Supporting Information

Au@CeO_2 Nanozyme-Based Targeted Delivery Platform for Computed Tomography-Guided Synergistic Antioxidant/Mild Photothermal Therapy of Inflammatory Bowel Disease

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1. Additional Experimental Section

1.1 Chemicals and Materials.

$CeCl_3 \cdot 7H_2O$ (≥99.9% trace metals basis) were purchased from Sigma-Aldrich Co (MO, USA). $HAuCl_4 \cdot 3H_2O$ (≥99.9% trace metals basis), sodium polyacrylate (PAAS) (Mw: 4500), Salicylic acid (SA), Lipopolysaccharide (LPS) and polyacrylic resin (Eudragit S100) were purchased from Aladdin Bio-Chem Technology Co. Ltd. (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Jiangsu Keyuan Biotechnology Co., Ltd. Calcein acetoxymethyl ester (Calcein-AM), propidium iodide (PI), 2',7'-dichlorofluorescein diacetate (DCFH-DA) and the assays for superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) was purchased from Beyotime Biotechnology. Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (New York, USA). The assays for myeloperoxidase (MPO), molondialdehyde (MDA) and LISA kits for TNF-α, IL-β, IL-6 and IL-10 were obtained from Solarbio (Beijing, China). Dextran sulfate sodium (DSS) was purchased from psaitong Biotechnology Co., Ltd (Beijing, China). All reagents and chemicals were used directly without further purification. Milli-Q water (18.2 MΩ cm) was used in all experiments.

1.2 Characterization

The morphologies of nanozymes were recorded by a Hitachi H-600 transmission electron microscope (TEM) with an acceleration voltage of 100 kV (Hitachi Ltd., Japan). The powder X-ray diffraction (XRD) analysis was carried out on a D8

ADVANCE diffractometer (Bruker Co., Germany) using Cu Ka (0.15406 nm) radiation. The X-ray photoelectron spectroscopy (XPS) measurements were performed using a VG ESCALAB MKII spectrometer (VG Scientific Ltd., UK). Zeta potential and hydrodynamic size measurements were carried out on Malvern Zetasizer (Malvern Instrument Ltd., UK). The element analysis of nanozymes was performed by an ELAN 9000/DRC ICP-MS system (Perkin Elmer Co., USA). UV-visible spectra were recorded by a Mini 1240 UV-visible spectrophotometer (Shimadzu Co., Japan). EPR spectra was recorded by a Magnettech ESR5000 (Bruker Co., Germany). The MTT assay was performed using a Versamax microplate reader (Bio-Tek Instruments, Inc., USA). Fluorescence images were obtained using a confocal laser scanning microscope (CLSM, Nikon Co., Japan). Flow cytometry was recorded by a BD flow cytometer FACSCelesta X (BD FACSCelesta™, USA).

1.3 Synthesis of Au@CeO_2 nanozyme

The Au@CeO_2 nanozyme was synthesized according to the literature [S1]. 50 mL of H_2O was placed under an ice bath, $CeCl_3$ solution (0.1 M, 1400 μL) and $HAuCl_4$ solution (0.024 M, 600 μL) were added and stirred for 5 min. Then, ammonia solution (64 μL of 25%-28 % ammonia solution added to 3 mL of H_2O) was quickly added and stirred rapidly for 25 s. Then, $CeCl_3$ solution (0.0467 M, 30,00 μL) was added. The material was separated by centrifugation (4 °C, 11000 rpm,10 min) and washed three times with H_2O.

1.4 Synthesis of AuCeP

3 mL PAA solution (3% wt/v) were added into 20 mL of Au@CeO_2 (1 mg/mL). The

mixture was continuously stirred at 25 °C for 2 h. Then, the material was centrifuged (11000 rpm, 10 min) and washed three times with 20 mL H_2O by centrifugation (11000 rpm, 10 min) to obtain PAA-coated Au@CeO_2 (abbreviated as AuCeP).

To obtain FITC labelled AuCeP, the Au@CeO_2 suspension (20 mL, 1 mg/mL) was mixed with FITC (10 mL, 1 mg/mL) and incubated for 24 h at room temperature protected from light. The FITC-labelled Au@CeO_2 was collected by centrifugation (11000 rpm, 10 min) and washed with deionized water until the supernatant was clear. The FITC-labelled Au@CeO_2 suspension (10 mL, 1 mg/mL) and 1.5 mL PAA solution (3% wt/v) were mixed and stirred for 2 h. The FITC- labelled AuCeP was collected by centrifugation.

1.5 Synthesis of AuCeP@Eudragit

10 mg AuCeP and 10 mg Eudragit S100 were dissolved in 5 mL of acetone. The mixture was added into 50 mL citrate buffer (0.1 M, pH 5.0) under sonication. After stirred in a fume hood for 2 h, the AuCeP@Eudragit were collected by centrifugation (3000 rpm, 15 min), and washed by 25 mL water (3 times). For in vitro fluorescence imaging, the 20 mg Au@CeO2 and 5 mg DiD were mixed in 20 mL H2O, and incubated for 24 h. The DiD/Au@CeO2 were collected by centrifugation (11000 rpm, 10 min), and washed by 20 mL H2O (3 times). The DiD/AuCeP@Eudragit were prepared according to previously described preparation strategy of AuCeP@Eudragit.

