Supporting Information

Oxygen-evolving hollow polydopamine alleviates tumour hypoxia for enhancing photodynamic therapy in cancer treatment

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Experimental section

Reagents and Apparatus

Methanol, alcohol, isopropanol, n-propanol, DMSO and DMF were purchased from Sinopharm Chemical Reagent Co., Ltd. DA HCl and Tris were purchased from Sigma-Aldrich. 4-Nitrophtalonitrile, Zinc acetate and 4-(dimethylamino)phenol were purchased from J&K Scientific (China). The $[Ru(dpp)_3]Cl_2$ was purchased by Alfa Aesar. ADPA was purchased form Macklin Inc. DCFH-DA, Lyso-Tracker Blue, Mito-Tracker Green, and DAPI were purchased from Beyotime Biotechnology. DMEM and Trypsin were purchased from Thermo Fisher Scientific.

The UV-Vis absorption was measured using an ultraviolet spectrophotometer (Agilent Cary 60, USA). The fluorescence property of samples was detected by a fluorescence emission spectrograph (Hitachi F4700, JPN). The pH of aqueous solution was confirmed by a FE20/EL20 pH-meter (Mettler Toledo, CH). ^1H NMR spectra was measured by a Bruker Advance 500 MHz NMR spectrometer (Bruker, GER). The mass spectra of Compound 1 and ZnPc was measured by a liquid chromatograph mass spectrometer (Shimadzu LCMS2020, JPN). The mass spectra of Compound 2 were measured using an UltrafleXtreme MALDI-TOF-MS spectrometer (Bruker, GER). The Raman spectrum was obtained by a Raman Spectrometer (Bruker, GER). The O_2

solubility in solution was measured by a Dissolved Oxygen Meters (Leici JPBJ-608, CHN). The microcosmic morphology characterization of nanomaterials was measured using TEM (Hitachi H-7650, Japan) and SEM (Hitachi SU5000, JPN). The DLS distribution of nanomaterials was detected by a Particle Sizing Systems (Malvern Zeta-sizer Nano ZS, UK). The intracellular ROS was detected by a laser confocal scanning microscope (Nikon Ti-E-A1R, JPN). The local temperature changes of chemical samples and tumor in mice were recorded by a thermal imager (FLIR E5, USA). The optical biopsy system for experimental small animals was measured by the IVIS Spectrum CT system (PerkinElmer, USA). The experimental tumor-bearing mice were anesthetized by an anesthetic gas machine (Rui ode R580S, CHN).

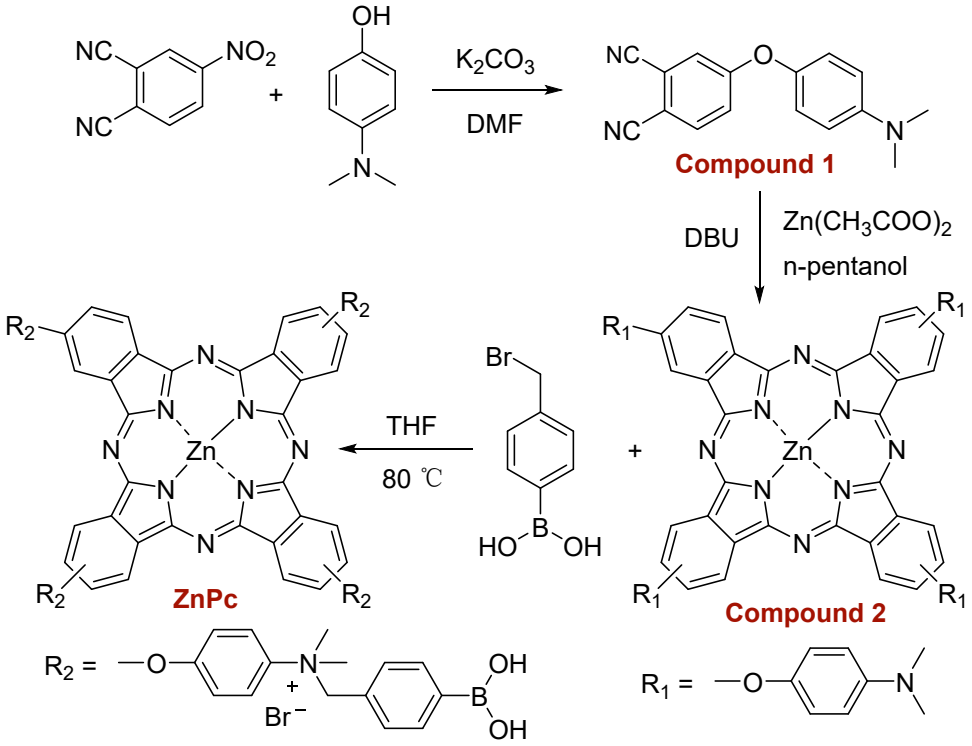
The Synthesis and Characterization of ZnPc

The synthetic route was showed in **Scheme S1**. All compounds were characterized by ^{1}H NMR spectra and mass spectrometer.

