Supporting information

Materials

Polyvinylpyrrolidone (PVP K30), chloroplatinic acid hexahydrate ($H_2PtCl6 \cdot 6H_2O$, 99%), Iron chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), Cupric chloride dihydrate ($CuCl_2 \cdot 2H_2O$), Nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$), Zinc chloride ($ZnCl_2$), Ruthenium (III) chloride hydrate ($RuCl_3 \cdot xH_2O$), Gold (III) chloride hydrate ($HAuCl_4 \cdot XH_2O$), Manganese Chloride Tetrahydrate ($MnCl_2 \cdot 4H_2O$) were purchased from Aladdin Reagent (China). TMB, CytC, Hydrogen peroxide (H_2O_2, 30%), DPPH, ABTS, and DCFD-A were bought from Sigma-Aldrich (USA). Reactive Oxygen Species Assay Kit, Apoptosis Detection kit (propidium iodide/annexin V-FITC) were obtained from Beyotime Institute of Biotechnology (China). All the aqueous solutions were prepared using purified deionized (DI) water purified with a purification system (Direct-Q3, Millipore, United States).

Synthesis of bimetallic nanozyme and QCN.

Briefly, 133 mg polyvinylpyrrolidone (molecular weight 30 KDa) was dissolved in 180 mL methanol. Then, 20 mL deionized water solution dissolved with 10 mg $H_2PtCl_6 \cdot 6H_2O$ and equimolar amounts of other metal chlorides (Ru, Mn, Fe, Cu, Zn, Ni, Au) was added to the above solution, and stirred at 70°C for 3 hours. After the reaction is complete, the reaction solution was rotary evaporated to remove excess methanol, and the residue was collected into a centrifuge tube. The reaction solution was washed several times with chloroform and n-hexane until neutral, and then dried in a vacuum oven to obtain the bimetallic nanozymes. The obtained bimetallic nanozymes were named RuPt nanozyme, MnPt nanozyme, FePt nanozyme, ZnPt nanozyme, NiPt, CuPt nanozyme, AuPt nanozyme, respectively.

Synthesis of QCN.

The oxidation nanozyme was developed through the coordination of quercetin (Que) with a ruthenium (Ru) doped platinum (Pt) nanozyme (RuPt nanozyme). Briefly, 1 mg Que was dissolved in 1 mL DMSO and then dropped into 9 mL double-distilled water containing 10 mg RuPt nanozyme. The mixture was stirred for 30 min, followed by dialysis for 24 h and lyophilization under vacuum.

Catalase-like Activity of bimetallic nanozyme and QCN.

In order to investigate the catalase-like activity of bimetallic nanozyme and QCN, 200 μL H_2O_2 (100 mM) and 200 μL of bimetallic nanozyme and QCN solution were added to 10mL buffer solution (0.01M) at 25°C. The change in dissolved oxygen content in the system was detected using a dissolved oxygen meter immediately after vibration mixing.

SOD-like activity of bimetallic nanozyme and QCN.

The SOD-like activity of bimetallic nanozyme and QCN was determined using xanthine oxidase method. Briefly, 30 μL of prepared nanozyme solutions were transferred into a 96-well plate. xanthine, PH7.4 phosphate buffer, Cytochrome C, xanthine oxidase working solution were added in sequence. The resulting solutions were immediately Measured the absorbance of each well at 450 nm for 2 minutes before and after, using a microplate reader, each group was parallel three times.

ABTS radical-scavenging activity of bimetallic nanozyme and QCN.

A discoloration test of the ABTS radical cations was utilized to measure the free radical scavenging capacity of the prepared NPs. First, ABTS radical cations ($ABTS^{\cdot+}$) was produced by mixing ABTS aqueous solution (7 mM) and potassium persulfate (2.45 mM) for 16 h. Then, different concentrations of bimetallic nanozyme and QCN (0-200μg/mL) were mixed to the above mixture. Finally, the absorbance value at 734 nm of the final solutions was measured and scavenging efficiency of ABTS is calculated as follows:

ABTS radical cations scavenging activity=$[1-(A_i-A_1)]/A_0 \times 100\%$.

A_0 is the absorbance of ABTS solutions without adding samples; A_i is the absorbance of the sample after reacting with ABTS; A_1 is the absorbance of solvent mixed with NPs.

DPPH radical cations scavenging activity of bimetallic nanozyme and QCN.

Different concentrations of bimetallic nanozyme and QCN were prepared and mix them with DPPH (125M) in the same volume of ethanol. The final bimetallic nanozyme and QCN concentrations were 0-200μg/mL and the final DPPH concentration was 62.5 M. After 30 min to complete the reaction, the absorption of DPPH at 517 nm was measured. The scavenging efficiency of DPPH was calculated by the following equation:

DPPH radical cations scavenging activity=$[1-(A_i-A_1)]/A_0 \times 100\%$.

A_0 is the absorbance of DPPH solutions without adding samples; A_i is the absorbance of the sample after reacting with DPPH solutions; A_1 is the absorbance of solvent mixed with bimetallic nanozyme and QCN.

Cell culture

Human embryonic kidney 293 (HEK293) cells were purchased from the American Type Culture Collection (ATCC) and cultured under 5% CO_2 at 37 °C in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (FBS).

Cell Cytotoxicity

HEK293 cells were seeded with 8000 cells/well on a 96 well plate and incubated for 24 hours. Afterward, cells were treated with varied doses QCN (0-200 μg/mL). After treatment for 24h and 48h, cell viability was measured by Cell Counting Kit8 assay.

