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Electronic Supplementary Information (ESI)

Harmonization of upconverting nanocrystals and photosensitizer for antimicrobial application†

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DETAILED METHODS

S1. Materials

$YCl_3 \cdot 6H_2O$ (99.99%), $YbCl_3 \cdot 6H_2O$ (99.99%), $TmCl_3 \cdot 6H_2O$ (99.99%), oleic acid (90%), 1-octadecene (90%), ammonium fluoride, sodium hydroxide and Poly(ethylene oxide)bis(carboxymethyl)ether (PEO-600-diacid, average M_n 600), sodium azide and 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) were purchased from Sigma-Aldrich (Steinheim, Germany). All chemicals were used as received without any further purification.

S2. Analytical instruments

^{1}H-NMR spectra were obtained in $CDCl_3$ at 400 MHz (Varian Company, USA). Chemical shifts (δ) are reported in parts per million (ppm) relative to the residual $CHCl_3$ peak (7.26 ppm). Coupling constant (*J*) are reported in Hertz (Hz). Mass spectra were obtained using matrix-assisted laser desorption ionization (MALDI) mass spectrometry with dithranol as a matrix. Absorption spectra were recorded in toluene at room temperature by a Hewlett-Packard 8453 spectrophotometer, and

absorption extinction coefficient (ε) was reported in L/mol·cm. Fluorescence spectra were measured in toluene at room temperature using a Perkin-Elmer LS45 luminescence spectrophotometer.

S3. Synthesis of upconverting nanocrystals

To make core $NaYF_4$:Yb^{3+},Tm^{3+}, the desired amount of $YCl_3 \cdot 6H_2O$, $YbCl_3 \cdot 6H_2O$ and $TmCl_3 \cdot 6H_2O$ were added to a mixed solution of oleic acid (24 mL) and octadecene (60 mL) in a three-neck round-bottom flask. The mixture was heated to 130°C with magnetic stirring for 40 min under vacuum until form a slightly yellow solution and followed by natural cooling to room temperature. Next, a solution of sodium hydroxide (10 mmol) and ammonium fluoride (16 mmol) in methanol (20 mL) was added drop-wise to the obtained solution and stirred for 40 min at room temperature. The mixed solution was then allowed to heat at 75°C for methanol evaporation. After methanol was evaporated, the reaction temperature was increased to 300 °C as fast as possible with continuous stirring for 90 min under the nitrogen gas flow. The resulting solution was cooled down to room temperature and precipitated by adding of ethanol. The precipitates were collected by centrifugation (GL21M Changsha Yingtai instrument, Hunan, China) at 5000 rpm (2688 rcf) and repeatedly washed with ethanol.

To make core/shell structure $NaYF_4$:Yb^{3+},Tm^{3+}/$NaYF_4$, $YCl_3 \cdot 6H_2O$ (2.0 mmol) was added to a three-neck round-bottom flask containing oleic acid (12 mL) and octadecene (30 mL). The reaction mixture was heated to 130 °C with magnetic stirring for 40 min under vacuum. When reaction temperature was cooled down to 80 °C, the dispersion of core structure nanocrystals in chloroform was allowed to add under nitrogen atmosphere. The mixed solution was heated to 110 °C in order to evaporate the chloroform and then cooled down to 50 °C. A methanol solution (20 mL) containing sodium hydroxide (5.0 mmol) and ammonium fluoride (8.0 mmol) was added into the reaction solution and further stirred for 30 min. Next, the reaction temperature was increased to 75°C to evaporate the methanol and rapidly increased to 300°C for 90 min under the gentle nitrogen flow. The obtained mixture was cooled down to room temperature and precipitated by addition of ethanol. The precipitation were isolated via centrifugation at 5000 rpm (2688 rcf) and washed with ethanol several times.

To make poly(ethylene oxide) coated core/shell $NaYF_4$:Yb^{3+},Tm^{3+}/$NaYF_4$, the core/shell structure upconverting nanocrystals (50 mg) were added into a glass vial containing PEO-600-diacid ligand (125 mg) and ethanol (5 mL). The vial was capped, sonicated and then heated at 75 ˚C overnight. The solution was left to cool down at room temperature before hexane was added drop-wise for precipitation. The PEO coated core/shell structure $NaYF_4$: Yb^{3+}, Tm^{3+} /$NaYF_4$ upconverting nanocrystals were collected by centrifugation at 8000 rpm (6880 rcf) and washed with ethanol repeatedly.

S4. Characterization

X-ray diffraction (XRD). XRD patterns of dried particles were recorded on a DMAX 2200/Ultima+ diffractometer (Rigaku, Tokyo, Japan) with a scan range of 10°- 80° at scanning rate of 1°/min, using Cu Kα radiation and operating at 40 kV and 30 mA.