The synthesis and characterization of Compound 1. 4-Nitrophtalonitrile (200 mg, 1.16 mmol), 4-(dimethylamino) phenol (190.17 mg, 1.39 mmol) and K_2CO_3 (239.48 mg, 1.73 mmol) were dissolved in DMF (10 mL). The reaction system reacted under N2 protection 65 ℃. 12 h later, the mixture solution was poured into the distilled water, and the precipitate was obtained by extraction filtration. The product was separated by column chromatography using ethyl acetate/ petroleum ether (1:3, v/v). The resulting compound 1 was dried in vacuo (256.2 mg, 84.2 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 8.05 (d, 1H, J = 10.0 Hz), 7.65 (d, 1H, J = 5.0 Hz), 7.27 (dd, 2H, J_1 = 2.6 Hz, J_2 = 8.8 Hz), 7.05-7.02 (m, 2H), 6.82-6.79 (m, 2H), 2.92 (s, 6H). LRMS (EI, $[M + H]^+$): m/z Calcd for $C_{16}H_{13}N_3O$, 263.11, found 263.11.

The synthesis and characterization of Compound 2. Compound 1 (500 mg, 1.90 mmol) and $(CH_3COO)_2Zn$ (174.2 mg, 0.95 mmol) were dissolved in 1-pentanol (15 mL). The reaction solution was heated up to 90 ℃ and reacted for 30 min under N_2 protection. After then, DBU (300 μL) was added into the reaction solution, and the reaction solution was heated up to 140 ℃ for further reacted for 12 h. The 1-pentanol was removed by vacuum distillation. The solid product was washed by methanol and then dried in vacuo. The product was further purified by column chromatography using CH_2Cl_2 and ethyl acetate as the eluent to give a resulting compound 2. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 9.37-9.28 (m, 4H), 8.92 (s, 4H), 7.81-7.73 (m, 4H), 6.99-6.92 (m, 12H), 6.77 (d, 4H, J = 9.0 Hz), 3.07-2.95 (m, 24H). MS (MALDI-TOF, $[M + H]^+$): m/z Calcd for $C_{64}H_{52}N_{12}O_4Zn$, 1118.58, found 1118.58.

The synthesis and characterization of ZnPc. Compound 2 (500 mg, 0.13 mmol) and 4-(bromomethyl) phenylboronic acid (461 mg, 2.15 mmol) were dissolved in anhydrous THF (50 mL). The mixture solution was refluxed (80 ℃) under N_2 protection for 48 h. After cooling to room temperature, the precipitation was obtained by centrifugation (9600 rpm, 5 min) and washed with THF (100 mL) for two times. After then, the filtered green solid was dissolved in methanol (5 mL), and $CHCl_3$ (200 mL) was added into the solution for recrystallization. The ZnPc was filtered and washed with $CHCl_3$ and then dried in vacuo (36.6 mg, 15.7%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 9.21-8.74 (m, 4H), 8.39-8.21 (m, 4H), 8.05-7.92 (m, 4H), 7.88-7.63 (m, 8H), 7.32-7.20 (m, 16H), 7.03-6.91 (m, 8H), 3.75-3.54 (m, 24H). HRMS (ESI): m/z Calcd for $C_{92}H_{84}B_4N_{12}O_{12}Zn[M-4Br]^{4+}$ 414.4, found 414.4.



Scheme S1 The synthesis route of ZnPc.

The Preparation of HZNPs

HZNPs (5 mg) were redispersed and stirred in distilled water (20 mL), and ZnPc (10 mg) dissolved in DMSO (5 mL) was added into the water solution. After reaction for 6 h at room temperature, the precipitate was collected by centrifugation. The crude HZNPs were washed with distilled water, and the amount of ZnPc loaded in HZNPs was determined by ICP to be 0.2891 mg mg^{-1}.

pH-triggered ZnPc Release

The phthalocyanines have two typical absorptions in B band (200~300 nm) and Q band (600~800 nm) because of the π-π electron transition. The ZnPc release behavior was measured via observe the UV absorbance change at 628 nm. The percentage release curve of ZnPc was construct at different pH conditions, and the release of ZnPc reached to 69.71% in the condition of pH was 5.5.

O_2 Release Behavior

The gas (mainly N_2 and O_2) dissolved in aqueous solution was removed by vacuum pump, and filled in with N_2. Then, H_2O_2 (200 μM) was added into the solution. The O_2 release behavior of different drugs ([HPDA] = 7.30 μg mL^{-1}; [ZnPc] = 2.97 μg mL^{-1}; [HZNPs] = 10.27 μg mL^{-1}) was investigated by $Ru(dpp)_3Cl_2$ under hypoxia condition. The fluorescence intensity of the samples was monitored by fluorescence emission spectrometer at different time point (λ_{ex} = 488 nm; λ_{em} = 630 nm).

$^1O_2$ Generation Ability

The $^1O_2$ generation ability of different drugs was measured by ADPA since the ADPA could be oxidated to its endoperoxide derivative in the presence of $^1O_2$. Various drugs were mixed with ADPA (60 μM) in distilled water containing H_2O_2 (200 μM). After light (665 nm) irradiation, the absorbance decrease of samples in enclosed hypoxic colorimeter was detected by UV-vis absorption at 378 nm with time prolong. The $^1O_2$ generate rate was calculated by the following **Equation S1**.

$$\mathrm{In}([\mathrm{ADPA}]_t/[\mathrm{ADPA}]_0) = -kt \quad (1)$$

$[ADPA]_t$ and $[ADPA]_0$ is the concentration of ADPA after and before the light irradiation, respectively. Values of k are the rate of $^1O_2$ generation and t is the light irradiation time.