Hemolysis Assays

Red blood cells were isolated from serum by centrifugation (1500rpm, 10 minutes) and then incubate different concentrations of RuPt nanozyme, Que, and QCN (25−400 μg/mL) with red blood cells at 37 ° C for 30 minutes (materials diluted with PBS). Finally, centrifuge the above mixed solution and take 100μL supernatant and measure its absorbance at 578nm via a microplate reader.

Hemolysis ratio=$[OD_{(sample)}-OD_{(PBS)}]/[OD_{(ddH2O)}-OD_{(PBS)}] \times 100\%$

Protection of Cells with H_2O_2 Stimulation by RuPt nanozyme, Que, and QCN.

HEK293 cells were seeded in a 96-well plate at a concentration of 8000 cells/well and incubated for 24 hours. Then, cells were treated with RuPt nanozyme, Que, and QCN at different concentrations (0−200 μg/mL) for 2 hours. Next, cells were stimulated with H_2O_2 (500 μM) for 24 h in the dark. Finally, CCK8 assay was used to detect cell cell viability.

HEK293 cells were seeded in six-well plates at a concentration of 15,000 cells/well and incubated for 24 hours. Then, cells were treated with RuPt nanozyme, Que, and QCN at a concentration of 50 μg/mL for 2 hours. Next, cells were stimulated with H_2O_2 (500 μM) for 24 h in the dark. Finally, Calcein AM (1μM) and 2 μM propidium iodide (PI) were applied to study cell viability (Calcein AM for live cells; PI for dead cells). Fluorescence images were taken on an inverted fluorescence microscope.

In addition, the apoptosis of HEK293 cells was evaluated by Annexin V Apoptosis Detection Kit. Cells were treated and collected using the same method, then washed three times with cold PBS and stained with Annexin V and PI. Finally, the collected cells were evaluated by flow cytometry.

Detection of Intracellular ROS

Intracellular ROS was measured by 2′,7′-dichlorofluorescein diacetate (DCFH-DA) staining. HEK293 cells were seeded in six-well plates at a concentration of 15,000 cells/well and incubated for 24 hours. Then, cells were treated with RuPt nanozyme, Que, and QCN at a concentration of 50 μg/mL for 24 hours. Then, the cells were washed with PBS to remove free RuPt nanozyme, Que, and QCN. Next, HEK293 cells were treated with H_2O_2 (1mM) for 30 min. Finally, cells were imaged by an inverted fluorescence microscope (emission wavelength: 520 ± 20 nm, excitation wavelength: 488 nm). In addition, the cells were treated and collected using the same method, stained with DCFH-DA, incubated at 37°C for 30 min, and washed three times with PBS. Finally, the collected cells were evaluated by flow cytometry.

AKI mouse model

The Balb/c female mice (6-8 weeks, 17-21 g) used in this study were purchased from the Experimental Animal Center of Zhengzhou University, and all animal studies were conducted in accordance with the protocol approved by the Animal Ethics Committee of the Experimental Animal Center of Zhengzhou University (license No. ZZU-LAC20230526[20]).

Glycerol-induced AKI mouse model: Balb/c mice were dehydrated for 15 hours but were able to freely obtain food. Afterwards, 50% glycerol was injected into the muscles of both hind limbs of the mice at a dose of 8mL/kg. After 2 hours of injection, an AKI mouse model was successfully established and used for subsequent experiments.

Cisplatin-Induced Acute Kidney Injury Model: cisplatin (20 mg/kg) injected into the abdominal cavity of all Balb/c mice. For the treatment group, mice were injected with RuPt nanozyme, Que, and QCN through the tail vein 2 hours after intraperitoneal injection of cisplatin. After 24 hours, each group of mice was euthanized, and blood samples and kidney tissue were collected and analyzed.

Therapeutic Effect in AKI Mice.

To analyze the therapeutic effects of RuPt nanozyme, Que, and QCN, we randomly divided mice into five groups: (i) healthy mice treated with PBS; (ii) AKI mice treated with PBS; iii) AKI mice treated with RuPt nanozyme; (iv) AKI mice treated with Que; (v) AKI mice treated with QCN. After treatment, kidney function and body weight were monitored.

Kidney Function Evaluation.

After 24 hours intravenous injection of RuPt nanozyme, Que, and QCN, the mice were euthanized, and kidney and blood samples were collected. The obtained kidney sections were stained with H&E, and the levels of BUN and CRE in blood samples were detected.

Detection of Biomarkers.

Kidneys from each group were stored at −80°C until assayed. Kidney homogenates were prepared according to the protocol for the different assays. SOD levels were assessed with a SOD assay kit (Solarbio, China). KIM-1 expression and HO-1 expression were measured using KIM-1 or HO-1 Elisa kit (mmbio, China). DNA damage was assessed using a DNA damage competition Elisa kit (mmbio, China).

Confocal imaging of superoxide production in kidneys.

To assess superoxide production histologically, kidneys obtained from mice were cryosectioned at −20°C. Frozen kidney tissue sections were washed with PBS and stained with 1 mM dihydroethidium (DHE) for 30 min to detect superoxide formation. Kidney tissue sections were then placed on glass slides and confocal imaged using a confocal microscope.

In vivo toxicity assessment.