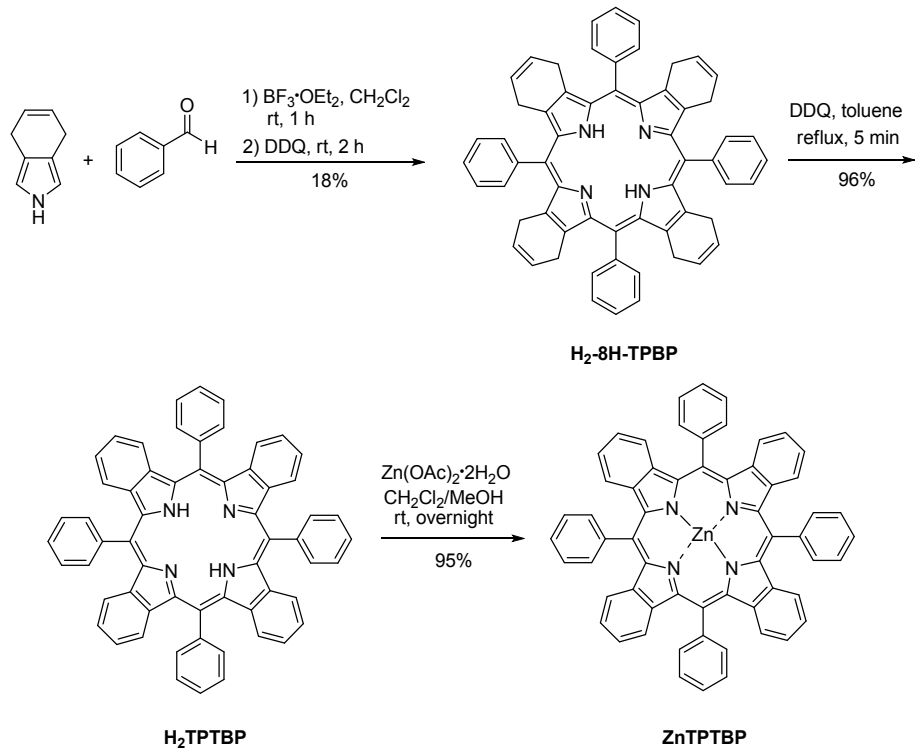
Inductively coupled plasma optical emission spectrometry (ICP-OES). The Y^{3+}, Yb^{3+} and Tm^{3+} contents in $NaYF_4$: Yb, Tm samples were analyzed using an iCAP 6500 ICP emission spectrometer (Thermo Scientific, MA, USA). Five milligrams of as-synthesized sample was digested in concentrated nitric acid at 80 °C for 20 h and diluted with milliQ water before analysis. Calibration was performed by analyzing serial dilutions of standard elements.

Transmission electron microscopy (TEM). Particle shapes and sizes were determined by TEM technique. The samples were prepared by drying a suspension drop on carbon coated copper grids. TEM photographs were obtained using a TECNAI 20 TWIN transmission electron microscope (FEI Company, OR, USA) operating at 120 kV. The average particles size was measured using SemAfore program.

Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR). ATR-FTIR spectra were analyzed on a Nicolet 6700 FT-IR spectrometer coupled with diamond ATR (Thermo Scientific, MA, USA). The spectra were recorded at resolution of 2 cm^{-1} and 64 numbers of scans.

Dynamic light scattering (DLS). The hydrodynamic diameters, polydispersity index (PDI) and zeta potentials of particles were measured by DLS. The measurements were performed on a S4700 Zetasizer nanoseries (Malvern Instruments, Worcestershire, UK) using a He−Ne laser beam at 632.8 nm and scattering angle of 173°.

S5. Synthesis of *meso*-Tetraphenyltetrabenzoporphyrinatozinc (ZnTPTBP).



Scheme S1. Synthesis of ZnTPTBP

Following a previous published procedure,[23] to a 250 mL round-bottomed flask equipped with a nitrogen inlet. 4,7-Dihydro-2*H*-isoindole (0.30 g, 2.5 mmol) and benzaldehyde (0.26 mL, 2.5 mmol) were dissolved in dichloromethane (250 mL). The solution was stirred for 10 min in the dark at room temperature and then treated with BF_3·OEt_2 (63 μL, 0.50 mmol) in one portion. After 1 h, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.63 g, 2.8 mmol) was added to the solution and the reaction was stirred for additional 2 h. The resulting solution was washed with 10% sodium sulfite (100 mL), water (100 mL) and brine (100 mL) and dried over anhydrous sodium sulfate. After removal of the solvent, the crude product was purified by a silica column (dichloromethane to 10% ethyl acetate in dichloromethane as eluents to obtain 5,10,15,20-tetraphenyloctahydrotetrabenzoporphyrins (H_2-8H-TPTBP) as a green solid (94 mg, 18%). MALDI-TOF-MS: obsd 822.652 ([M^+]), calcd 822.372 ([M^+]; M = $C_{60}H_{46}N_4$). Otherspectroscopic data are consistente with those described in the literature.

Following to a published procedure,[23] a solution of H_2-8H-TPTBP (90 mg, 0.11 mmol) and dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (125 mg, 0.550 mmol) in toluene (18 mL) was refluxed for 5 min. The resulting solution was washed with 10% sodium sulfite (10 mL), water (10 mL) and brine (10 mL), and dried over anhydrous Na_2SO_4. After removal of the solvent,

the crude product was purified by a silica column using dichloromethane then 10% ethyl acetate in dichloromethane as eluents to give 5,10,15,20-tetraphenyltetrabenzoporphyrins (H_2TPTBP) as a green powder (86 mg, 96%). ^{1}H-NMR δ –1.17 (s, 2H), 7.02–7.07 (m, 4H), 7.09–7.16 (m, 4H), 7.73–7.97 (m, 16H), 8.06 (d, *J* = 7.2 Hz, 4H), 8.37 (d, *J* = 7.2 Hz, 8H); MALDI-TOF-MS obsd 814.592 ([M^+]), calcd 814.310 ([M^+]; M = $C_{60}H_{38}N_4$). Other spectroscopic data are consistent with those described in the literature.