1.6 Photothermal effect of AuCeP

The AuCeP solution (100 μg/mL) was irradiated with 808 nm laser of different powers (0.5, 0.8, 1.0, 1.5 W/cm^2). The temperature was recorded every 30 seconds

using an infrared camera. In addition, the AuCeP solutions with different concentrations (100, 200, 300, 400 μg/mL) were irradiated with 808 nm laser (1.0 W/cm^2). The photothermal stability was tested by irradiating AuCeP (100 μg/mL) for 420 s using an 808 nm laser with a power density of 1.0 W/cm^2. The laser was then switched off and the solution naturally cooled. This cycle was repeated 5 times and the temperature was recorded every 30 seconds using an infrared camera. The photothermal conversion efficiency (η) of AuCeP can be calculated by the following equations:

$$\theta = (T - T_{surr})/ (T_{max} - T_{surr})$$

$$\eta= [hS (T_{max} - T_{surr}) - Q_{Dis}] / [I (1 - 10^{-A808})] \times 100\%$$

$$hS= (\Sigma miCp,i)/\tau s$$

$$t= \tau s \times (-ln\theta)$$

Where h is the heat transfer coefficient, S is the irradiated area, T_{max} is the equilibrium temperature, T_{surr} is the ambient temperature of the surroundings, I is the laser power density, A is the absorbance of AuCeP at 808 nm, m is the mass of the sample, Cp is the thermal capacity of the sample, t is cooling time after irradiation, and τs is the sample system time constant [S2].

1.7 In vitro ROS scavenging ability of AuCeP

1.7.1 SOD-like enzyme activity

Firstly, the reaction system of xanthine and xanthine oxygenase in the organism was simulated to produce superoxide anion radical·O_2^-. After the system was constructed, AuCeP (25, 50, 100, 150, 200 μg/mL) were added and incubated with the reaction system. Then the color developer WST-8 was added and mixed, and left for 30 min at

37 °C. Then, the absorbance of the samples at 450 nm was measured using a microplate reader. Additionally, 0.3 mM xanthine, 0.025 U/mL XOD, 0.1 mM EDTA, AuCeP (25, 50, 100, 150, 200 μg/mL) and 100 mM DMPO was mixed in PBS solution (pH =7.4). EPR spectra from the samples were obtained 1.5 min after mixing.

1.7.2 CAT-like enzyme activity

H_2O_2 can react with $Ti(SO_4)_2$ to form a yellow titanium peroxide complex, which shows characteristic absorption at 415 nm. The H_2O_2 solution (3 mM) and AuCeP solution (100 μg/mL) were mixed and the reaction was continued for different times. After incubated for 5, 10, 20 and 30 min, 200 μL of sample were taken out, and mixed with 800 μL of $Ti(SO_4)_2$ solution, respectively. Then, the absorbances of samples at 415 nm were measured using a microplate reader.

1.7.3 ·OH scavenging capacity

Salicylic acid (SA) is capable of trapping ·OH and reacts to form 2,3-dihydroxybenzoic acid, which exhibits a characteristic absorption peak at 510 nm. The AuCeP (100 μg/mL) was added to a mixed solution containing $FeCl_2$ (0.6 mM) and salicylic acid (0.6 mM), followed by the addition of H_2O_2 (5 mM). The mixture was then incubated through different conditions. Then, the absorbance of samples at 510 nm was measured using a microplate reader.

1.8 Cell Culture and cytotoxicity assay of AuCeP

The RAW264.7 and CT-26 cells were cultured in a CO_2 incubator at 37 °C with 5% CO_2. The RAW264.7 cells were treated with DMEM supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (100 U/mL penicillin and 100 μg/mL

streptomycin). The CT-26 cells were treated with 1640 supplemented with 10 % fetal bovine serum (FBS) and 1% antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin).

All CT-26 and RAW264.7 cells were cultured in 96-well plates (1×10^4 cells) for 24 h. Afterwards, different amount of AuCeP were added and co-cultured with the cells for 24 h. Cytotoxicity was evaluated using MTT assay. Similarly, the CT-26, RAW264.7 cells and fresh medium containing AuCeP (100 μg/mL) were co-incubated for 24 h. After that, the cells were treated with laser irradiation (808 nm, 1.0 W/cm^2) for 10 min, and cytotoxicity was evaluated using MTT assay.

1.9 The intracellular uptake capacity of AuCeP

The RAW264.7 cells were inoculated and incubated overnight. The cells were incubated with FITC-labelled AuCeP (100 μg/mL) for 0.5 h, 2 h, 4 h and 8 h. The cells were washed three times with PBS and stained with Hoechst for 15 min to label the nuclei. Finally, the cells were imaged by fluorescence inverted microscope.

The RAW264.7 cells were inoculated overnight. The medium was refreshed, and the LPS (100 ng/mL) was added to each well to induce inflammation. After 6 h, the FITC-labelled AuCeP was added to the wells and incubated for 2 h. Normal cells were used as control. Finally, intracellular uptake capacity was photographed by fluorescence inverted microscope, and intracellular uptake levels was quantified by flow cytometry.

