Electronic Supplementary Information

Metal–organic framework-based biomimetic cascade bioreactor for highly efficient treatment of hyperuricemia with low side effects

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EXPERIMENTAL SECTION

Materials and instrumentation

Zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), 2-methylimidazole (2-MI), uricase (UOX, 10 units mg^{-1}), tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) dichloride were purchased from J&K Scientific (Beijing, China). Catalase (CAT, 10,000-40,000 units mg^{-1}) was obtained Sigma-Aldrich Co., LLC. (Shanghai, China). 3-[4,5-dimethylthiazol-2-yl-]-2,5-diphenyltetrazolium-bromide (MTT), 2',7'-Dichlorofluorescin diacetate (DCFH-DA) were provided by Beyotime Institute of Biotechnology (Shanghai, China). All cell culture components were obtained from Gibco® (Invitrogen) except as otherwise noted. Ultrapure water (18.2 MΩ cm) obtained from Milli-Q system was used throughout the experiments.

Flow cytometry was carried out using a BD FACSCanto TM II Flow Cytometer. The ζ potential and particle size were measured by dynamic light scattering (DLS, Litesizer 500, Anton Paar, Austria). Transmission electron microscope (TEM, Tecnai G2, FEI) was employed to analyze the morphology of NPs. The cell viability and uric acid/H_2O_2 quantification were implemented using a microplate reader (ThermoFisher Scientific). Dissolved oxygen was measured by a real-time dissolved oxygen meter (AZ8403, Japan). The fluorescence signal was monitored by fluorescence spectrophotometer (Agilent).

Preparation of CAT@UOX@ZIF-8

UOX@ZIF-8 was firstly prepared using the one-pot biomineralization method. In detail, 3 mg UOX and 3 mg BSA were first dissolved in 2 mL of 2-MI (2.5 M). Then, 140 μL of $Zn(NO_3)_2 \cdot 6H_2O$ solution (0.5 M) was quickly added to the mixture. After stirring for 20 min, the mixture was centrifuged at 8000 rpm for 10 min. The obtained precipitate was washed 3 times with ddH_2O and redispersed in ddH_2O. For electrostatic assembly of CAT on the surface of UOX@ZIF-8, PEI was coated on the surface by simply mixing 1 mL of PEI (5 mg mL^{-1}) with 2 mL of UOX@ZIF-8, followed by incubation for 15 min. After centrifugation and washing, the obtained PEI-UOX@ZIF-8 (1.5 mL) was further mixed with 1.0 mL of CAT (2 mg mL^{-1}) and

incubated for 20 min under stirring. The obtained CAT@UOX@ZIF-8 was collected via centrifugation at 8000 rpm, followed by washing with ddH_2O for 3 times.

Preparation of RBC-CAT@UOX@ZIF-8

To prepare RBC membrane, whole blood was adopted from the orbital venous plexus of Kunming mice. The whole blood was centrifuged at 3000 rpm for 10 min to remove the upper serum and impurities. After washing with an appropriate amount of 1 × PBS, the supernatant was discarded. This operation was repeat for several times until the supernatant is clear. The obtained erythrocyte precipitation was added to 40 times volume of precooled 0.1 × PBS solution and lysed at 4 ºC for 30 min. after centrifugation at 8000 rpm for 5 min, the supernatant was discarded and pink precipitate (RBC membrane) was collected.

For preparation of RBC-CAT@UOX@ZIF-8, RBC membrane and CAT@UOX@ZIF-8 were mixed in a mass ratio of 1: 1. In this process, the cell membrane was coated on the CAT@UOX@ZIF-8 surface by ultrasound at 4 ºC for 5 minutes. The obtained RBC-CAT@UOX@ZIF-8 was collected by centrifugating at 8000 rpm for 10 min. The obtained precipitate was washed 3 times with PBS and redispersed in PBS for further use. As a control, RBC-UOX@ZIF-8 was prepared using the same method.

Synthesis of FITC labeled enzyme.

Firstly, 50 μL FITC in DMSO (10 mg mL^{-1}) was gradually added to 1 mL enzyme solution (10 mg mL^{-1} UOX or CAT containing 50 mM sodium carbonate buffer, pH 8.5). This reaction was carried out overnight at 4 ºC. The labeled proteins were then dialyzed with sodium carbonate buffer (50 mM, pH = 8.5) to remove free FITC and stored at 4 ºC for further use.