Zinc complex of H_2TPTBP was prepared by a standard metallation method.[24] Compound H_2TPTBP (80 mg, 0.098 mmol) was dissolved in dichloromethane (32 mL) and then reacted with a solution of $Zn(OAc)_2{\cdot}2H_2O$ (108 mg, 0.49 mmol) in methanol (4 mL) at room temperature overnight. After removal of the solvent, the reaction mixture was redissolved in dichloromethane (30 mL), washed with water (30 mL), dried over anhydrous Na_2SO_4, and concentrated to dryness. Purification by column chromatography [silica, CH_2Cl_2] followed by sonicating-centrifugating process in hexanes to give ZnTPTBP as a green powder (82 mg, 95%). ^{1}H-NMR δ7.17 (dd, *J* = 6.0, 3.2 Hz, 8H), 7.29 (dd, *J* = 6.0, 3.2 Hz, 8H), 7.87 (t, *J* = 7.2 Hz, 8H), 7.95 (t, *J* = 7.2 Hz, 4H), 8.31 (d, *J* = 7.2 Hz, 8H); MALDI-TOF-MS m/z obsd 875.598[M^+], calcd 876.223 [M = $C_{60}H_{36}N_4Zn$]; λ_{abs} (*ε*, toluene) 470 (1.9×10^5), 613, 656 nm; λ_{em} (λ_{ex} = 470 nm, toluene) 666, 731 nm. Other spectroscopic data are consistent with those described in the literature.

S6. Cytotoxicity of ZnTPTBP

Cell culture. The A-375 human cancer skin cells (ATCC CRL-1619) were cultured in Dulbecco's Modified Eagle's Medium (HyClone, UT, USA) supplemented with 10% v/v fetal bovine serum (HyClone, UT, USA), 100mM sodium pyruvate (HyClone, UT, USA), 10mM HEPES (HyClone, UT, USA), 100 U/ml penicillin (General Drugs House Co., Ltd., Bangkok, Thailand) and 0.4 mg/ml streptomycin (M & H Manufacturing Co., Ltd., SamutPrakan, Thailand) at 37 ^{o}C under 5% CO_2 atmosphere (Thermoelectron 311 MA, incubator)

Cytotoxicity assay. *In vitro* cytotoxicity of PEO-UCs, ZnTPTBP and ZnTPTBP-UCs with and without a 980 nm NIR irradiation was performed on cancer human skin cell (A-375). Cells were seeded onto sterile 96-well plates at a density of $8\text{x}10^3$ cells per well and then allowed to adhere at 37 ^{o}C under 5% CO_2 overnight. Cells incubated with various concentrations of PEO-UCs, ZnTPTBP and ZnTPTBP-UCs (3-1000 μg/mL) for 24 h were used as non-NIR irradiation condition. For NIR irradiation condition, cells were incubated with PEO-UCs, ZnTPTBP and ZnTPTBP-UCs for 4 h then exposed to NIR at 560 J/cm^2. After that, the cells were further incubated until complete 24 h. Each concentration was carried out in triplicate wells. Cell viability was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The absorbance at 540 nm of dissolved formazan

products from the survival cells was measured by an Anthos 2010 microplate reader (Becthai Bangkok Equipment and Chemical Co., Ltd., Bangkok, Thailand). This assay was performed in two independent experiments.

SUPPLEMENTARY TABLE

Table S1. The relative contents of the three metals (from ICP-OES analysis) in the Tm^{3+}, Yb^{3+}-doped β-$NaYF_4$ nano-crystalline products obtained at various amounts of Tm^{3+} used in the synthesis.

Amounts of Tm^{3+} used in the synthesis (mol%)	Y^{3+} (mol%)	Yb^{3+} (mol%)	Tm^{3+} (mol%)
0.2	71.58±5.67	28.15±5.66	0.27±0.02
0.5	69.71±0.11	29.71±0.13	0.58±0.04
1.0	68.61±0.14	30.15±0.10	1.24±0.07
1.5	69.81±0.08	28.51±0.16	1.68±0.09

Table S2. Integrated peak area from upconversion emission spectra.

Wavelength (nm)	Peak area			Ratio of area (Folds)	
	Core-UCs	[Core/shell]-UCs	PEO-UCs	[Core/shell]-UCs: Core-UCs	PEO-UCs: Core-UCs
800	2434.04	11167.184	10146.529	4.6	4.2
635	412.719	2379.078	2250.108	5.8	5.5
542	111.682	758.652	695.036	6.8	6.2
450 and 475	1283.4	7468.462	7008.78	5.8	5.5
350,365	399.434	1929.586	1854.608	4.8	4.6
290	19.324	98.560	95.596	5.1	4.9