QCN (20mg/Kg) and PBS were intravenously injected into two groups of mice (n=5) twice a week. After 21 days, organ and blood samples were collected from both groups. Then, H&E staining was performed on the heart, liver, spleen, lungs, and kidneys of the mice, and blood data from both groups were measured. The relevant blood parameters are as follows: alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CRE), blood urea nitrogen (BUN), white blood cells (WBC), lymphocytes (LYM), red blood cells

(RBC), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), hematocrit (HCT), mean cell volume (MCV), mean hemoglobin concentration (MCHC), platelets (PLT), platelet distribution width (PDW), average Platelet volume (MPV) and red blood cell distribution width (RDW).

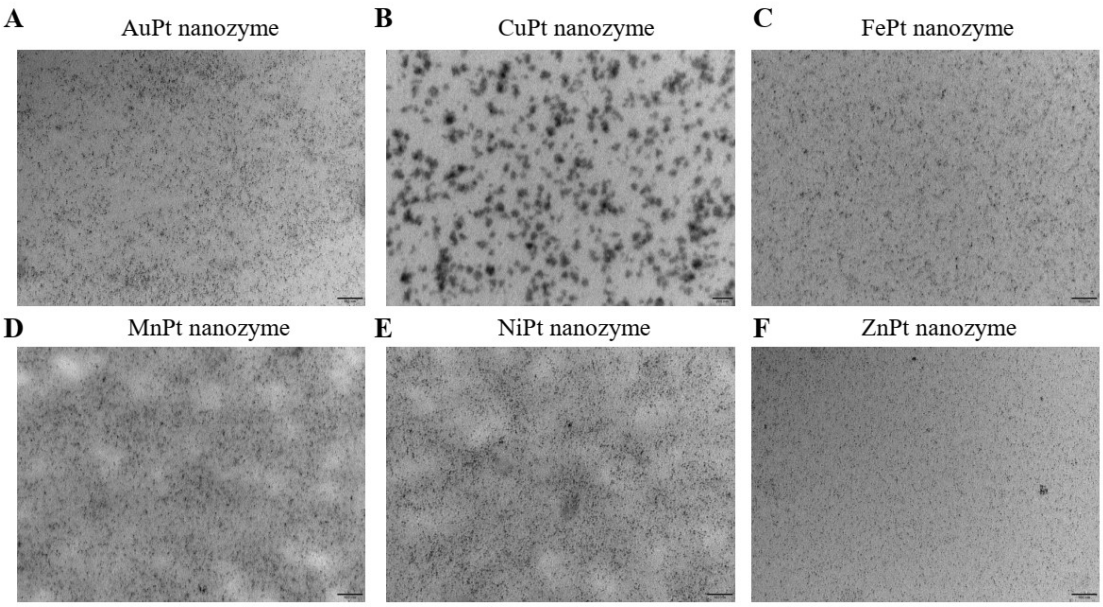

Figure S1. TEM image of AuPt nanozyme, CuPt nanozyme, FePt nanozyme, MnPt nanozyme, NiPt nanozyme, and ZnPt nanozyme.

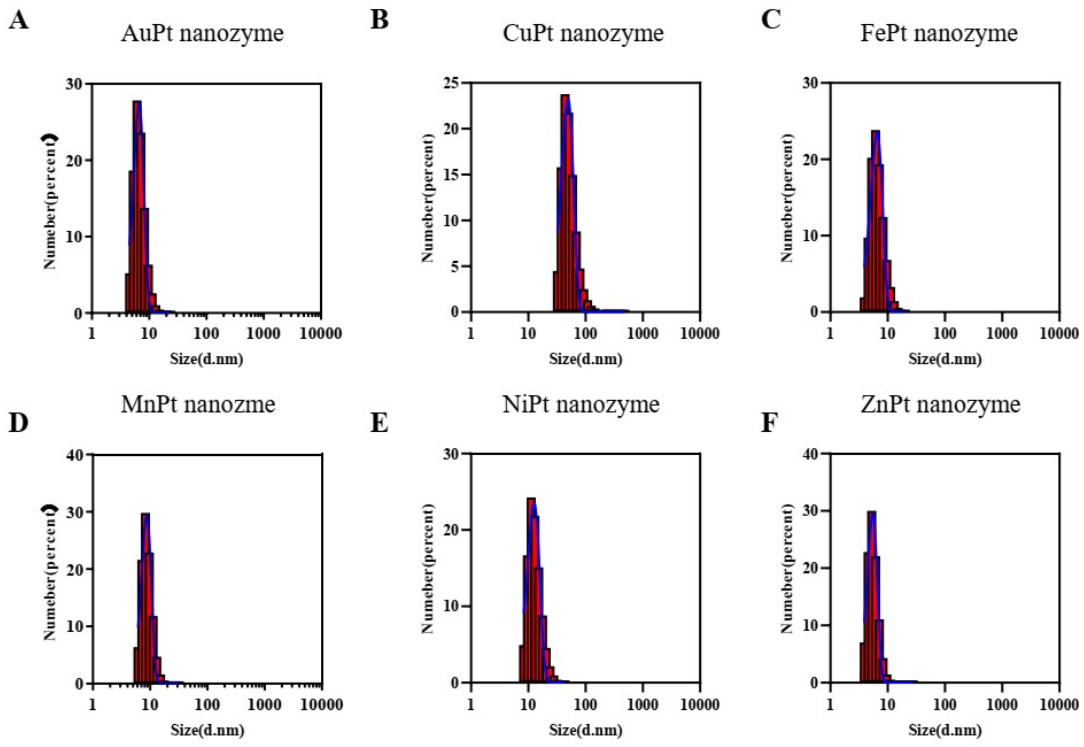

Figure S2. Size distribution of AuPt nanozyme, CuPt nanozyme, FePt nanozyme, MnPt nanozyme, NiPt nanozyme, and ZnPt nanozyme.

