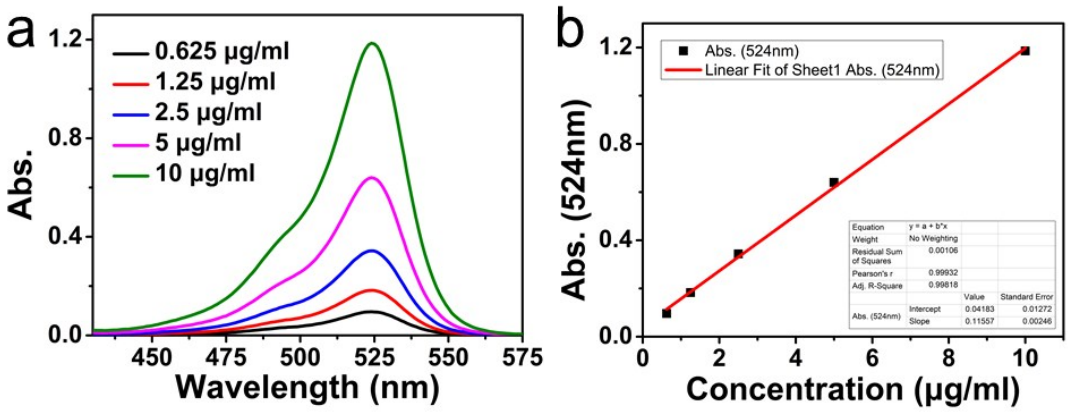

Fig. S4 UV−vis absorption (a) and standard curve (b) of IBDP in mixed solvents of DMF/Water = 9:1.

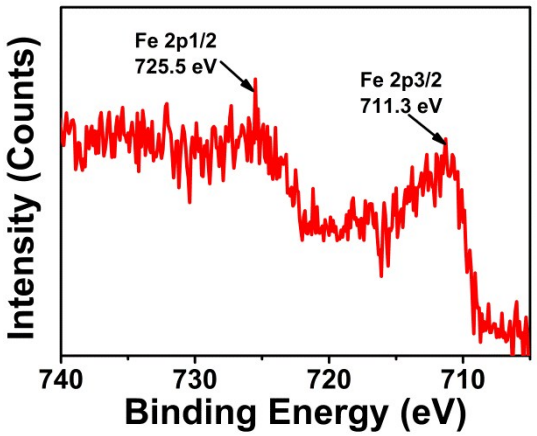

Fig. S5 Fe 2p XPS spectrum of the Fe-IBDP NPs.

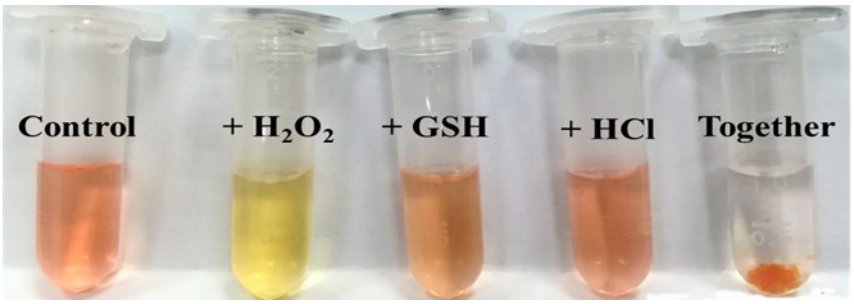

Fig. S6 Photograph of samples of the Fe-IBDP NPs after being incubated with 1mM H_2O_2, 5mM GSH, 1mM HCl (pH = 5.0) and all the three conditions together in a 37°C air bath for 5 hours.

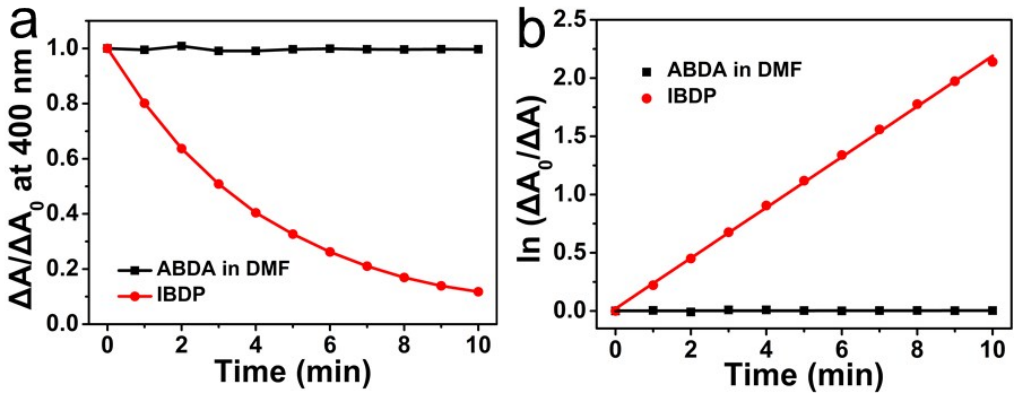

Fig. S7 (a) The measurement of $^1O_2$ production of IBDP. (b) Time-dependent $^1O_2$ generation kinetics. ΔA_0 = the difference value between absorbance of ABDA + IBDP and IBDP without irradiation. ΔA = the difference value between real-time absorbance of ABDA + IBDP and IBDP with different irradiation time.