1.10 The cytoprotective capacity of AuCeP

To assess the protective effect of AuCeP and AuCeP +mPTT against oxidative damage, the RAW264.7 cells and AuCeP were co-cultured for 24 h, followed by

exposure to 1 mM H_2O_2 for 6 h. For the AuCeP +mPTT group, cells were treated with laser irradiation (808 nm, 1.0 W/cm^2) for 10 min, and finally cell viability was assessed using MTT assay. The concentration of H_2O_2 was fixed at 1 mM, and the same treated cells were stained with Calcein-AM/PI, and imaged by fluorescence inverted microscope.

To assess the intracellular ROS scavenging ability of AuCeP and AuCeP +mPTT, the RAW264.7 cells and AuCeP were co-cultured for 24 h, followed by exposure to 1 mM H_2O_2 for 6 h. For the AuCeP +mPTT group, the cells were treated with laser irradiation (808 nm, 1.0 W/cm^2) for 10 min. After being washed, the cells were stained with DCFH-DA probe for 30 min and then imaged by fluorescence inverted microscope.

To further assess the effects of AuCeP and AuCeP +mPTT on mitochondrial function, the RAW264.7 cells and AuCeP were co-cultured for 24 h and followed by exposure to 1 ng/mL LPS for 6 h. For the AuCeP +mPTT group, the cells were treated with laser irradiation (808 nm, 1.0 W/cm^2) for 10 min. After being washed, the cells and JC-1 staining solution were co-incubated for 20 min. Then, the cells were washed twice with JC-1 staining buffer. Finally, the cells were imaged with a fluorescence inverted microscope.

1.11 Antioxidant and anti-inflammatory capabilities

To assess the antioxidant and anti-inflammatory capacity, the RAW264.7 cells and AuCeP were co-cultured for 24 h, followed by exposure to LPS (100 ng/mL) for 6 h. Then, the cells were treated with laser irradiation (808 nm, 1.0 W/cm^2) for 10 min. The

incubation was continued for 24 h. Then, oxidative markers (MDA, GPx, and SOD) in the cells were measured using assay kits, and the levels of inflammatory factors (TNF-α, IL-1β, and IL-6) were determined using ELISA and biochemical assay kits.

1.12 In vivo imaging of DSS-induced acute colitis mouse

For in vivo study, all animal procedures were performed in accordance with the regulations for the Administration of Affairs Concerning Experimental Animals of the People's Republic of China and approved by the Animal Ethics Committee of Shenyang Medical University of TCM (Permit No. SYYXY2024121301). The C57/BL6 male mice (6-8 weeks old, 20 g average weight) were fed water containing 2.5% DSS for 7 days. For in vivo fluorescence imaging, the DSS-induced acute colitis mice were treated with free DiD, DiD/AuCeP and DiD/AuCeP@Eudragit by oral administration, respectively. The healthy mice were treated with DiD/AuCeP@Eudragit as a control. The mice were sacrificed at 1, 6, and 12 h post-oral administration, and the colons were collected for in vitro fluorescence imaging. The fluorescence images were recorded by a Davinch Invivo HR imaging system (Davinch K, Korea) under an excitation wavelength of 600 nm emission and emission wavelength of 700 nm. For in vivo CT imaging, healthy mice and DSS-induced acute colitis mice were treated with AuCeP@Eudragit (5 mg/Kg) by oral administration, respectively, and CT imaging was performed before (0), and 1, 6, and 24 h after oral administration of AuCeP@Eudragit.

1.13 The therapy of DSS-induced acute colitis mouse

C57/BL6 male mice (6-8 weeks old, average weight 20 g) were randomly divided

into 5 group (n=5). The mice in G1 group (control group) were fed water only, and mice in other five groups (DSS groups, G2 to G6) were fed water containing 2.5% DSS, and were orally administered 600 μL PBS (G1, G2 and G3), or 600 μL AuCeP (5 mg/Kg AuCeP in PBS, G4) at 2, 4, 6, and 8 days, or 600 μL AuCeP@Eudragit (5 mg/Kg AuCeP@Eudragit in PBS, G5 and G6) at 2, 4, 6, and 8 days, respectively. After administered AuCeP@Eudragit or PBS for 6 h, the mice in G3 and G6 were irradiated by 1.0 W/cm2 808 nm NIR laser for 10 min. Body weight, fecal blood and fecal morphology of the mice were monitored daily during the treatment period. The mice were sacrificed at the ninth day, and the colons were collected for subsequent analysis.

1.14 *In vivo* safety assessment

To evaluate the biosafety of AuCeP@Eudragit, healthy mice were orally administered daily by AuCeP@Eudragit with dose of 5 mg/Kg for 7 days. The abdomens of mice were irradiated by an 808 nm NIR laser (1 W/cm^2, 10 min) at 6 h post-oral administration of AuCeP@Eudragit, respectively. The mice were sacrificed at 8 days. Main organs and bloods were collected for subsequent analysis.