Calculation of drug-loading efficiency

The drug-loading efficiencies of enzyme (UOX or CAT) were calculated by the following formula:

$$\text{LE}\ (\%) = (W_T - W_F)/W_{NP} \times 100\%$$

Where W_T was the total weight of enzyme fed, W_F was the weight of non-encapsulated enzyme, and W_{NP} was the weight of nanoparticles. The weight of enzyme was quantified by measuring the fluorescent signal of labeled FITC.

Degradation kinetics of UA

UA was dissolved in 100 mM boric acid buffer (pH 8.5) to prepare 1 mM UA working solution. Then, UOX, RBC-UOX@ZIF-8 and RBC-CAT@UOX@ZIF-8 were added to working solution respectively. The final concentration of UOX was about 0.2 U mL^{-1}. The reaction system was incubated at 37 ºC and a certain volume of reaction solution was taken out for measuring the absorbance value at 290 nm.

Kinetic studies of H_2O_2 generation

The kinetics of H_2O_2 generation and elimination were monitored according to the following procedure. Firstly, 3, 3', 5, 5'-tetramethylbenzidine (TMB) solution (1.0 mg mL^{-1}, dissolved in ethanol) was mixed with HRP (dissolved in a buffer solution containing 0.1 M citric acid and 0.2 M Na_2HPO_4, pH 5.2) to obtain a substrate solution containing 1.6 mM TMB and 0.005 U mL^{-1} HRP. 100 μL of the substrate solution was injected into a 96 well plate. Meanwhile, 20 μL of reaction solution of UA with UOX, RBC-UOX@ZIF-8 or RBC-CAT@UOX@ZIF-8 was taken at a fixed time and transferred to the aforesaid 96 well plate. After reaction for 5 minutes, 20 μL of H_2SO_4 solution (160 mM) was added to terminate the enzymatic reaction in each well. Finally, the absorbance at 450 nm was recorded by a microplate reader.

Detection of dissolved oxygen.

RBC-UOX@ZIF-8 or RBC-CAT@UOX@ZIF-8 was added into 2 mM UA working solution. Then, the dissolved oxygen was directly measured using a dissolved oxygen meter.

Cytotoxicity measurements

The cytotoxicity of RBC-UOX@ZIF-8 or RBC-CAT@UOX@ZIF-8 against MCF-

7 cells was determined using MTT method. Initially, MCF-7 cells were seeded in 96-well plates at a density of about 8000 cells per well. After culture overnight, the original culture medium was replaced by fresh culture medium containing gradient concentrations of RBC-UOX@ZIF-8 or RBC-CAT@UOX@ZIF-8. After incubation for another 24 h, the culture medium was removed, followed by adding 100 μL of MTT solution (0.5 mg mL^{-1}) into each well. After incubation for 4 h, MTT solution was replaced by 150 μL of DMSO and the absorbance at 490 nm was measured using a microplate reader.

Intracellular ROS analysis

To evaluate the total intracellular ROS content, MCF-7 cells were cultured in 6-well culture plate for 24 hours. Then the original culture medium was replaced by fresh culture medium containing the mixture of UA with UOX, RBC-UOX@ZIF-8 or RBC-CAT@UOX@ZIF-8. After incubation for 6 h, the cells were collected by digestion and further incubated with DCFH-DA for 20 minutes. Finally, the quantitative fluorescence signals were monitored by flow cytometry.

***In vivo* biodistribution study**

All animal experiments have been approved by the Ethical Committee of Fujian Medical University (No. FJMU IACUC 2021-0353) and conducted by following the guidelines from the Ethics Committee and national regulations of China. Kunming mice (purchased from Shanghai SLAC laboratory animal Co. Ltd.) were randomly divided into two groups of three. The mice were intravenously injected with 200 μg of FITC-RBC-CAT@UOX@ZIF-8 or PBS (as a control). Afterwards, the mice were euthanized 24 hours after administration, and the main organs (including heart, liver, spleen, lungs, kidney and blood) were collected. The organs were homogenized in 1 mL of PBS using an automatic sample rapid grinder. The supernatant was collected by centrifugation, and the fluorescence signal at 488 nm was measured. To calculate the fluorescence signal in blood, the blood weight was estimated to be 6% of the body weight of mice.