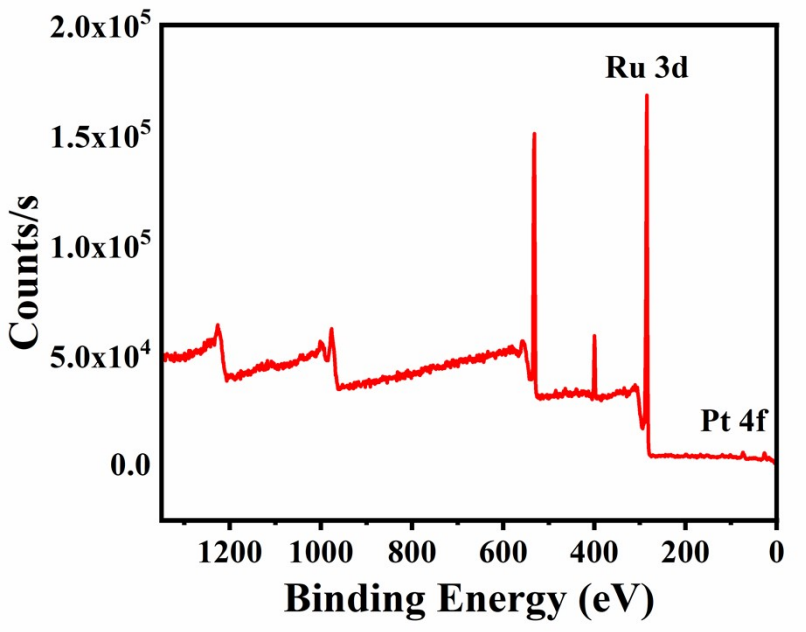

Figure S3. XPS analysis of QCN.

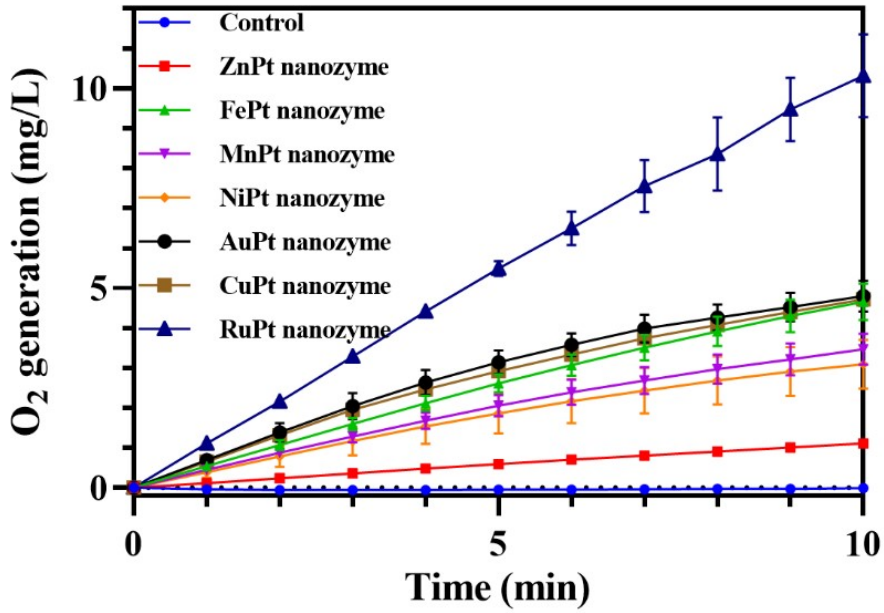

Figure S4. CAT-like activity of AuPt nanozyme, CuPt nanozyme, FePt nanozyme, MnPt nanozyme, NiPt nanozyme, RuPt nanozyme and ZnPt nanozyme.

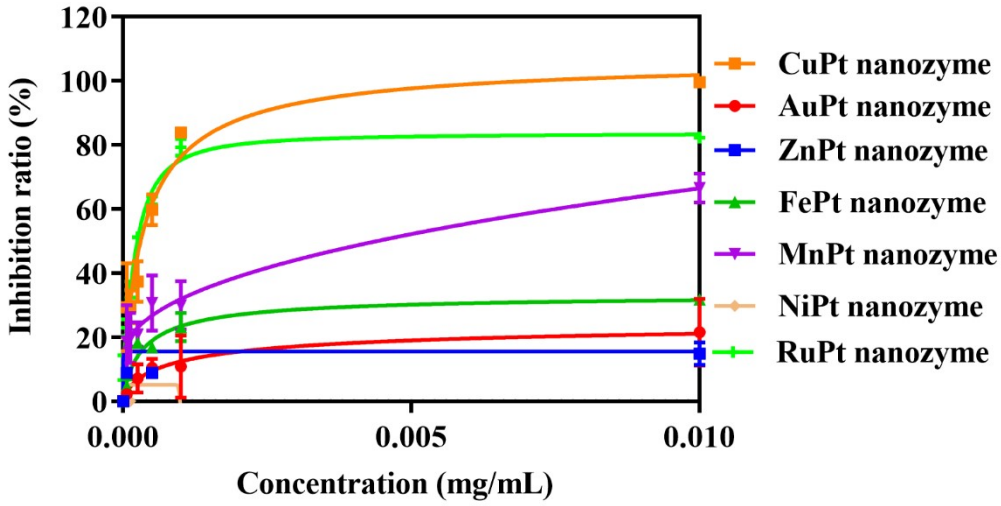

Figure S5. SOD-like activity of AuPt nanozyme, CuPt nanozyme, FePt nanozyme, MnPt nanozyme, NiPt nanozyme, RuPt nanozyme and ZnPt nanozyme.