To evaluate the long-term biological safety of the material. The C57/BL6 mice were orally administered by AuCeP or AuCeP@Eudragit). After 28 days of normal feeding, blood was collected for hematological analysis, and tissues was collected for histological analysis with H&E staining.

1.15 Statistical analysis

All results were statistically analyzed by student's t-test and presented as the means

± standard deviations (SD). Statistically significant differences were defined as follows: significance level *P < 0.05 was considered statistically significant.

2 Additional Figures S1-S16

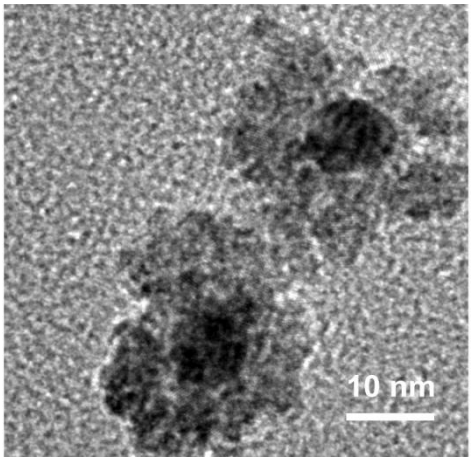

Fig S1. TEM image of Au@CeO_2 nanozymes.

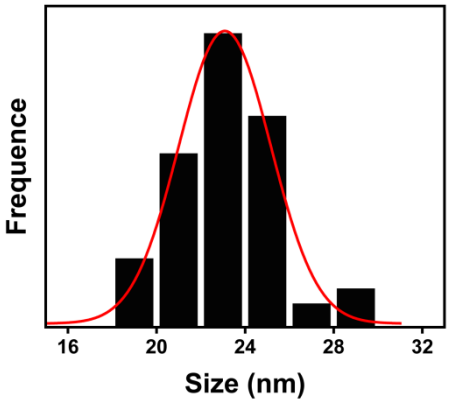

Fig S2. The size distribution of as-synthesized Au@CeO_2 nanozymes.

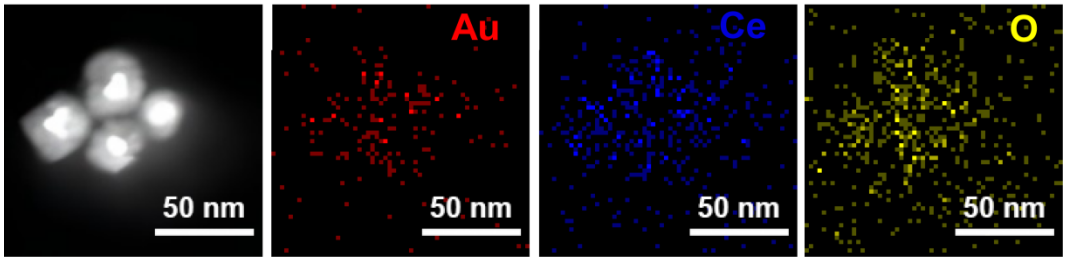

Fig S3. HAADF-STEM image and EDX elemental mapping of Au, Ce, O of Au@CeO_2 nanozymes.

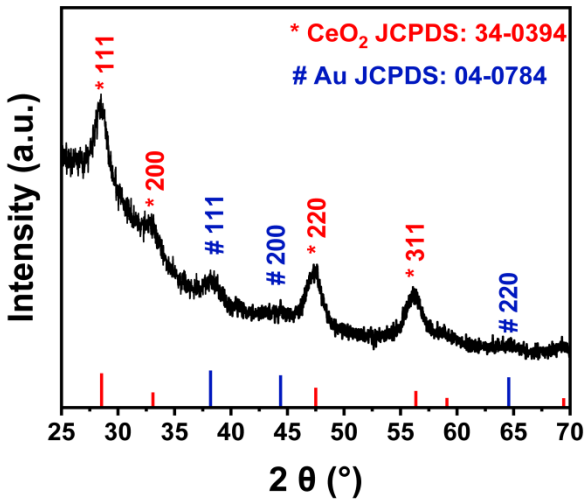

Fig S4. XRD pattern of Au@CeO_2 nanozymes.

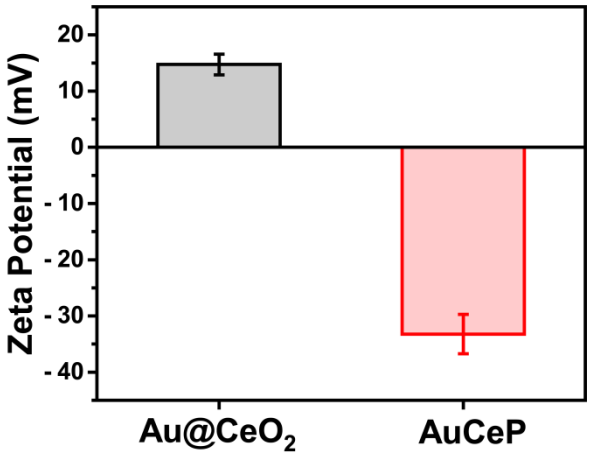

Fig S5 The zeta-potential of Au@CeO_2 nanozymes and AuCeP.