In vivo studies of uric acid degradation and H_2O_2 elimination

Male Kunming mice of 8-10 week old from Shanghai Lingchang Biotechnology Co., Ltd. were used in this work. The animal experiments were approved by the Institutional Animal Care and Use Committee at Fujian Medical University. Hyperuricemia was induced in mice according to the previously reported method[1]. Food and water were withheld overnight prior to the study. Thirty male Kunming mice were randomly divided into five groups six: normal control group, hyperuricemia model group, UOX group, RBC-UOX@ZIF-8 group and RBC-CAT@UOX@ZIF-8 group. Except for the normal control group, the mice in other groups were given hypoxanthine dissolved in 0.5% carboxymethylcellulose sodium by gavage (500 mg kg^{-1}) and oxonic acid potassium by subcutaneous injection (100 mg kg^{-1}). Three treatment groups were injected with UOX, RBC-UOX@ZIF-8 or RBC-CAT@UOX@ZIF-8 in caudal vein 1 h after modeling while the hyperuricemia group was given PBS. The blood of fundus venous plexus was taken at the specified time after modeling. The blood UA or hydrogen peroxide levels were then analyzed using a commercial UA detection kit or hydrogen peroxide detection kit (Beyotime).

For H&E staining analysis, the mice were euthanized 24 h after the treatment, and the main organs (heart, liver, spleen, lungs and kidney) were taken for H&E staining analysis.

For blood biochemical analysis, blood samples were adopted 24 h after the treatment. After natural coagulation at 4 ºC, serum samples were collected via centrifugating at 3000 rpm for 10 minutes. The obtained supernatants were directly used for biochemical test.

Statistical analysis

All data in this article were presented as mean ± standard deviation (SD), while statistical significance was analyzed by Student's t-test (*$p < 0.05$, **$p < 0.01$, and ***$p< 0.001$).

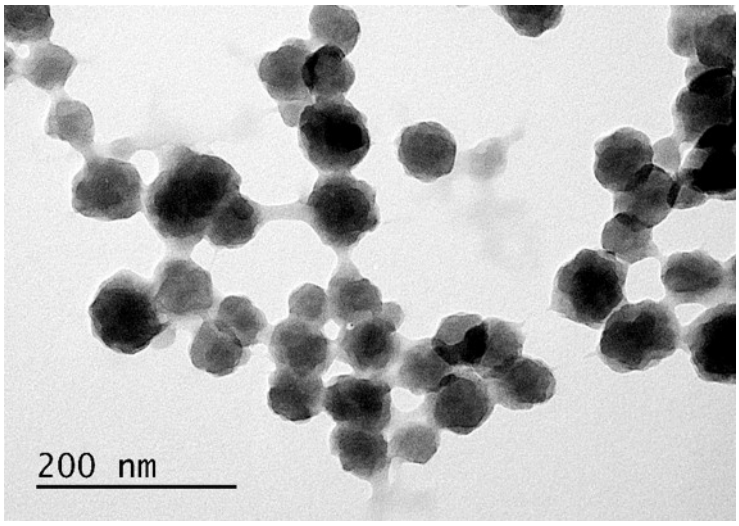

Fig. S1 TEM image of UOX@ZIF-8

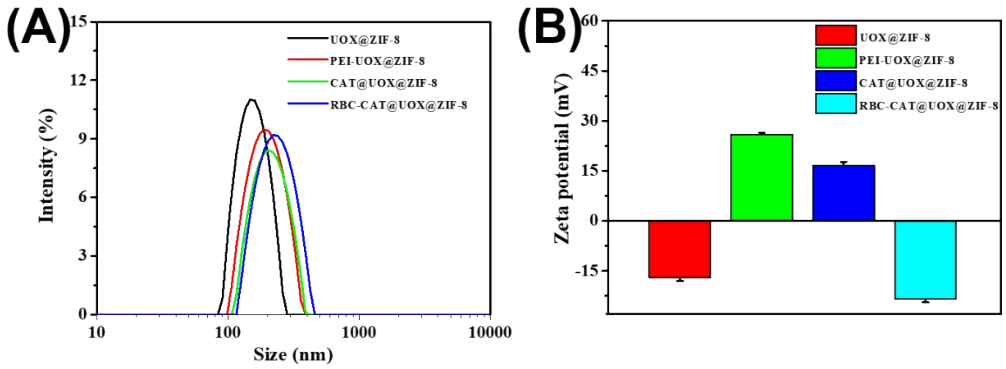

Fig. S2 DLS analysis (A) and zeta potential (B) of UOX@ZIF-8, PEI-UOX@ZIF-8, CAT@UOX@ZIF-8 and RBC-CAT@UOX@ZIF-8.