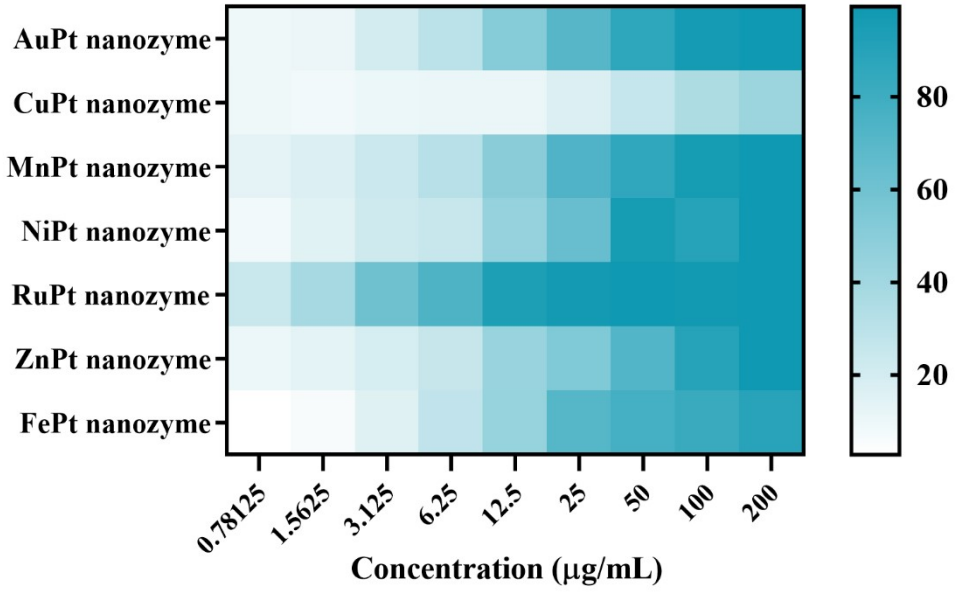

Figure S6. ABTS radical scavenging ratio of AuPt nanozyme, CuPt nanozyme, FePt nanozyme, MnPt nanozyme, NiPt nanozyme, RuPt nanozyme and ZnPt nanozyme.

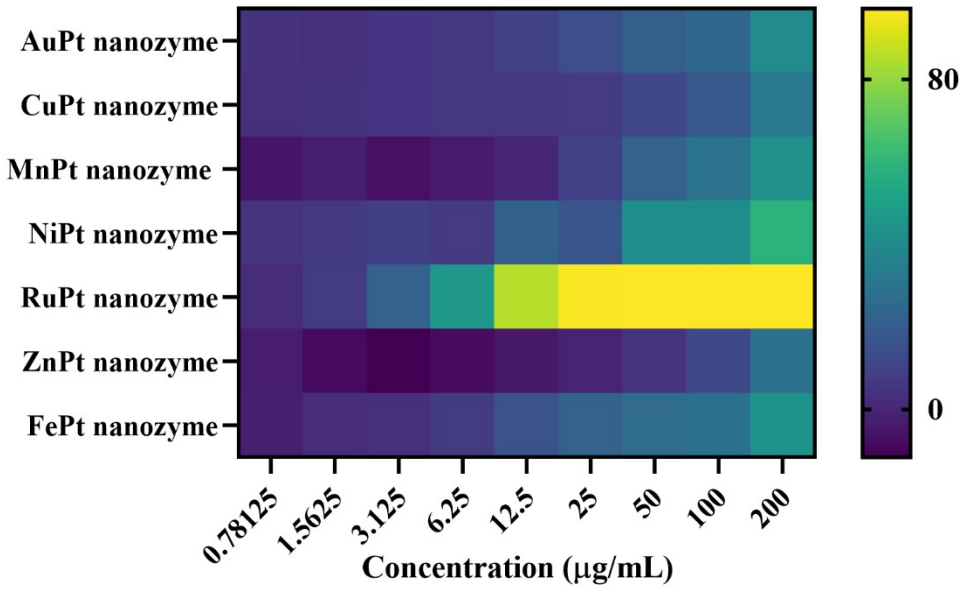

Figure S7. DPPH radical scavenging ratio of AuPt nanozyme, CuPt nanozyme, FePt nanozyme, MnPt nanozyme, NiPt nanozyme, RuPt nanozyme and ZnPt nanozyme.