The Photothermal Conversion Efficiency

The photothermal conversion property of distilled water, HPDA, ZnPc and HZNPs was investigated by thermal imager under laser irradiation. The corresponding temperature and real-time photothermal images were recorded by a thermal camera. To calculate the *η* of HPDA, the HPDA redispersed in distilled water were irradiated until its temperature reached a steady state. Then, the natural cooling curve of HPDA was recorded and the η was calculated by **Equation S2**.

$$\eta = \frac{\mathrm{hs}\ (T_{\max} - T_{surr}) - Qs}{I\ (1 - 10^{-A_{808}})} \times 100\% \quad (2)$$

The maximum temperature (T_{max}) was 65.0 °C and environmental temperature (T_{surr}) was 23.0 °C. The laser power (I) was 1.0 W cm^{-2}. The UV-vis absorbance of HPDA at 808 nm (A_{808}) was 0.3073. *h* is the heat transfer coefficient, and the *s* is the surface area of the container. The *hs* could be calculated by **Equation S3**.

$$\mathrm{hs} = \frac{m_D C_D}{\tau_s} \quad (3)$$

τ_s could be calculated by **Equation S4**.

$$t = -\tau_s In(\theta) \quad (4)$$

A dimensionless parameter θ is calculated by **Equation S5**.

$$\theta = \frac{T - T_{surr}}{T_{\max} - T_{surr}} \quad (5)$$

m_D is 0.5 g and C_D is 4.2 J/g °C. τ_s was calculated 385.73 s and *hs* was 5.44 mW/°C. The heat dissipated of the container was determined independently to be Q_s (7.52 mW). The *η* of the HPDA in distilled water was calculated to be 38.2%.

Cells and Animals

The 4T1 cells were purchased by the National Collection of Authenticated Cell Cultures (China). The cells were cultured in DMEM containing 10% (v/v) inactivated FBS. The following cell experiments were carried out under normoxic conditions (5% CO_2, 37 °C) or hypoxic conditions (5% CO_2, 5% O_2, 37 °C).

Female BALB/c mice (6~7 weeks, weighting about 18 g) were purchased from the Huachuang Sino (China). Animal experiments were approved by Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, and all procedures were in accordance with the instruction of the Institutional Animal Care and Use Committee (AEWC-20210413-133). The mice were subcutaneously injected with 4T1 cells to establish the tumor xenograft model. The formula $V = (L \times W^2)/2$ was used for tumor volume calculation (V: tumor volume; L: tumor length; W: tumor width).

Intracellular O_2 Detection

The intracellular O_2 level of different drug treated group was investigated by $Ru(dpp)_3Cl_2$. In brief, the 4T1 cells were first incubated in culture dish for 24 h. Different drugs were added into the culture dish ([HPDA] = 9.72 μg mL^{-1}; [ZnPc] = 3.96 μg mL^{-1}; [HZNPs] = 13.68 μg mL^{-1}), and incubated for another 12 h. After then, the $Ru(dpp)_3Cl_2$ (3.0 μM) was added into DMEM and incubated for 30 min. The fluorescence intensity was obtained with FCM, and cell images were acquired using a laser confocal microscopy.

Intracellular $^1O_2$ Detection

The commercial SOSG have excellent selectivity to $^1O_2$ in cells while no obvious response to hydroxyl radical and superoxide radical. In the presence of $^1O_2$, no fluorescence SOSG could react with $^1O_2$ and form strong green fluorescent substance. After treated with different drugs (HPDA, ZnPc and HZNPs), the 4T1 cells were further incubated for 6 h under hypoxia condition. The dose of ZnPc was 1.5 μM, and the dose of other drugs were all calculated according to ZnPc. The original DMEM in pore plate was removed, and fresh DMEM containing SOSG (3.0 μM) was added into the plate. 45 min later, the 4T1 cells were irradiated by the LED light (665 nm) for 3 min. After washing with PBS, the fluorescence property of various drugs treated group was investigated by FCM and laser confocal microscopy (E_x: 504 nm; E_m: 525 nm).

The Metabolic Distribution *in vivo*

The drugs (HZNPs and ZnPc) were injected into tumor-bearing mice by tail vein for three days. The dose of ZnPc was 2.00 mg kg^{-1}. The dose of HPDA in HZNPs was 4.92 mg kg^{-1}, and the dose of ZnPc in HZNPs was 2.00 mg kg^{-1}. The metabolic distribution in main organs (heart, liver, spleen, lung, kidney), blood and tumors eviscerated from sacrificial tumor-bearing mice was investigated by the IVIS Lumina LT system.