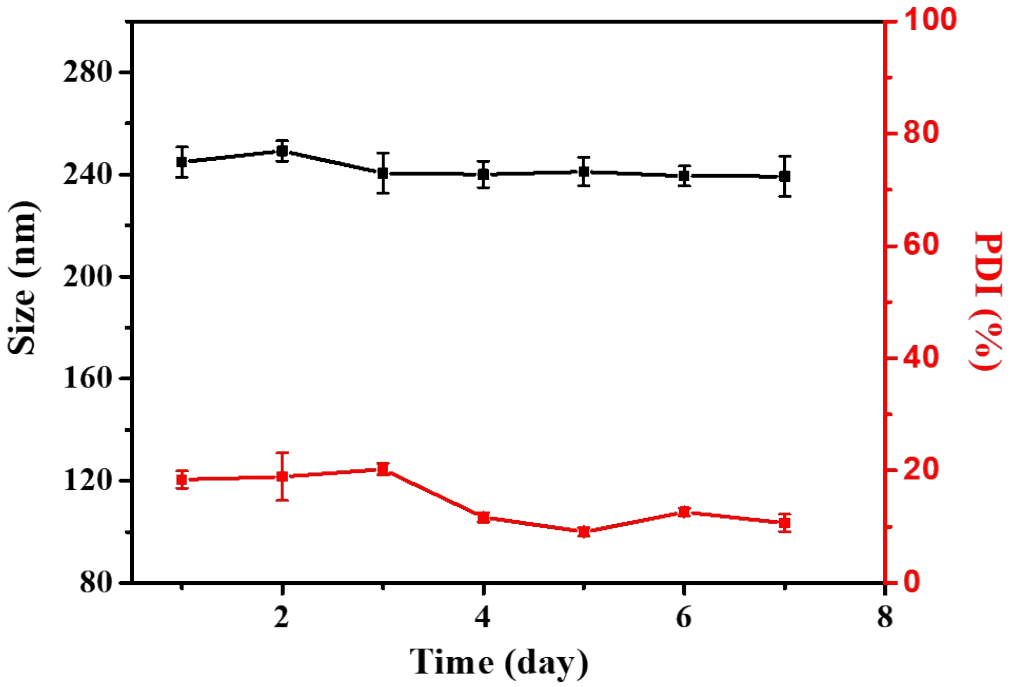

Fig. S3 Hydrodynamic size distribution and PDI changes of RBC-CAT@UOX@ZIF-8 in 7 days.