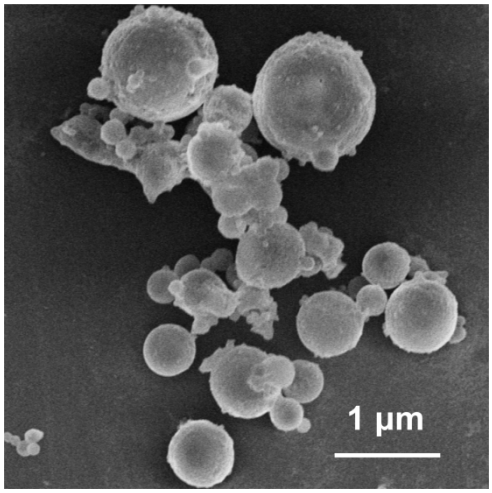

Fig S6. SEM image of AuCeP@Eudragit.

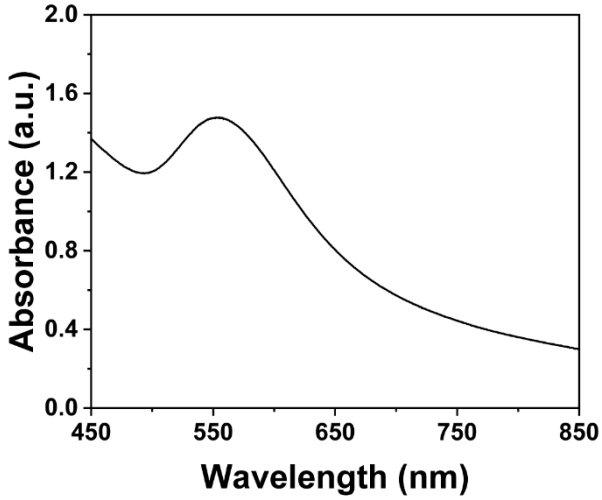

Fig S7 UV-vis-NIR spectrum of AuCeP.

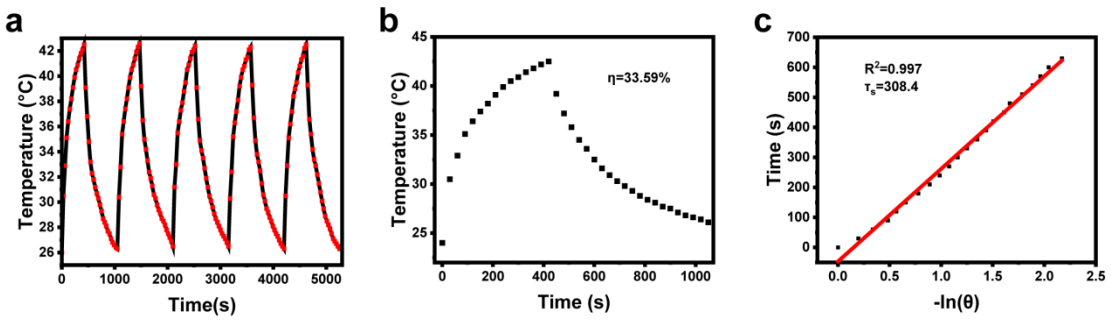

Fig S8. (a) Heating and cooling curves of AuCeP (100 μg/mL) irradiated with 808 nm NIR laser (1.0 W/cm^2) over five ON/OFF cycles. (b) Heating and cooling curve of AuCeP (100 μg/mL) irradiated with 808 nm NIR laser (1.0 W/cm^2). (c) Linear time data versus -lnθ obtained from the cooling period.