SUPPLEMENTARY FIGURES

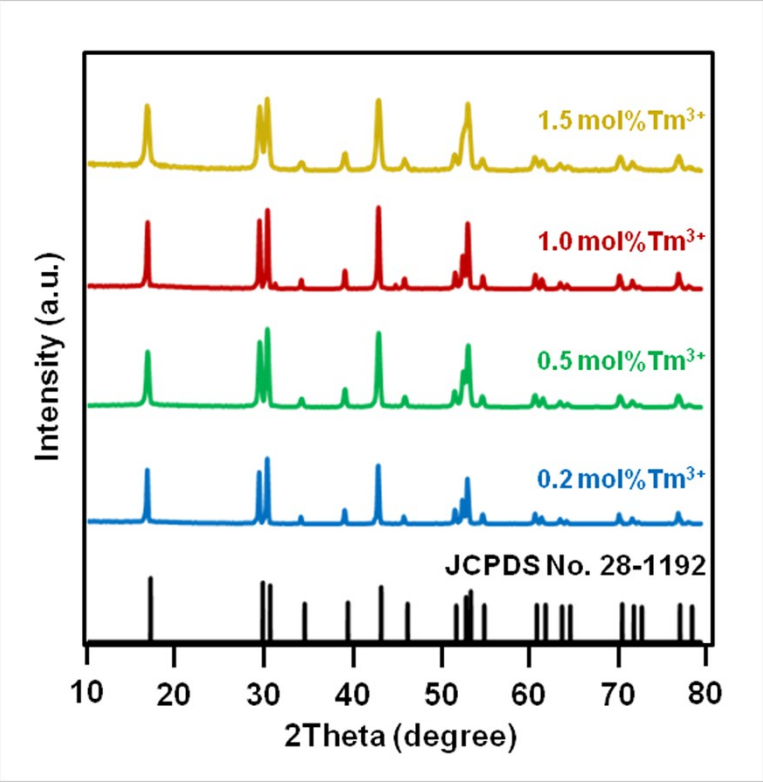


Figure S1. XRD patterns of core structure $NaYF_4$: 30mol%Yb^{3+}, *x*mol%Tm^{3+} upconverting nanocrystals (Core-UCs) with different Tm^{3+} ratios, *x*= 0.2, 0.5, 1.0 and 1.5 comparing with the standard pattern of β-$NaYF_4$ (JCPDS file No. 28-1192).

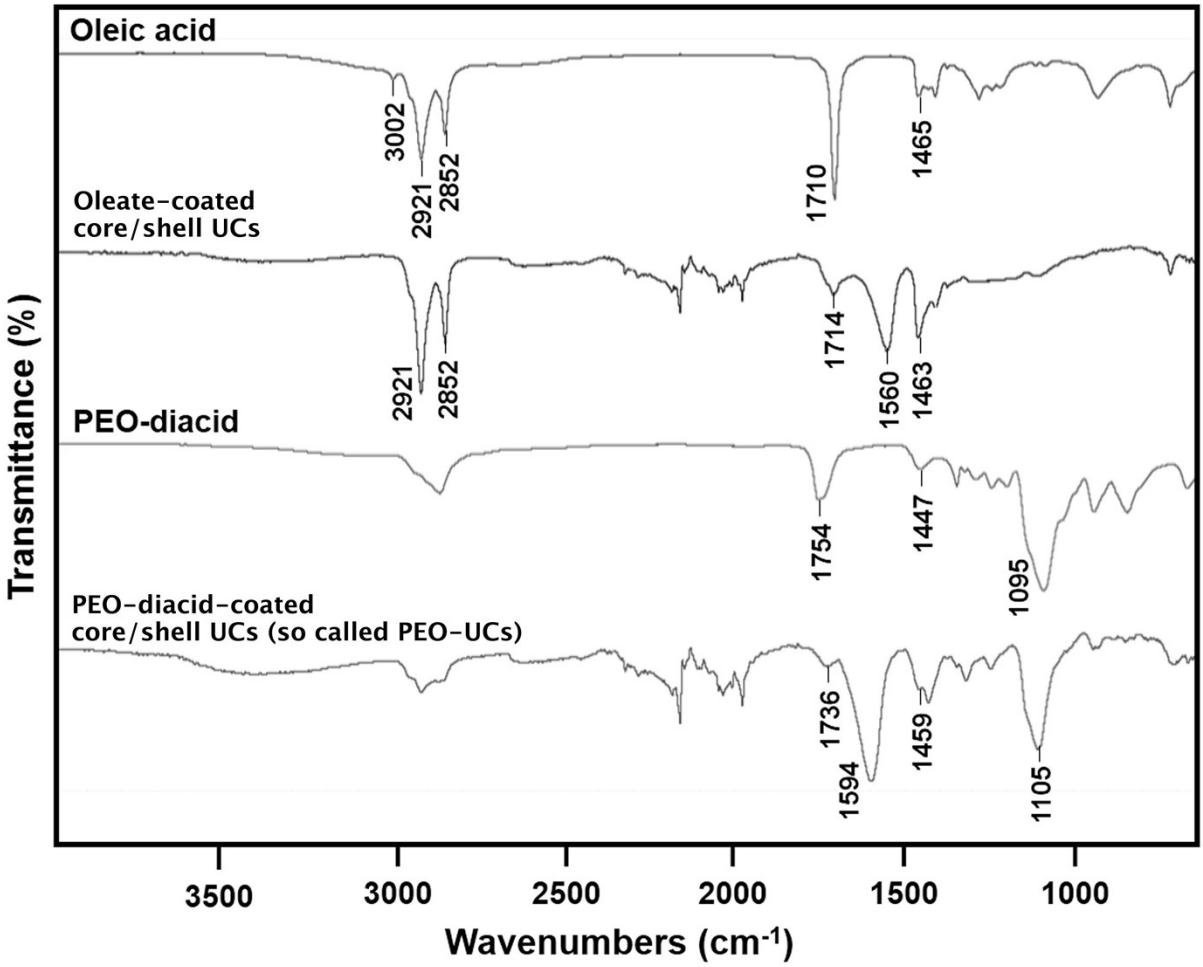


Figure S2. ATR-FTIR spectra of oleic acid ligand, oleate-UCs or oleate-coated core/shell UCs, PEO diacid ligand, and PEO-UCs or PEO diacid coated core/shell UCs.