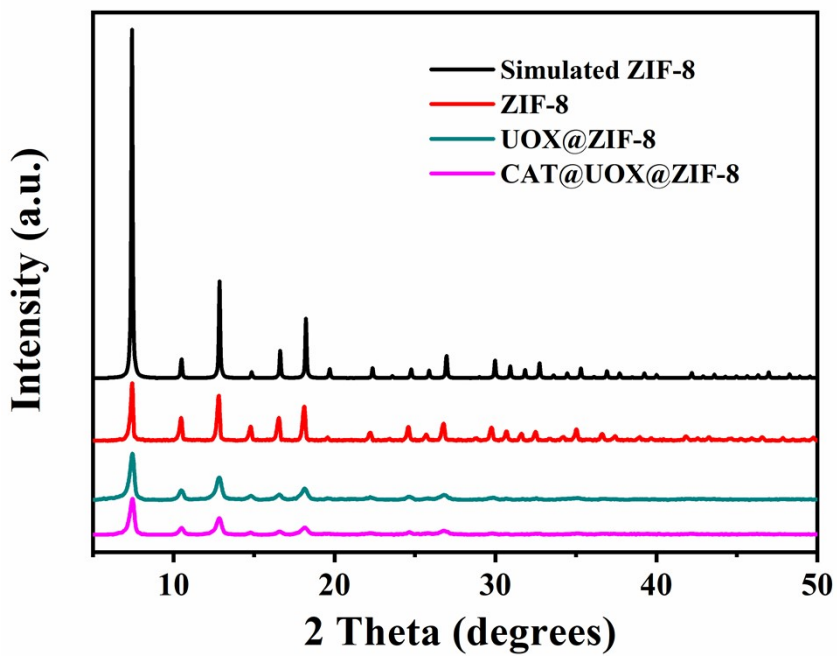

Fig. S4 The PXRD patterns of ZIF-8, UOX@ZIF-8, and CAT@UOX@ZIF-8 similar to the published simulation patterns of ZIF-8 crystal structure.

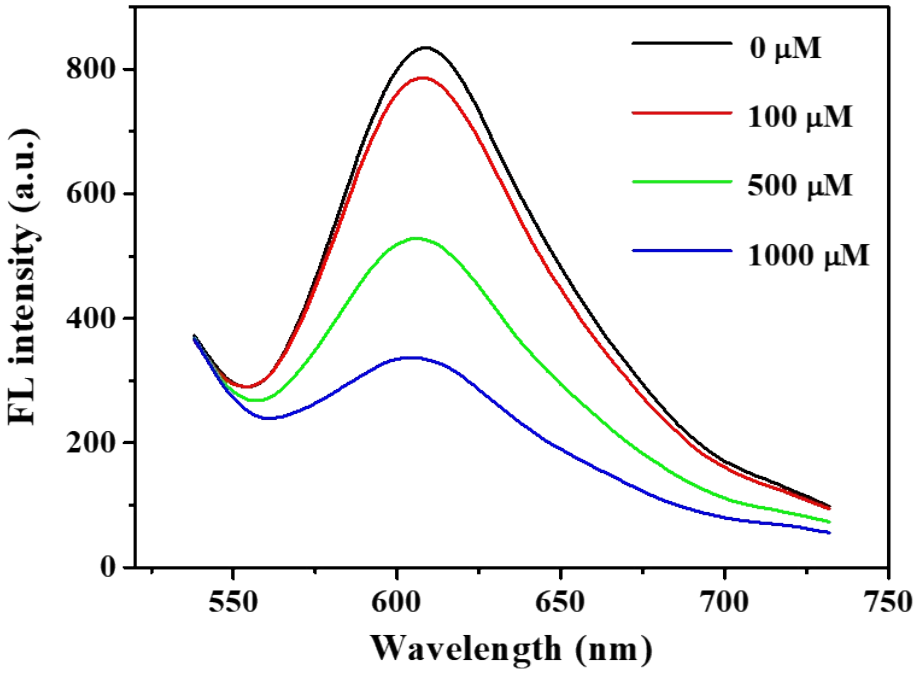

Fig. S5 Fluorescence spectra of RDPP after incubation with RBC-CAT@UOX@ZIF-8 in the presence of different concentrations of H_2O_2. *Note*: the fluorescence intensity of RDPP is inversely proportional to the concentration of O_2.

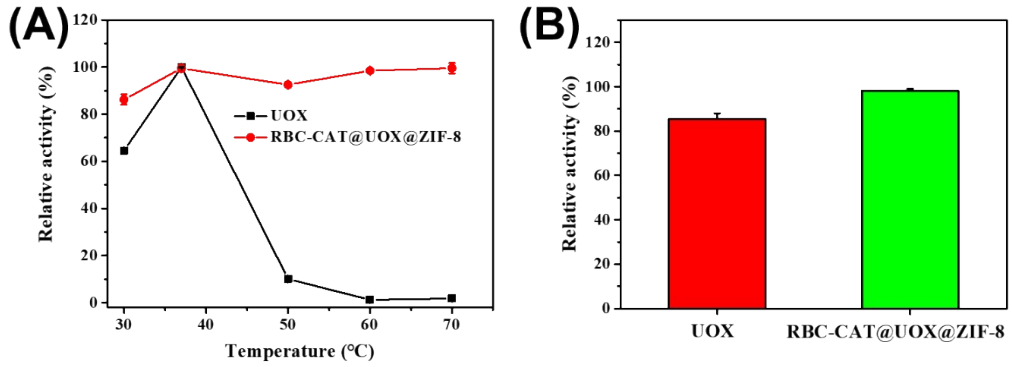

Fig. S6 The UA degradation activity of UOX and RBC-CAT@UOX@ZIF-8 under different temperature (A) and after treatment with 1.0 mg mL^{-1} trypsin (B).