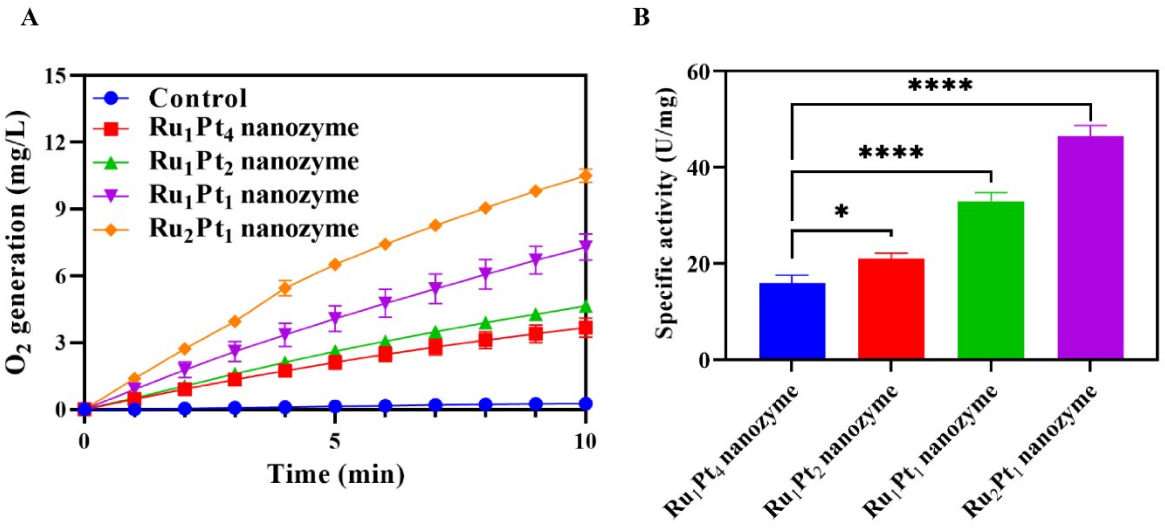

Figure S8. CAT-like activity of Ru_1Pt_4 nanozyme, Ru_1Pt_2 nanozyme, Ru_1Pt_1 nanozyme, Ru_2Pt_1 nanozyme.

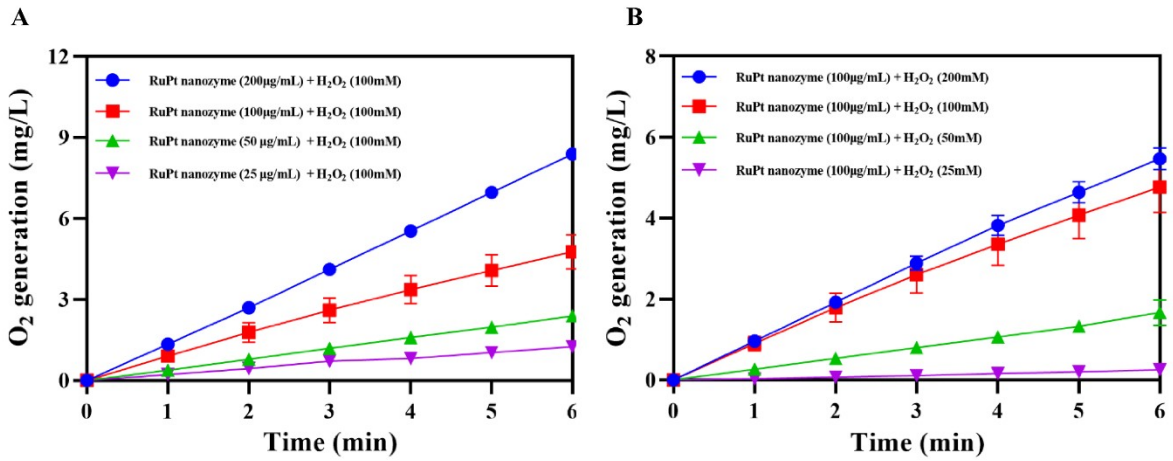

Figure S9. O_2 generations of H_2O_2 solutions in different concentrations of RuPt nanozyme (A) or H_2O_2 (B).

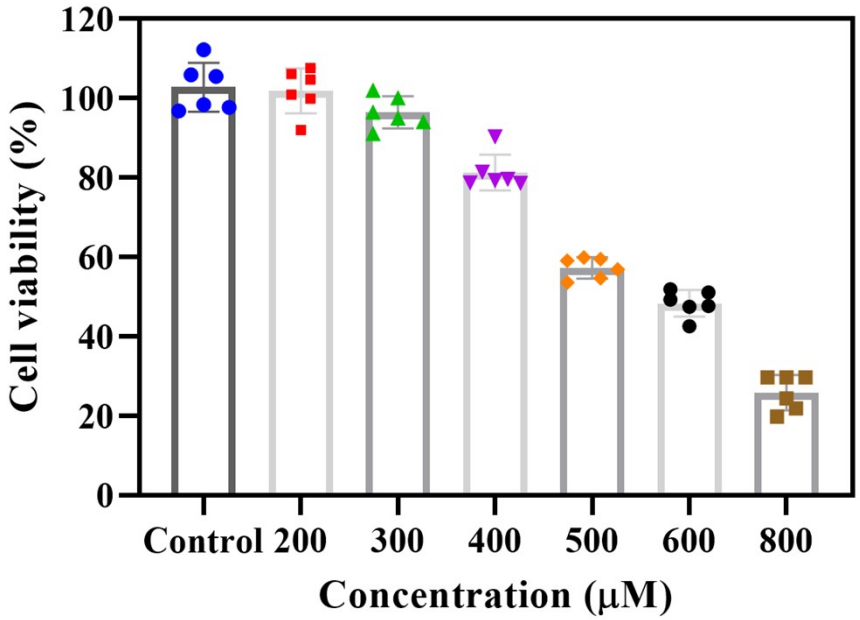

Figure S10. Cell viability of HEK293 cells damaged by different concentrations of H_2O_2 as determined by CCK8 assay (n=6).