Photothermal Performance *in vivo*

The tumor-bearing mice were randomly divided into six groups, including control, HPDA plus 808 nm laser irradiation, ZnPc plus 665 nm laser irradiation, HZNPs plus 808 nm laser irradiation, HZNPs plus 665 nm laser irradiation and HZNPs plus 808 nm + 665 nm laser irradiation. The mice were treated by drugs via tail intravenous injection at intervals of one day. The dose of HPDA was 4.92 mg kg^{-1}; the dose of ZnPc was 2.00 mg kg^{-1}; the dose of HZNPs was 6.92 mg kg^{-1}. During the 14 days' treatment, the body weight and tumor volume of mice were measured and recorded,

respectively. After then, all mice were killed via dislocation of the neck to obtain the main organs and tumors. These organs and the tumor tissue were stained with an H&E kit. The tumours were used for immunohistochemical staining by HIF-1α.

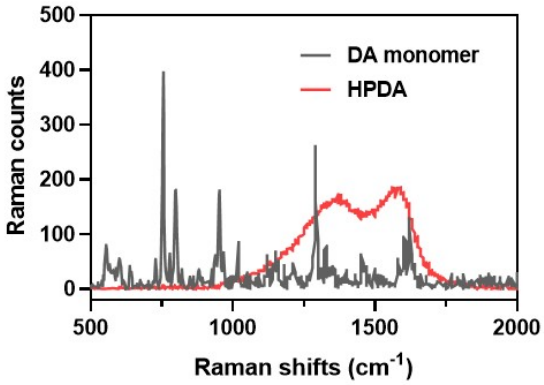


Fig. S1 The Raman spectra of DA monomer and HPDA.

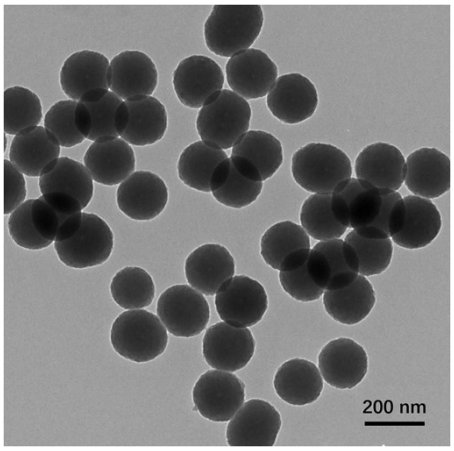


Fig. S2 The TEM image of PDA nanospheres.

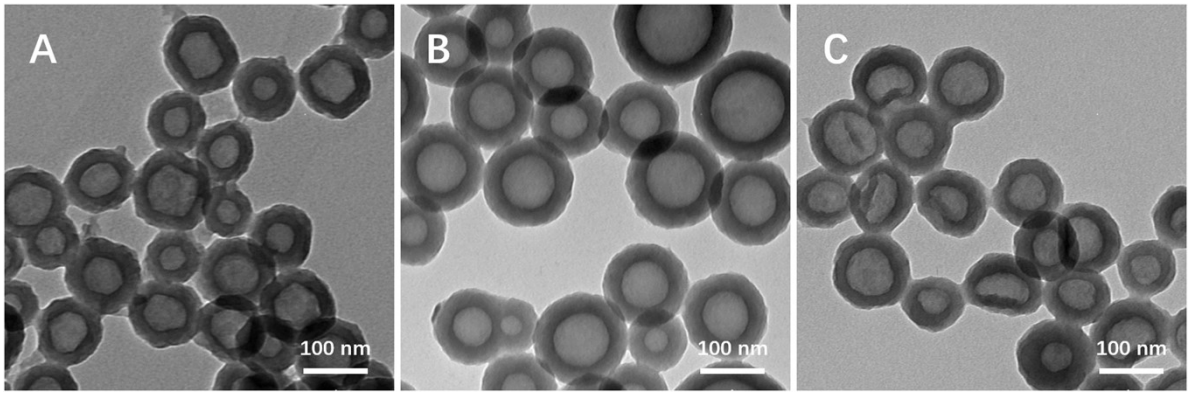


Fig. S3 The TEM micromorphology of HPDA polymerization in oxygenated (A) methanol/water, (B) alcohol/water and isopropanol/water, respectively.

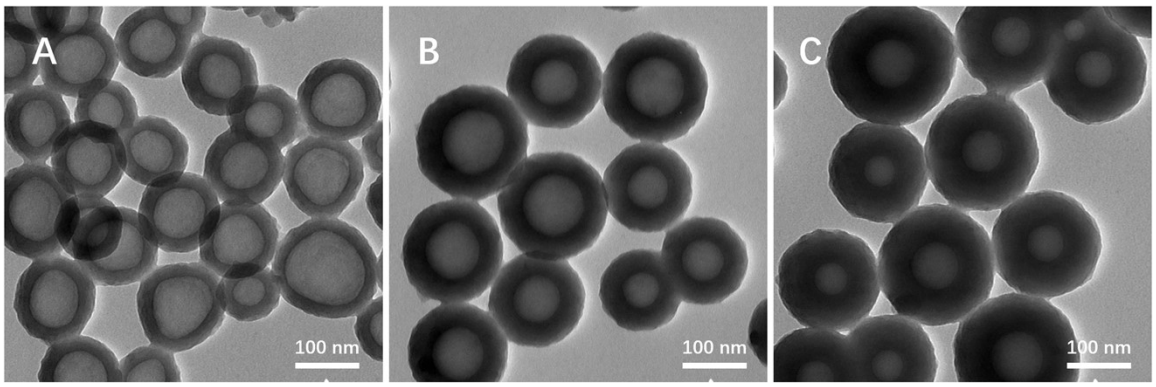


Fig. S4 The TEM images of HPDA formation in the mixing solution of oxygenated water/oxygenated n-pentanol at different time point (A, 45 min; B, 90 min; C, 180 min).