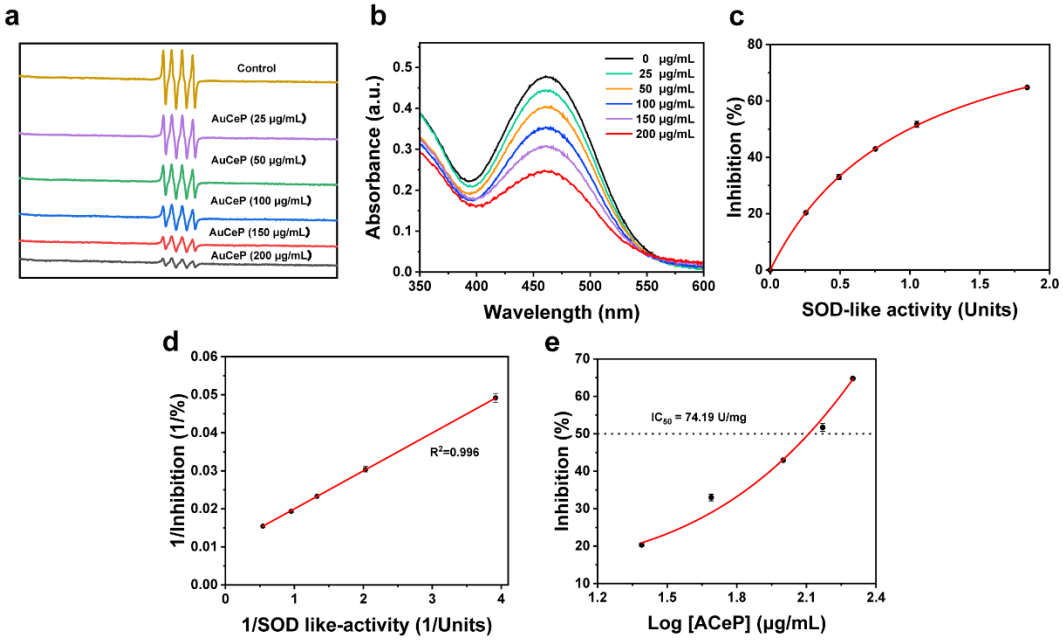

Fig S9. (a) EPR spectra for $\cdot O_2^-$ scavenging ability of AuCeP. (b) Uv-vis-NIR absorption spectra of WST-8 treated with different concentration of AuCeP. (c) Inhibition rate increased as the higher SOD-like activity of AuCeP. (d) Linear correlation between 1/SOD-like activity of AuCeP and 1/inhibition rate. (e) The inhibition rates corresponding to different concentrations of AuCeP were measured respectively and the half-inhibitory concentration (IC_{50}) was calculated.

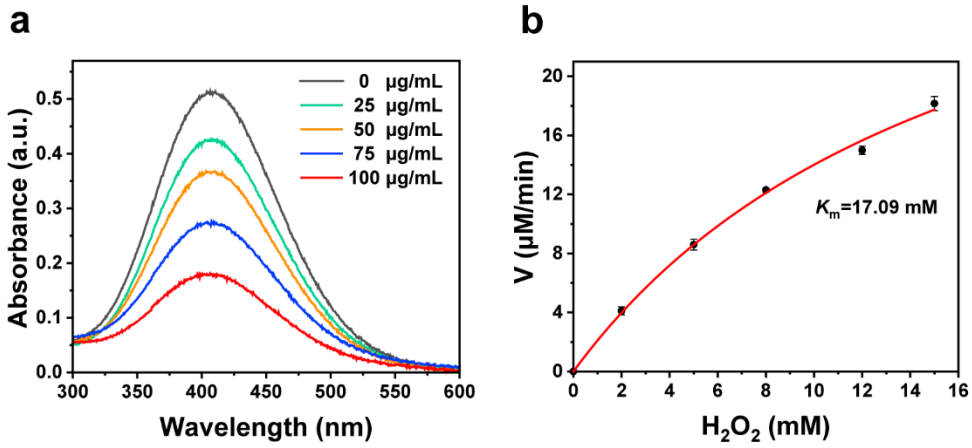

Fig S10. (a) Uv-vis-NIR absorption spectra of H_2O_2 treated with different concentration of AuCeP. (b) Steady-state kinetic profiles of the CAT-like activities. The Michaelis-Menten and corresponding Lineweaver-Burk plots at various concentrations of H_2O_2 (2, 5, 8, 10, 15 mM) for AuCeP.

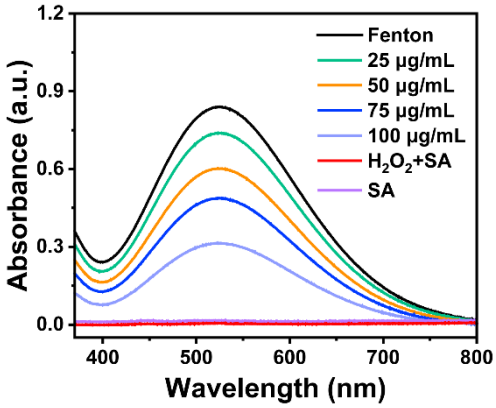

Fig S11. Uv-vis-NIR absorption spectra of SA after different treatments.

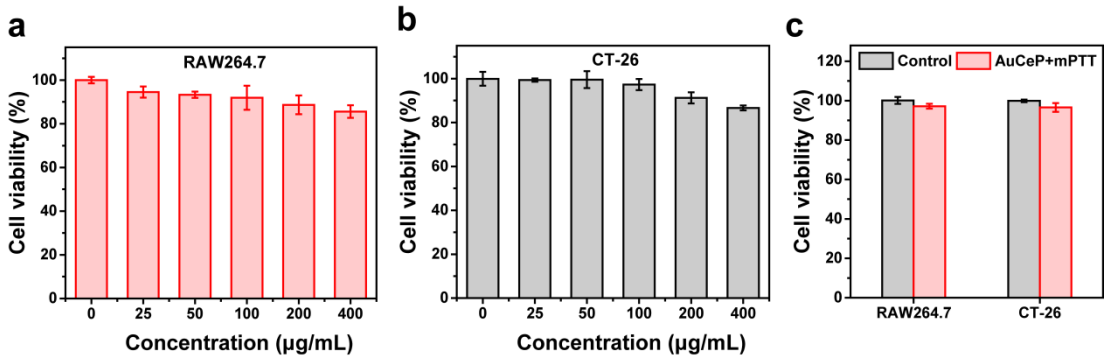

Fig S12. Cell viabilities of (a) RAW264.7 cells and (b) CT-26 cells after incubation with different concentrations of AuCeP for 24 h. (c) Cell viabilities of RAW264.7 cells and CT-26 cells after incubation with100 μg/mL AuCeP for 24 h, and then irradiated by1 W/cm^2 808 nm NIR laser for 10 min.