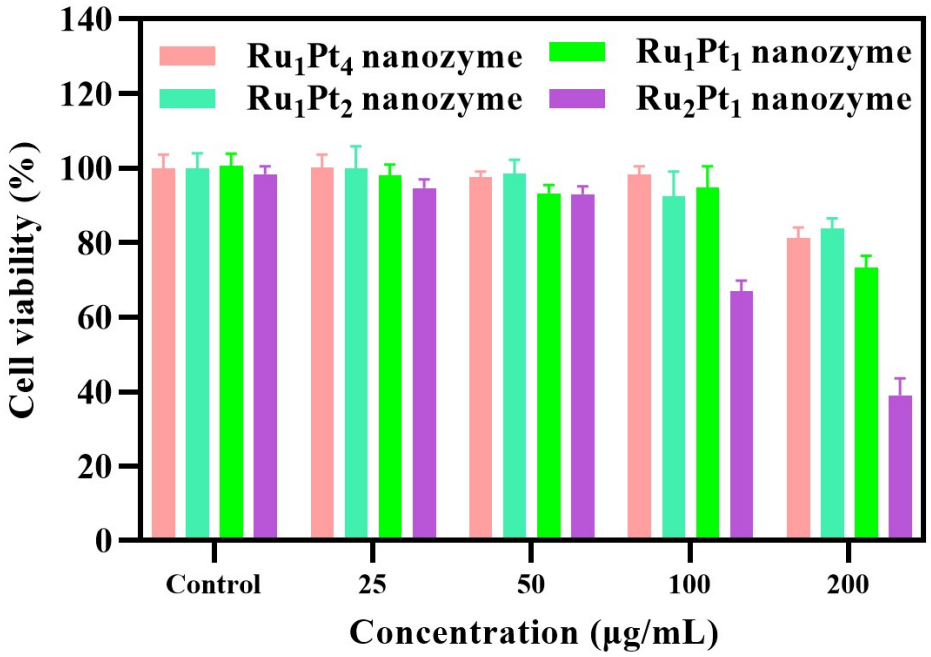

Figure S11. Cell viability of HEK293 cells treated with various concentrations of Ru_1Pt_4 nanozyme, Ru_1Pt_2 nanozyme, Ru_1Pt_1 nanozyme, Ru_2Pt_1 nanozyme as determined by CCK8 assay (n=5).

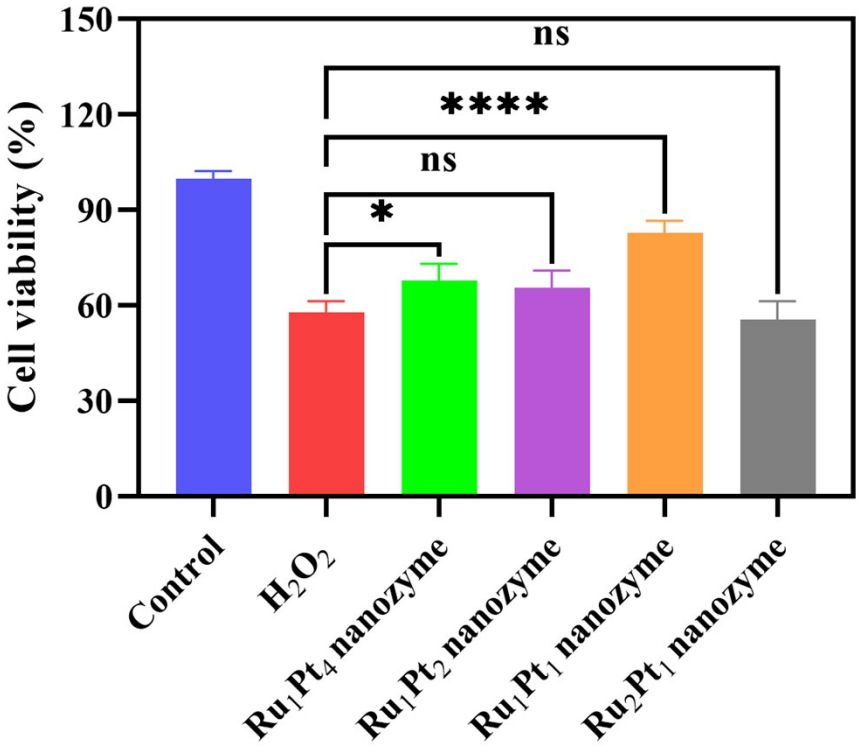

Figure S12. Cell viability of HEK293 cells under H_2O_2 with/without various concentrations of Ru_1Pt_4 nanozyme, Ru_1Pt_2 nanozyme, Ru_1Pt_1 nanozyme, Ru_2Pt_1 nanozyme as determined by CCK8 assay (n=5).