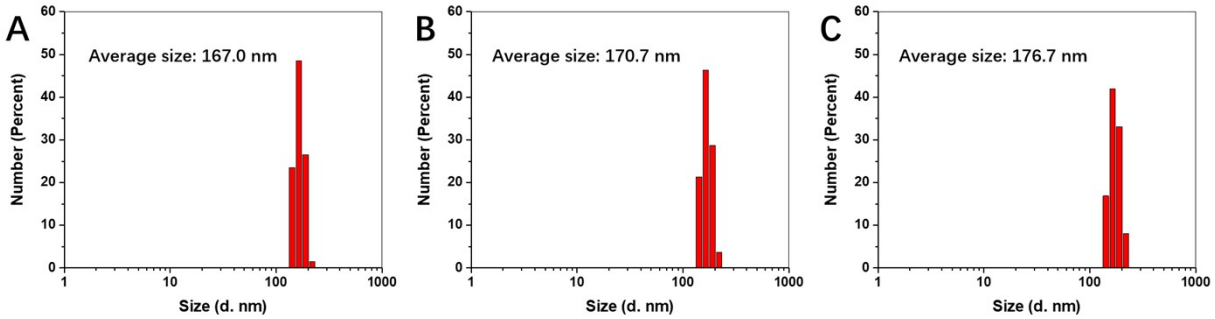


Fig. S5 The DLS distribution of HZNPs in (A) water, (B) PBS, and (C) cell culture medium (DMEM + 10% bovine serum).

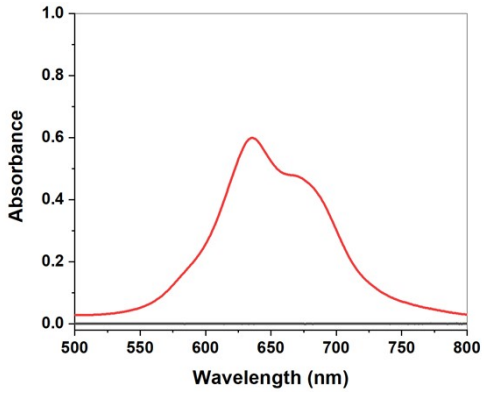


Fig. S6 The absorbance of free ZnPc in distilled water.