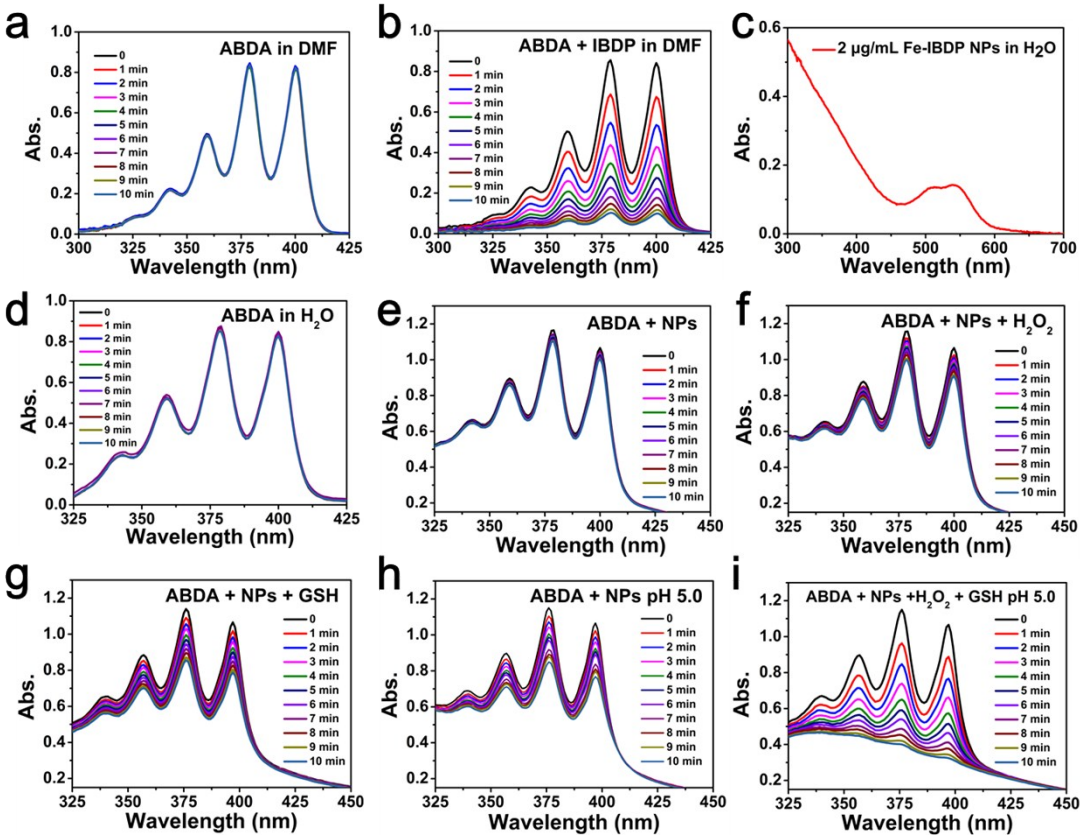

Fig. S8 Ultraviolet absorbance variations of ABDA in DMF with (b) and without (a) IBDP under light irradiation (540 nm, 10 mW cm^{-2}). (c) UV-vis absorption of the measured concentration Fe-IBDP NPs (2 μg mL^{-1}). ultraviolet absorption variations of ABDA in H_2O with (e) or without (d) the Fe-IBDP NPs, ABDA and nanoparticles with H_2O_2 (f), GSH (g), HCl (pH 5.0) (h) and with all the three conditions together (i) under light irradiation (540 nm, 10 mW cm^{-2}).

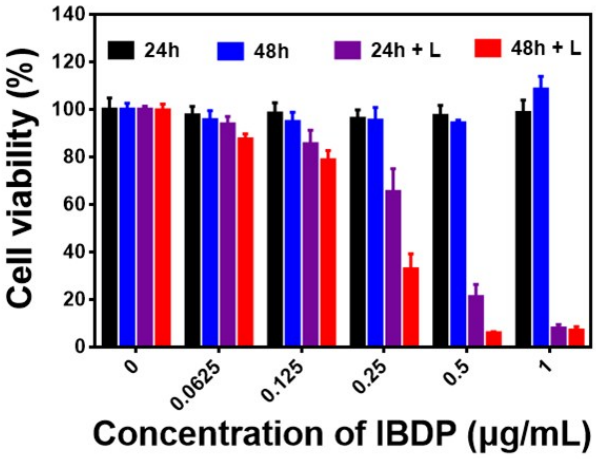

Fig. S9 Viability of HeLa cells cultured with different concentrations of Fe-IBDP NPs with or without light irradiation for 24 h and 48 h.

References

1 W. Wang, L. Wang, Z. Li and Z. Xie, *Chem Commun (Camb)*, 2016, **52**, 5402-5405.