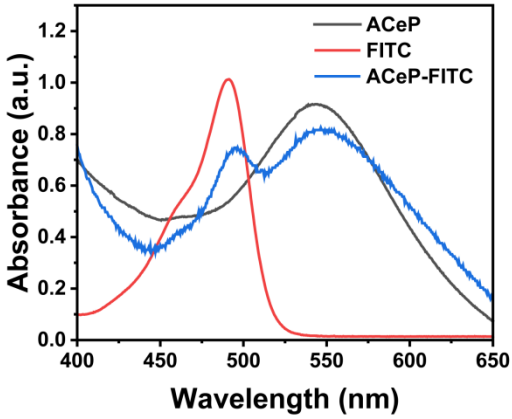

Fig S13. UV-vis-NIR spectrum of AuCeP, FITC and AuCeP-FITC.

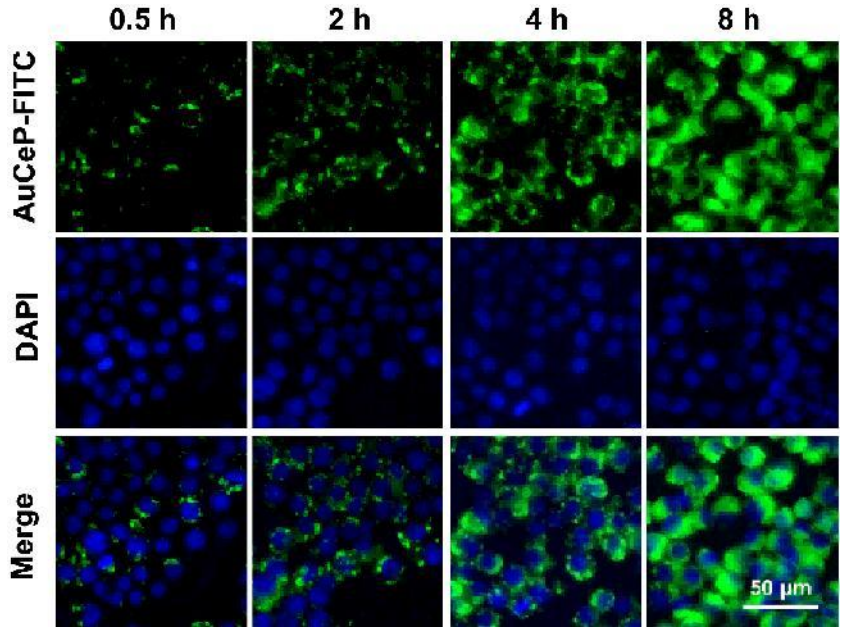

Fig S14. Fluorescent images of RAW264.7 cells receiving the treatment of AuCeP-FITC at different time points of 0.5, 2, 4, and 8 h.

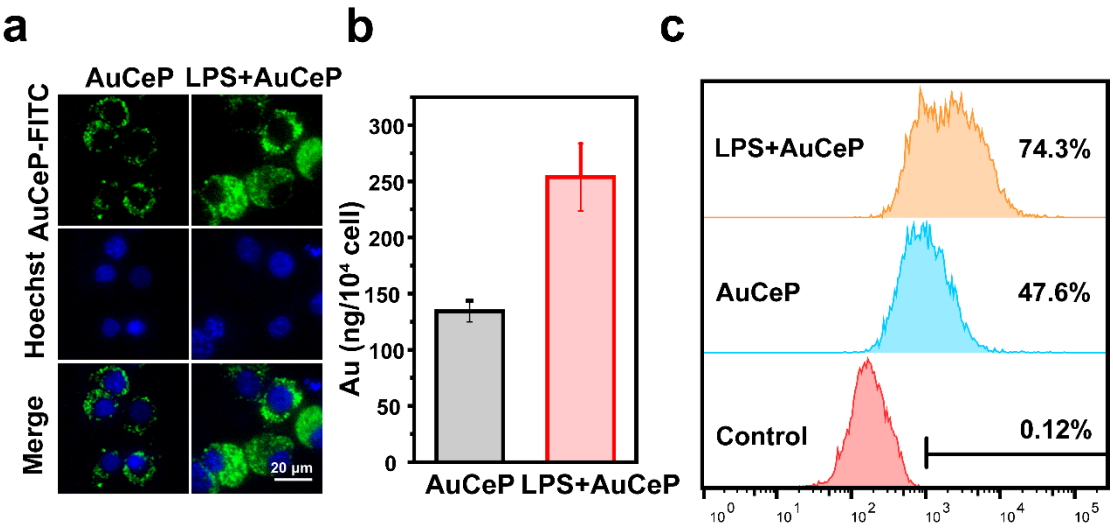

Fig S15. (a) Cellular uptake of AuCeP-FITC in control and LPS-activated RAW264.7 cells. (b) Au content in control and LPS-activated RAW264.7 cells after the AuCeP-FITC treatment. (c) Flow cytometry of AuCeP-FITC in control and LPS-activated RAW264.7 cells.