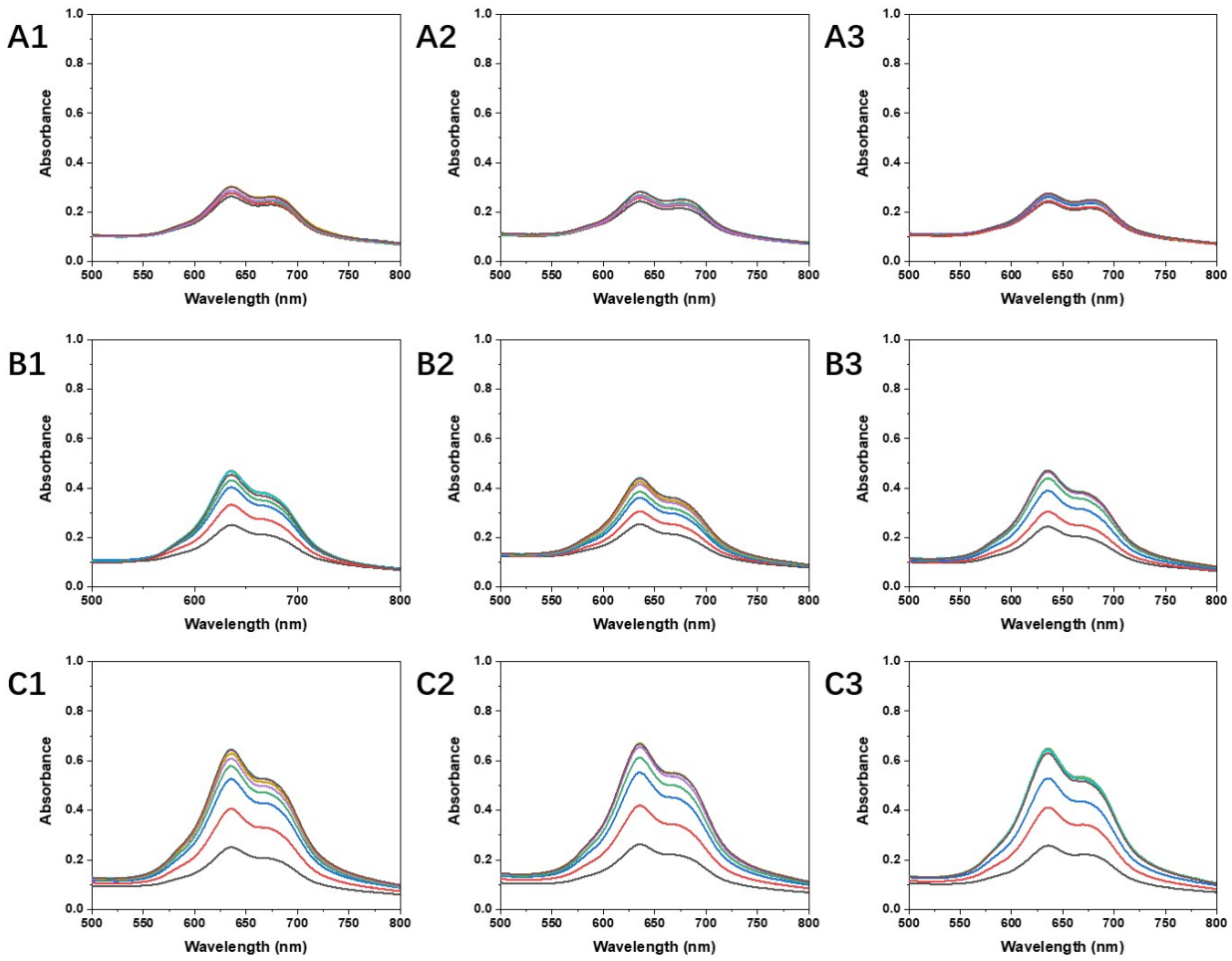


Fig. S7 The absorbance of HZNPs in acidic aqueous solution (A, pH = 7.4; B, pH = 6.5; C, pH = 5.5) at different time points.

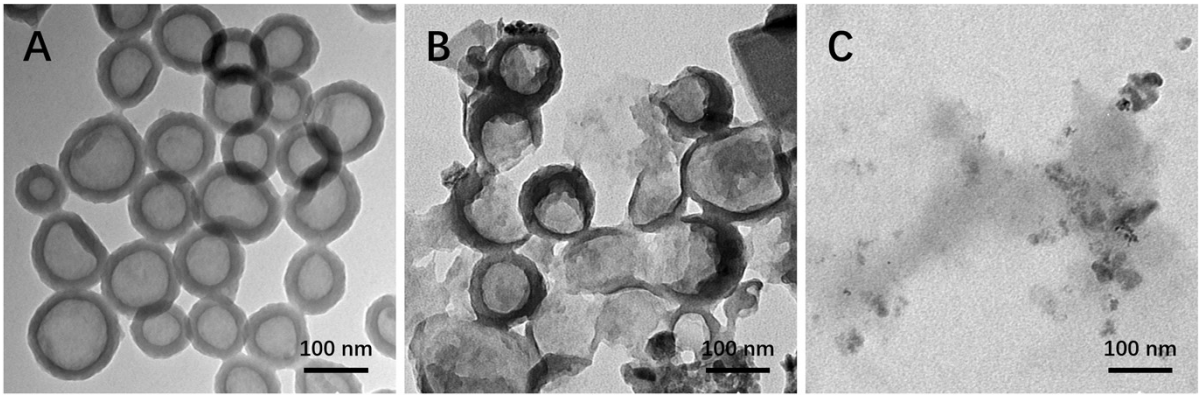


Fig. S8 The H_2O_2-triggered (200 μM) disintegration of HZNPs with time prolong (A, 0 h; B, 12 h; C, 48 h).

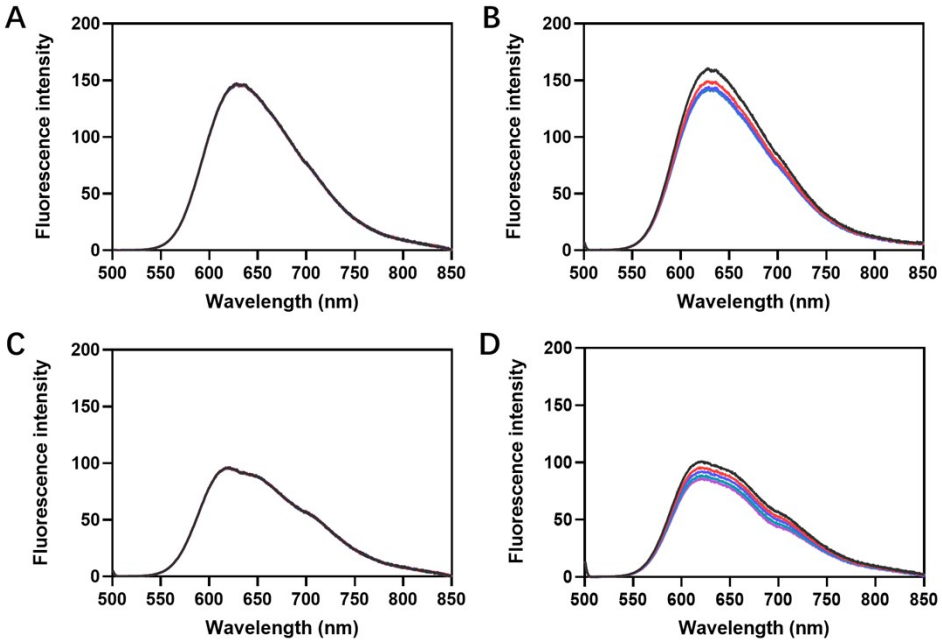


Fig. S9 The O_2-dependent fluorescence quenching behavior of Control (A), HPDA (B), ZnPc (C), HZNPs (D) under normoxic condition (H_2O_2, 200 μM).