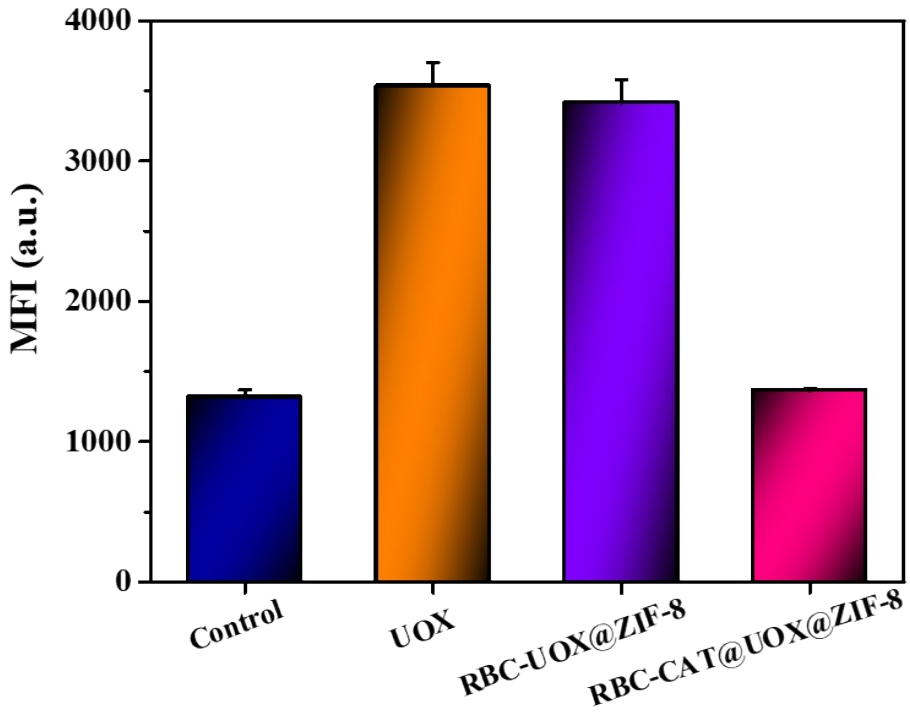

Fig. S7 Quantification of intracellular ROS from Fig. 3B.