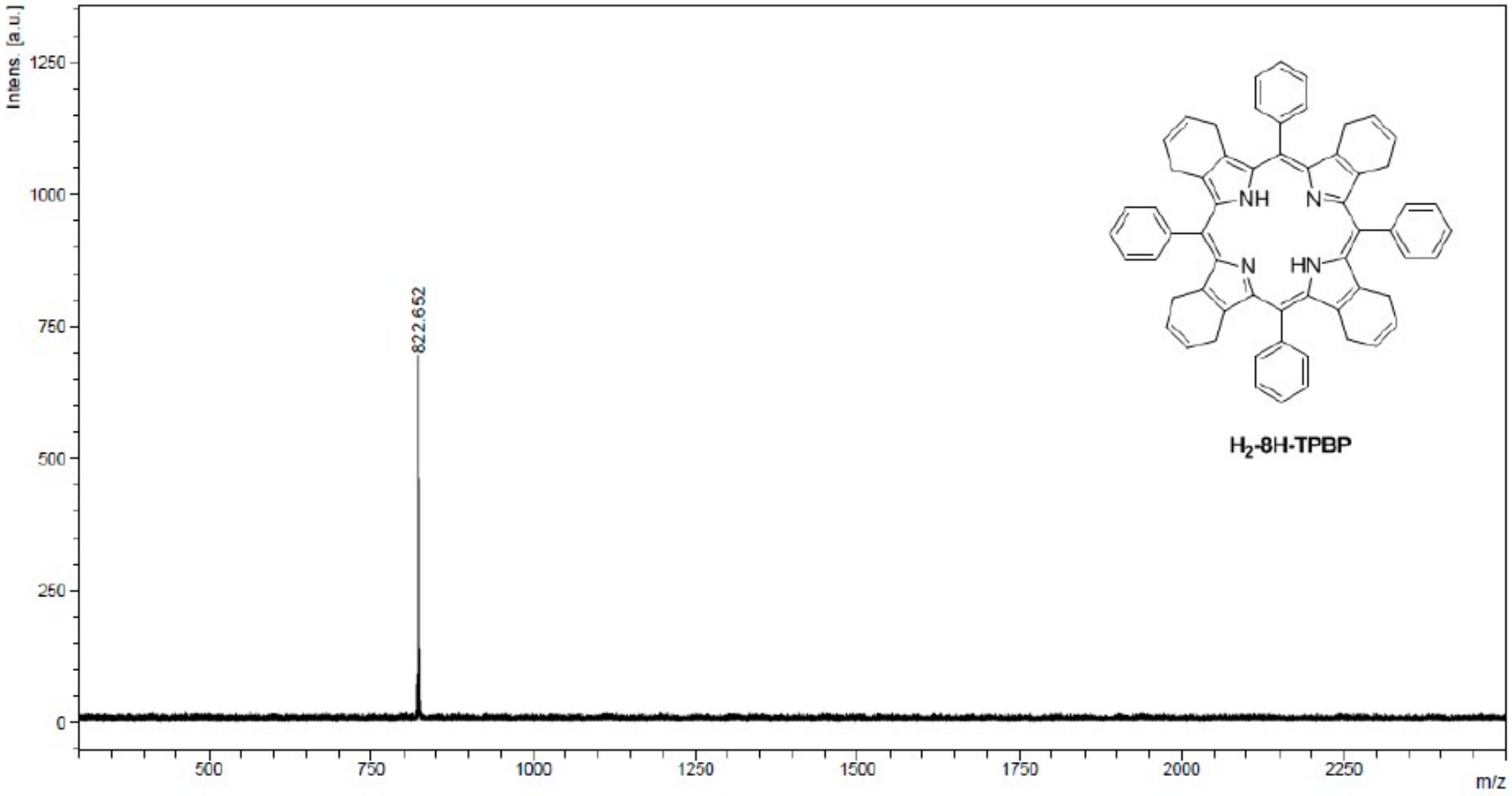


Figure S3. Mass spectrum of H_2-8H-TPTBP

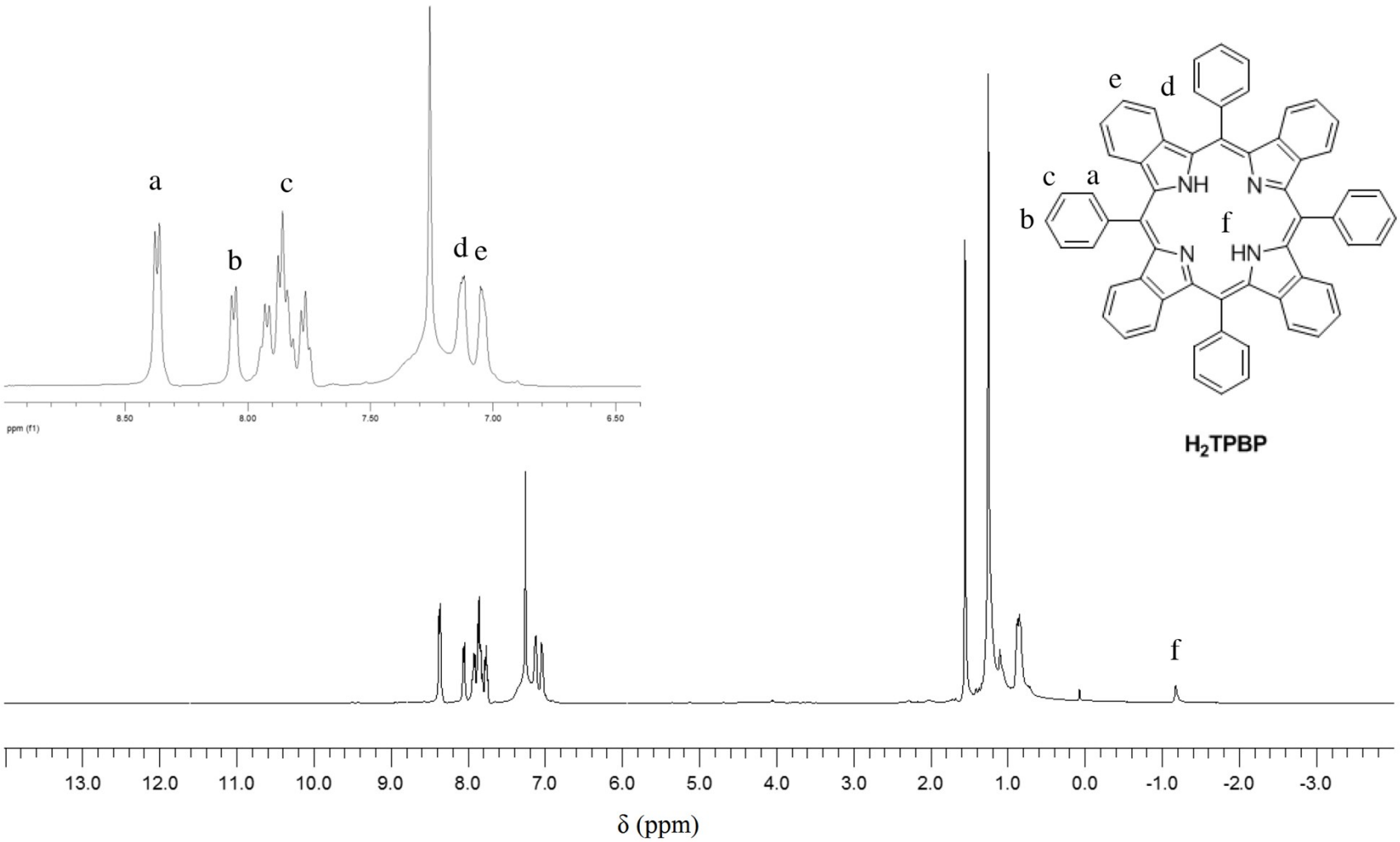


Figure S4. ^{1}H-NMR spectrum of H_2TPTBP

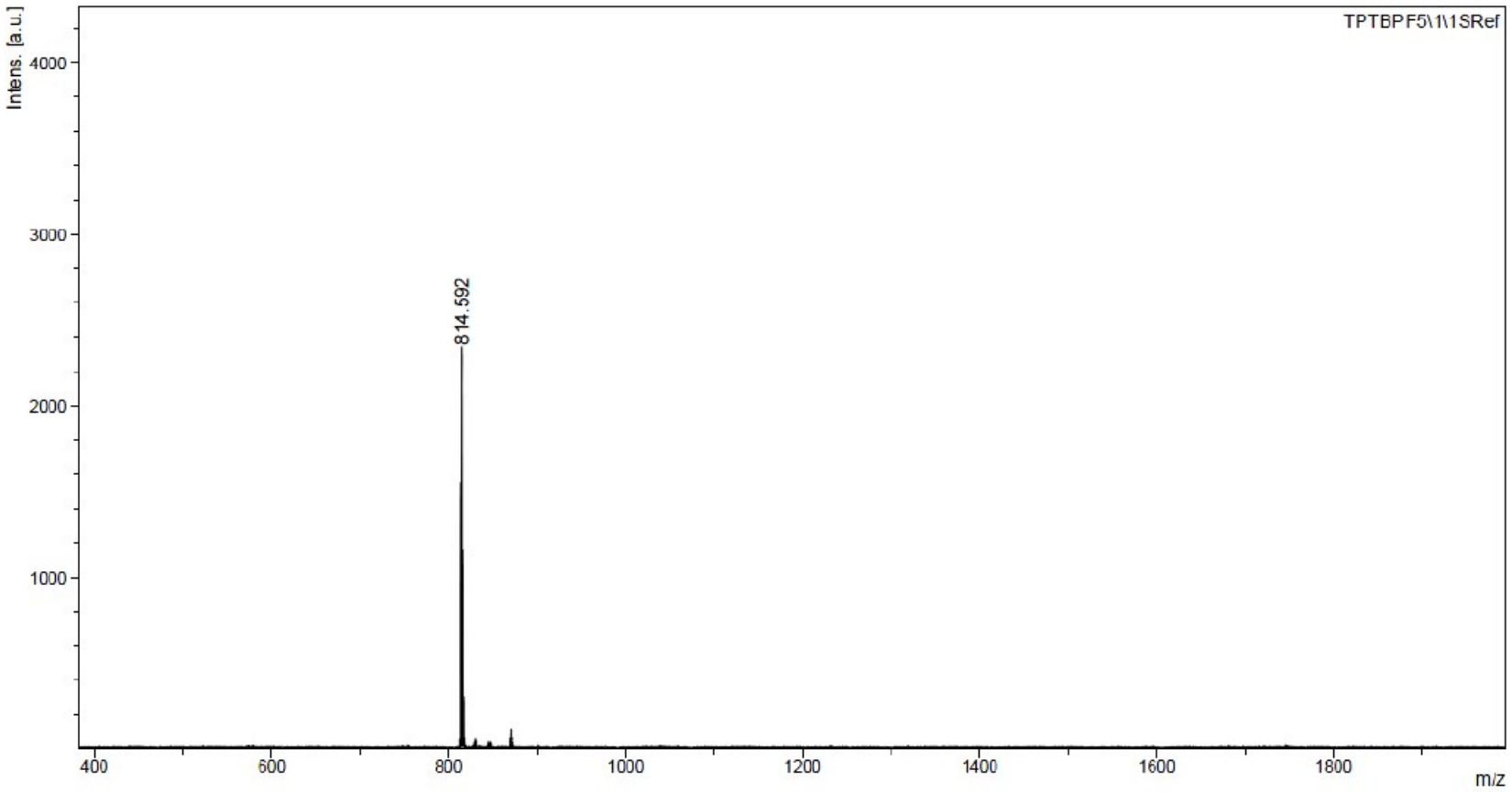