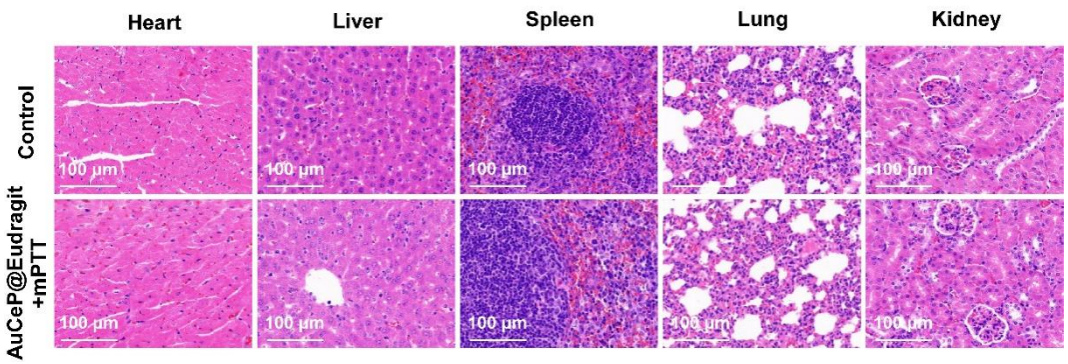

Fig S16. H&E staining of major organs of healthy mice treated with the AuCeP@Eudragit +mPTT and normal reared mice (control), respectively.

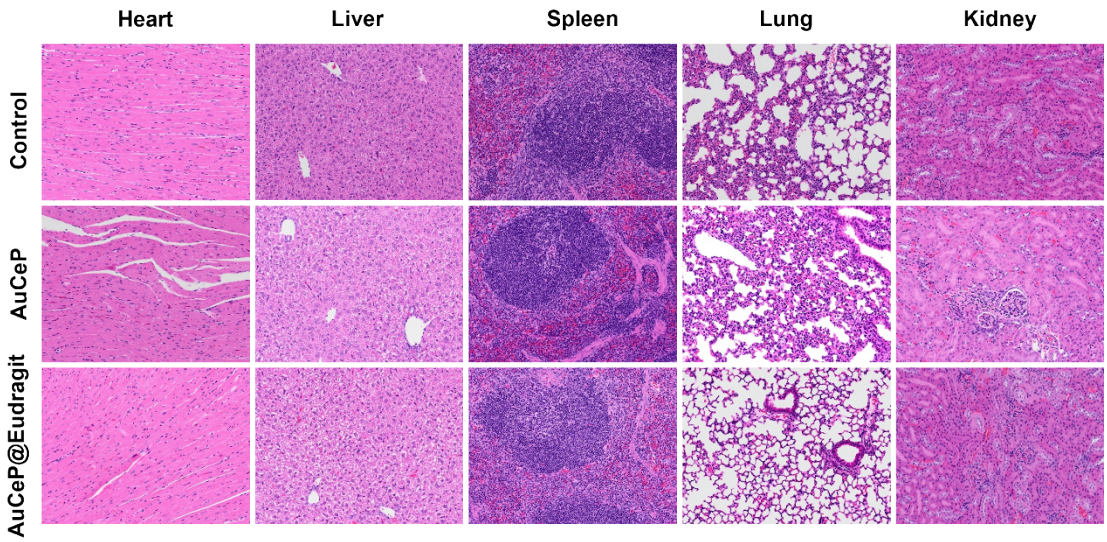

Fig S17. H&E staining of major organs of healthy mice treated with the AuCeP and AuCeP@Eudragit +mPTT after 28 days and normal reared mice (control), respectively.

3 Additional Tables S1

Table S1 Hematology analysis of healthy mice treated with normal reared mice (control) or the AuCeP@Eudragit +mPTT.

Hematological	Units	Control	Treatment
WBS	$\times 10^9$/L	4.55	4.43
RBC	$\times 10^{12}$/L	9.40	9.79
HGB	g/L	136.04	137.06
MCV	fL	49.75	49.34
MCH	pg	14.47	14.00
PLT	g/L	745.94	720.45
PDW	$\times 10^9$/L	7.43	7.15
MCHC	fL	290.84	283.64

Table S2 Hematology analysis of healthy mice treated with normal reared mice (control), the AuCeP or the AuCeP@Eudragit +mPTT.

Hematological	Units	Control	AuCeP	AuCeP@ Eudragit
WBS	$\times 10^9$/L	5.9	5.4	6.6
RBC	$\times 10^{12}$/L	9.13	8.51	10.05
HGB	g/L	141	125	151
MCV	fL	48.4	46.1	47.0
MCH	pg	14.9	14.6	15.0
PLT	g/L	798	795	854
PDW	$\times 10^9$/L	16	15.8	16.1
MCHC	fL	308	318	319

4. Additional References

1. J. Li, S. Song, Y. Long, L. Wu, X. Wang, Y. Xing, R. Jin, X. Liu, H. Zhang, *Advanced Materials*, 2018, **30**, 1704416.
2. W. Y. Zhen, Y. Liu, L. Lin, J. Bai, X. D. Jia, H. Y. Tian and X. Jiang, *Angewandte Chemie-International Edition*, 2018, **57**, 10309-10313.