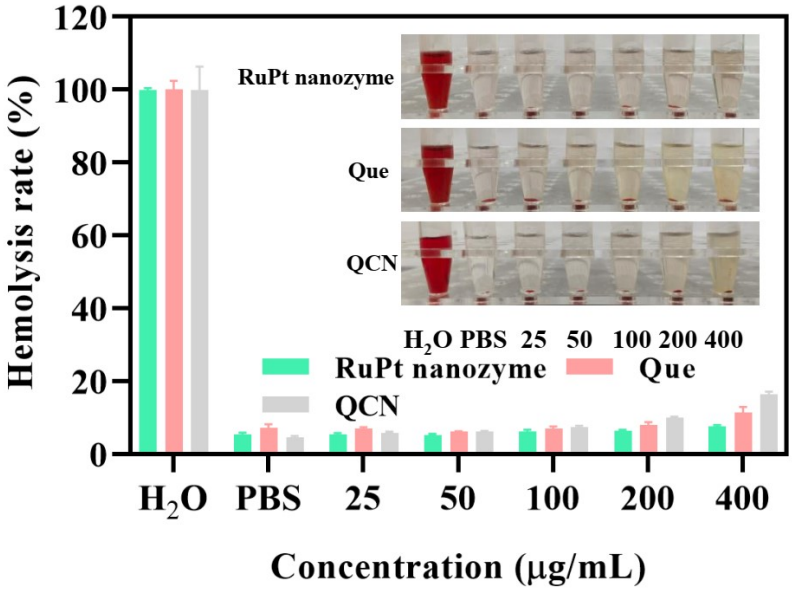

Figure S13. Hemolysis test of RuPt nanozyme, Que, QCN on red blood cells.

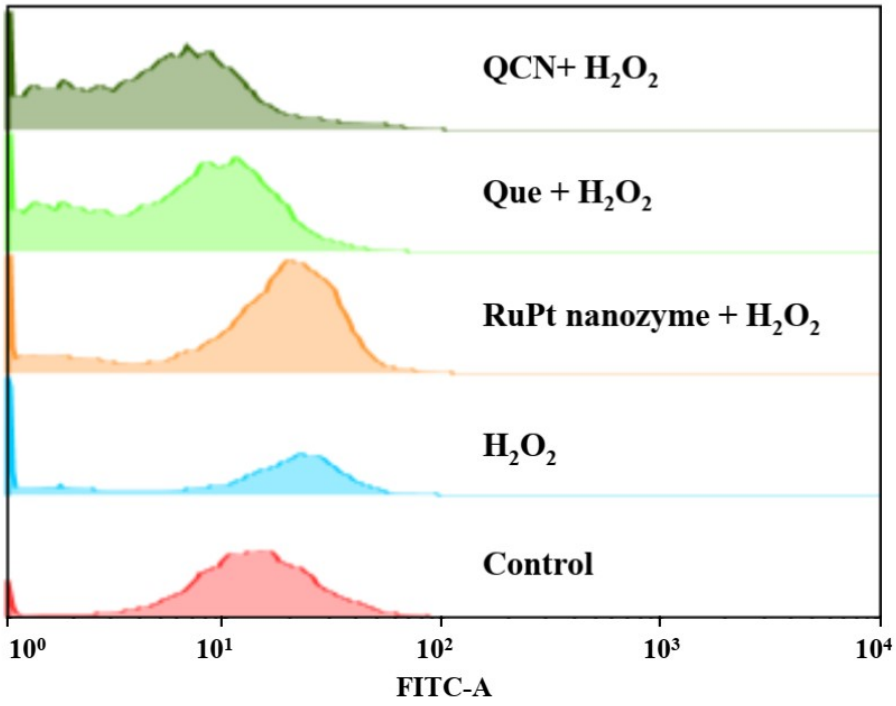

Figure S14. ROS levels were assessed in HEK293 cells after different treatments.

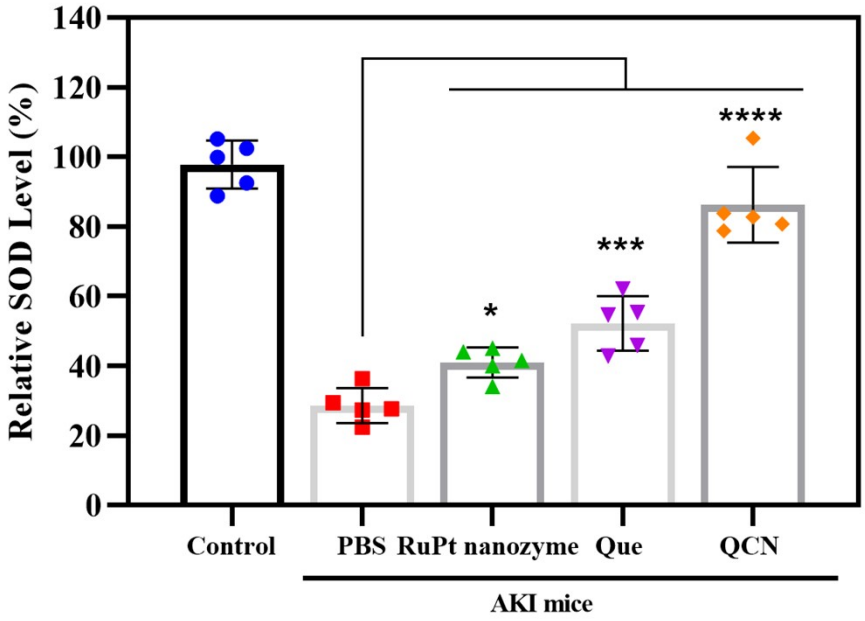

Figure S15. SOD levels measured in renal tissue homogenates from each group.