Figure S5. Mass spectrum of H_2TPTBP

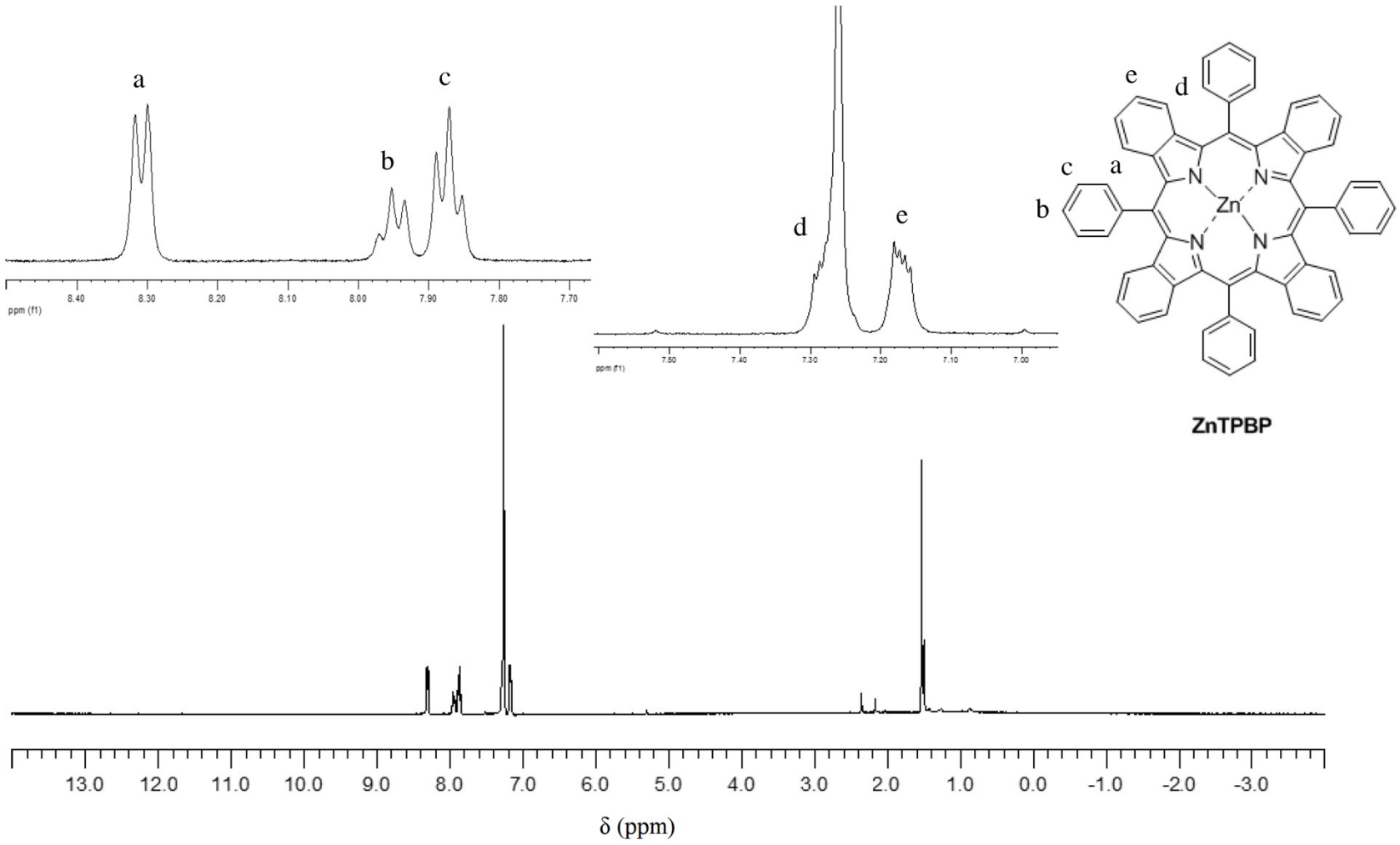


Figure S6. ^{1}H-NMR spectrum of ZnTPTBP

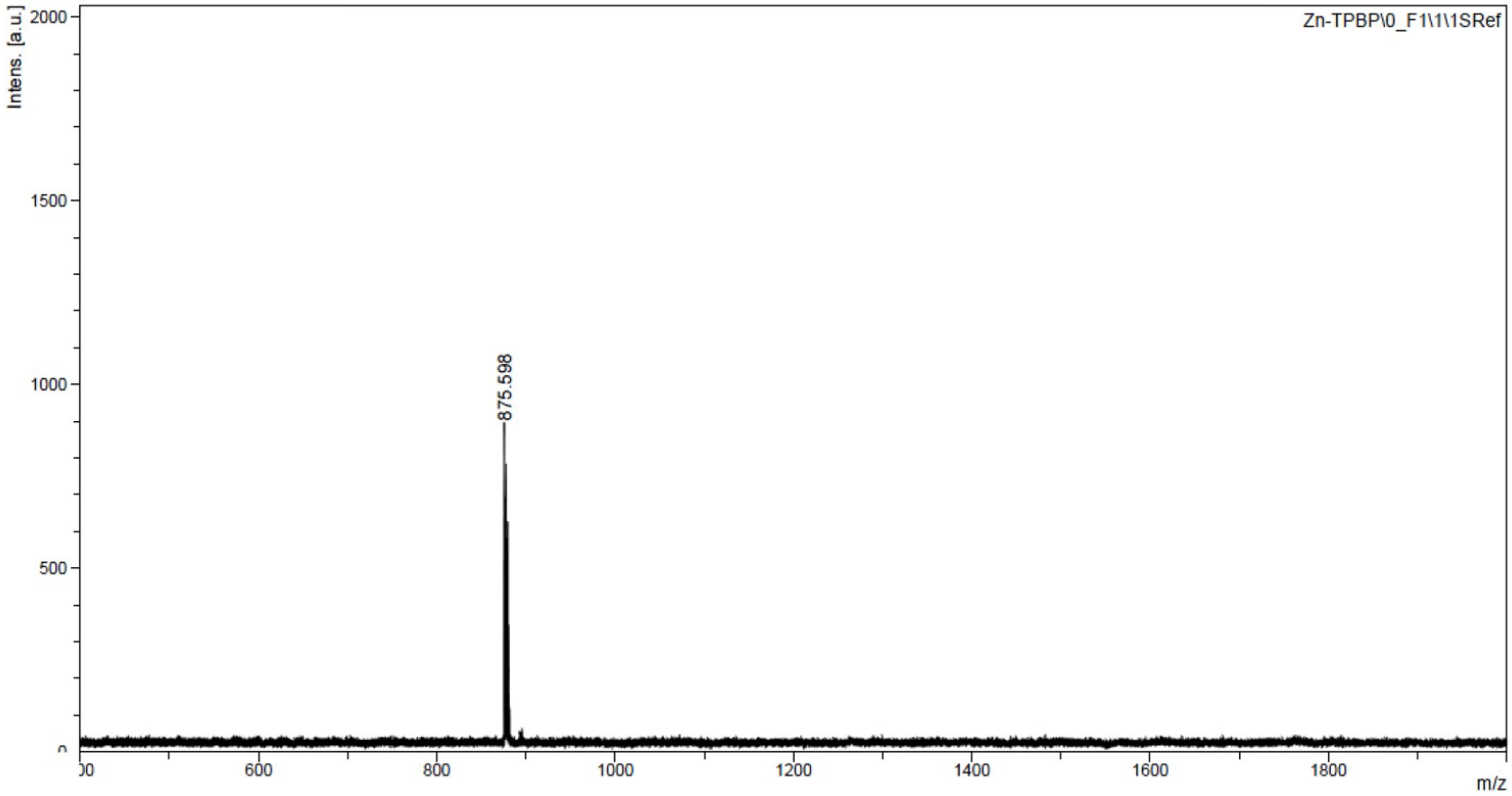


Figure S7. Mass spectrum of ZnTPTBP

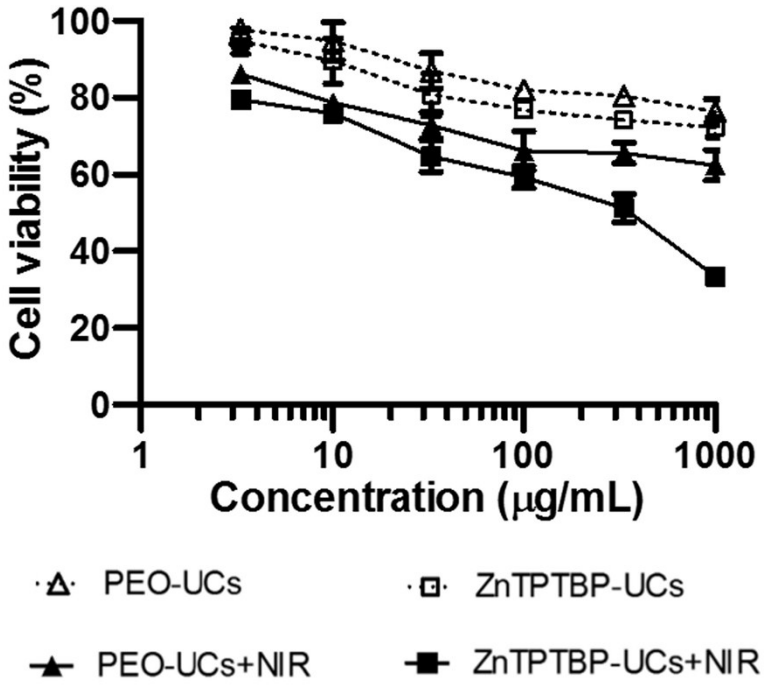


Figure S8. *In vitro* cytotoxicity of PEO-UCs (triangle) and ZnTPTBP-UCs (square) on A-375 cell line. Data represent means ± SD (n = 6) from two independent experiments. Mann-Whiney U test indicated that ZnTPTBP-UCs + NIR gave significantly lower cell viability than the PEO-UCs + NIR, at the P value of ≤ 0.05. Without NIR irradiation, cytotoxicity of ZnTPTBP-UCs was not significantly different from that of PEO-UCs (Mann Whitney U test at the P value of ≤ 0.05).