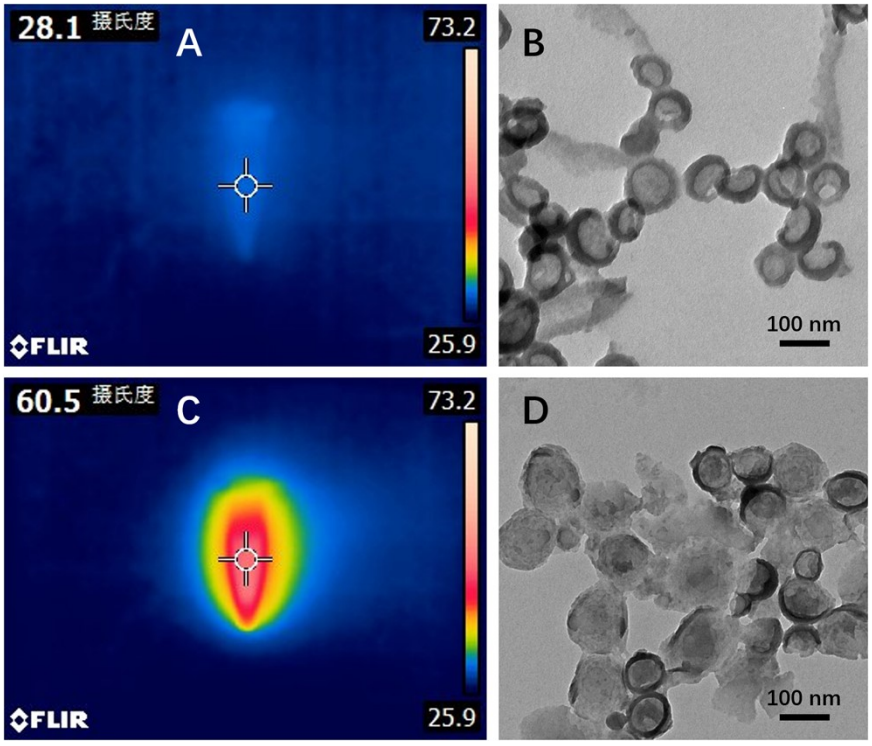


Fig. S10 (A) Thermal and (B) TEM images of HZNPs in H_2O_2 (200 μM) aqueous solution at room temperature. (C) Photothermal and (D) TEM images of HZNPs in H_2O_2 (200 μM) aqueous solution upon laser (808 nm, 1.0 W cm^{-2}) irradiation for 30 min.

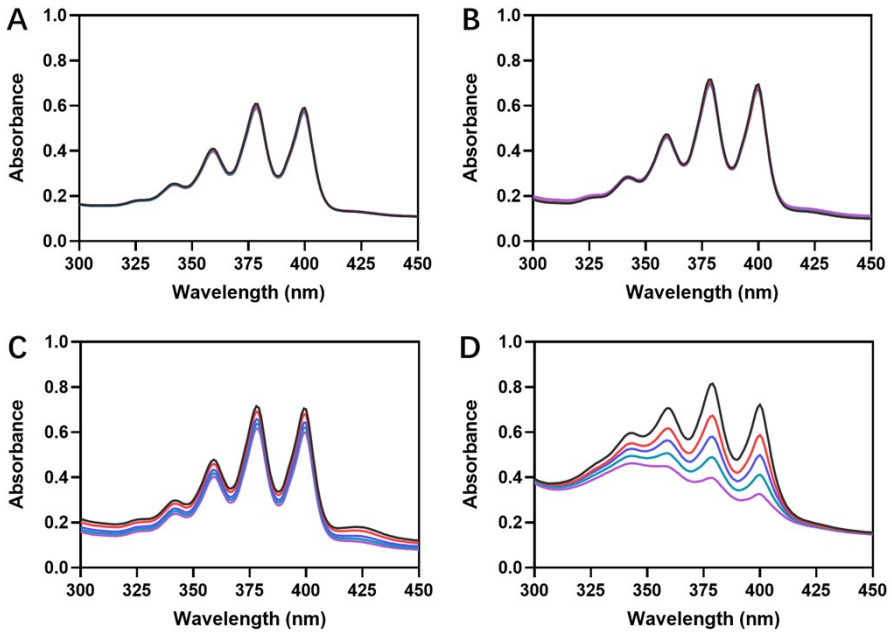


Fig. S11 The photo-bleaching of ADPA by Control (A), HPDA (B), ZnPc (C), HZNPs (D) under normoxic condition (H_2O_2, 200 μM).

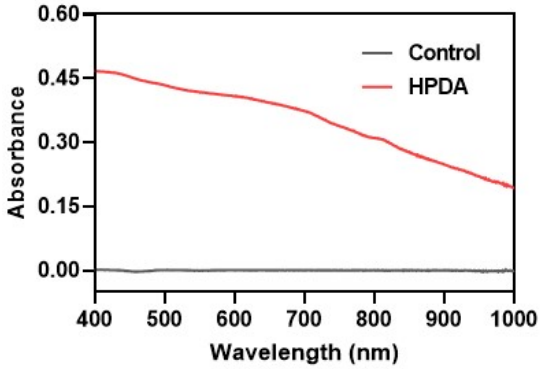


Fig. S12 The UV-vis absorbance of HPDA in distilled water.