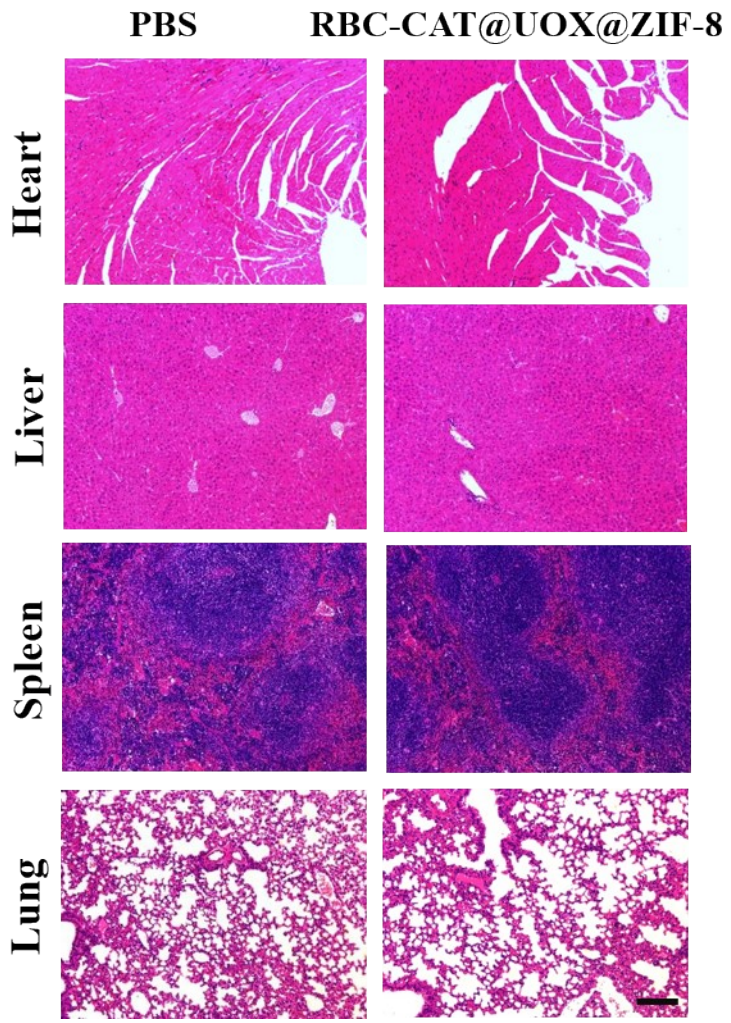

Fig. S8 H&E-stained histological sections from major organs 24 h after intravenous administration of PBS or RBC-CAT@UOX@ZIF-8 into healthy mice. Scale bar: 200 μm.

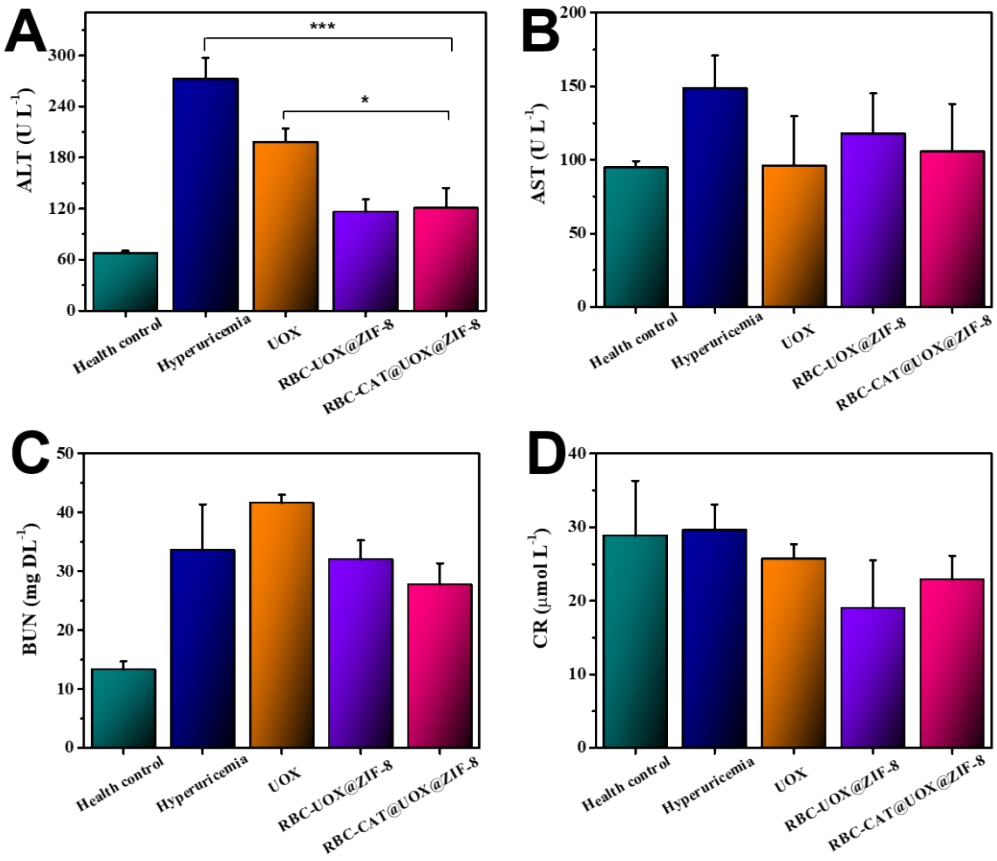

Fig. S9 Blood chemistry tests of hyperuricemia model mice injected with UOX, RBC-UOX@ZIF-8, and RBC-CAT@UOX@ZIF-8 via tail vein (n=3). Healthy mice were employed as the control. The indicators determined include alanine amiotransferase (ALT), aspartate amiotransferase (AST), blood urea nitrogen (BUN) and creatinine (CR).

Reference

1 X. Liu, Z. Zhang, Y. Zhang, Y. Guan, Z. Liu, J. Ren and X. Qu, *Adv. Funct. Mater.*, 2016, **26**, 7921.