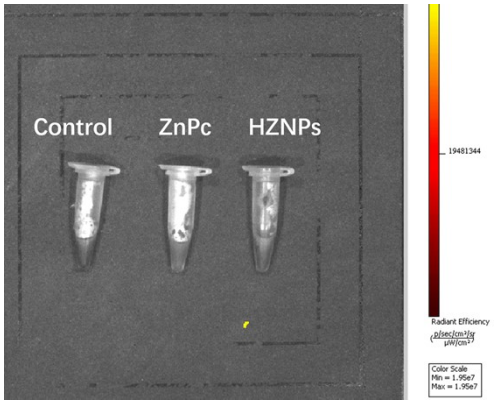


Fig. S13 The metabolism distribution of ZnPc and HZNPs in blood.

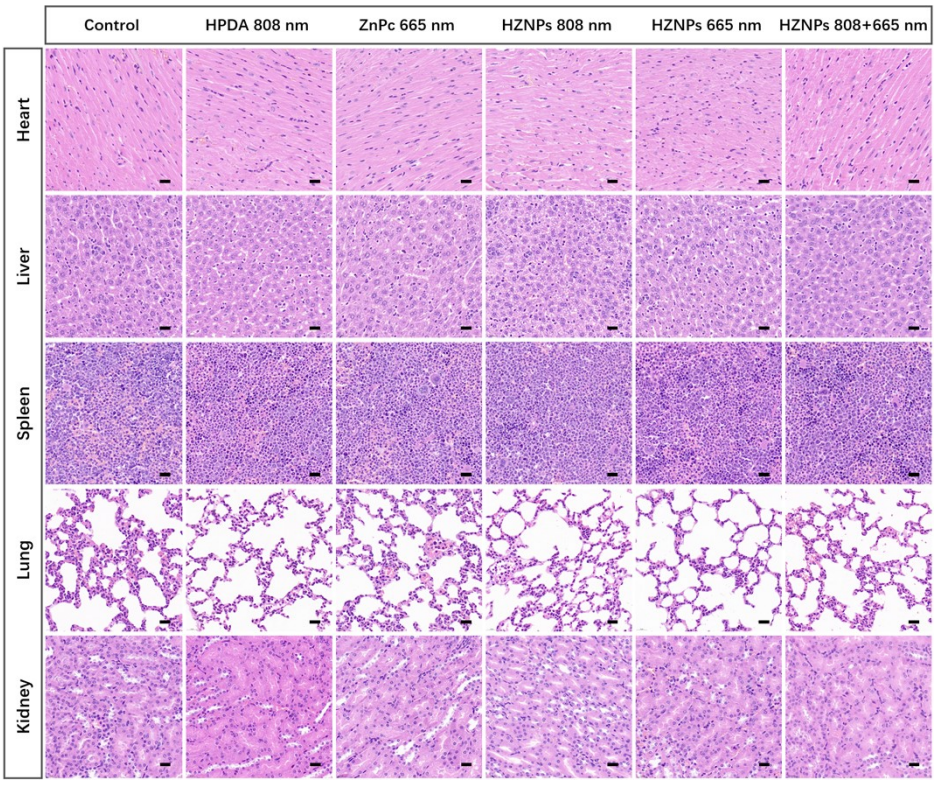


Fig. S14 The H&E staining of main organs (Heart, Liver, Spleen, Lung, and Kidney) slides sacrificing from tumour-bearing mice after 14 days' treatment (Bar = 20 μm).

Table S1. The loading amounts of ZnPc in HZNPs was detected by ICP

Sample	$M_{(Zn, wt)}$	$M_{(ZnPc, g/mol)}$	$m_{(HZNPs, g)}$	$m_{(ZnPc, g)}$	Loading efficiency (mg/mg)
1	1.17 %	1657.60	1.20×10^{-3}	3.56×10^{-4}	0.2967
2	1.11 %	1657.60	1.50×10^{-3}	4.22×10^{-4}	0.2814

Table S2. IC50 values of each formulation

Compound	IC50			
	Dark	808 nm	665 nm	808 + 665 nm
HPDA	＞ 100 μg	6.28 μg	-	-
ZnPc	50.42 μM	-	2.43 μM	-
HZNPs	＞ 100 μg	-	-	5.53 μg

Table S3. The relative amount of Zn element in main organs and tumours of tumour-bearing mice

Groups	Heart	Liver	Spleen	Lung	Kidney	Tumour
Control	4.26±0.51	76.55±3.04	8.47±0.71	7.07±0.62	6.29±1.51	＜2.00
ZnPc	4.08±0.21	87.60±9.90	10.15±0.64	6.53±0.28	8.69±0.40	3.15±0.58
HZNPs	4.99±1.33	82.40±7.64	8.82±0.17	6.91±0.04	7.27±1.12	8.16±0.99