Supporting Information

Constructing Metal-Organic Framework Nanodots as Bio-inspired Artificial Superoxide Dismutase for Alleviating Endotoxemia

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Experimental Section

Materials: Copper nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$, 99%), polyvinylpyrrolidone (PVP, average mol wt 10,000), N,N-dimethylformamide (DMF, 99.8%), Glutathione, L-methionine and glutathione reductase were purchased from Sigma-Aldrich. Trifluoroacetic acid (CF_3COOH, 99%) and Riboflavin were purchased from Alfa Aesar. Tetrakis (4-carboxyphenyl) porphyrin (TCPP, 97%) was purchased from TCI Shanghai. Nicotinamide adenine dinucleotide phosphate (NADPH) and nitrotetrazolium blue chloride (NBT) were purchased from Sangon Biotechnology Inc. (Shanghai, China). All chemicals were used as received without further purification. The Milli-Q water was obtained from the Milli-Q System.

Measurements and characterizations: SEM images were recorded using a Hitachi S-4800 field emission SEM. TEM images were recorded using a FEI TECNAI G2 20 high resolution transmission electron microscope operating at 200 kV. FT-IR characterization was carried out on a BRUKE Vertex 70 FT-IR spectrometer. X-ray photoelectron spectroscopy (XPS) was recorded using a Perkin Elmer PHI 5600. The crystalline structures of the as-prepared samples were evaluated by X-ray diffraction (XRD) analysis on a on a D8 Focus diffractometer (Bruker) using Cu Kα radiation (λ = 0.15405 nm). The UV-Vis absorption spectra were recorded using a JASCO V550 UV-Visible spectrophotometer (JASCO International Co., LTD., Tokyo, Japan). Flow cytometric analysis was performed on BD FACS Aria.

Synthesis of Cu-TCPP nanosheets: The Cu-TCPP nanosheets were prepared according to the literature with little modification.[19] Firstly, Cu $(NO_3)_2 \cdot 3H_2O$ (72 mg), PVP (200 mg) and Trifluoroacetic acid (1.0 M × 200 μL) were mixed with ethanol and DMF (V:V=1:3, 240 mL). After that TCPP (80 mg) dissolved in ethanol and DMF (V:V=1:3, 80 mL) were added. Then, the solution was heated to 80 °C and maintained the reaction for 3 h after the ultrasound treatment for 15 min. The as-synthesized red nanosheets were washed 3 times with ethanol and collected by centrifuging at 8,000 r.p.m. for 10 min.

Synthesis of CTMDs: CTMDs were prepared by combining probe and bath sonication liquid phase exfoliation of the as-synthesized nanosheets in water. The pulverized nanosheets powder, with initial concentration of 50 mg per 30 mL, was dispersed in a glass vial containing water. Subsequently, the Cu-TCPP nanosheets solution was sonicated for 6 h in ice-bath at 500 W with ultrasound probe. The dispersion was allowed to sonicate in an ultrasonic bath for another 4 h at 300 W with an ice-bath. Then the resulting solution was centrifuged for 30 min at 13,000 rpm. Finally, the supernatant containing CTMDs was collected gently in a clean glass vial stored at 4 °C for future use.

SOD-like activity of CTMDs: The SOD activities of CTMDs were assayed by measuring inhibition of the photoreduction of nitro blue tetrazolium (NBT). The solutions containing riboflavin (20 μM), methionine (0.013 M), NBT (75 μM), and composites of various concentrations were prepared with 25 mM pH 7.4 phosphate buffer. The mixtures were illuminated by a lamp with a constant light intensity for 3 min at 25 °C. After illumination, immediately the absorbance was measured at 560 nm. The entire reaction assembly was enclosed in a box lined with aluminum foil. Identical tubes with the reaction mixture were kept in the dark and served as blanks. The inhibition percentage was calculated according to the following formula: inhibition (%) = $[(A_0-A)/A_0] \times 100$, where A_0 is the absorbance of the control and A is the absorbance of the sample.

Calculation of the IC_{50} of the native SOD: According to the previous literature, the IC_{50} of the native Cu-Zn SOD was 1.3×10^{-3} μM. The molecular weight of Cu-Zn SOD was about 32000. The mass concentration could be calculated to be 4.16×10^{-2} μg mL^{-1}. Thus, the IC_{50} of CTMDs was approaching 12.6 % of the native SOD activity (IC_{50}, a generally used indicator for comparing the efficiencies of enzymes and enzyme mimics).

GPx-like activity of CTMDs: The GPx-like catalytic activity of CTMDs was studied using the GR-coupled assay by following the decrease in the concentration of NADPH spectrophotometrically at 340 nm on JASCO V550 UV-Visible spectrophotometer under time drive mode. In a typical assay, the reactants were added in the following order, CTMDs (with the Cu content 3.5 ug mL^{-1}), GSH (2 mM), NADPH (0.4 mM), GR (1.7 units), H_2O_2 (240 μM) in 25 mM pH 7.4 phosphate buffer at 25 °C. The control reactions were performed in the absence of at least one of the reactants. The steady-state kinetics of CTMDs (with the Cu content 0.3 ug mL^{-1}) was studied by varying the concentration of H_2O_2 (0-360 μM) and GSH (0-3.0 mM) at a time and at the fixed concentration of GR and NADPH in 25 mM pH 7.4 phosphate buffer.

Cell culture: The RAW264.7 (mouse macrophages), HEK293T (human embryonic kidney), L929 (mouse fibroblast cells) cells were supplied by American Type Culture Collection (ATCC). The cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

Cellular internalization of CTMDs: The cellular uptake degree of CTMDs in cells could be qualified by inductively coupled plasma mass spectrometry (ICP-MS) with high detection sensitivity. Cells were preincubated with CTMDs of different concentrations (0, 2.5, 5, 10, 20, 40 μg mL^{-1}) for 12 h, then the cells were washed with PBS buffer solution for 3 times, trysinized with trysin containing 0.25% EDTA, counted and then digested with HNO_3 and H_2O_2 at 85°C. Cu content was then analyzed by ICP-MS.

In vitro cytotoxicity assays: RAW264.7, L929 , HEK293T cells were seeded in 96-well assay plates for 24 h at a density of 10000 cells $well^{-1}$. Then, CTMDs, at the indicated concentrations (0, 5, 10, 25, 50, 75, 100, 150 μg mL^{-1}), were added to the cell culture medium and incubated for 24 h. Then, the fresh culture medium contained 10% MTT solution was added to each well and cultured for 4 h to determine the toxicity. Absorbance values at 490 nm were examined with Bio-Rad model-680 microplate reader. The following equation was used to estimate the cell viability.

$$\text{Cell Viability (\%)} = (OD_T / OD_C) \times 100\%$$

Where OD_T procured in the presence of CTMDs and $OD_{Control}$ was procured in the absence of CTMDs.

Intracellular Determination of ROS and RNS: For the ROS scavenging study *in vitro*, RAW264.7 cells were seeded in 24-well and 6-well plates and cultured for 24 h. 2',7'-dichlorofluorescein diacetate (DCFH-DA) could be used to monitor the generation of ROS, which was a nonfluorescent compound that reacted with intracellular free radicals and produced the fluorescent product dichloro-fluorescein (DCF). The fluorescent intensity of DCF correlated with the quantity of ROS. Firstly, the adhesive cells were cultured with CTMDs (12.5 μg mL^{-1}) for 4 h at 37 °C. Before use, the adherent cells were rinsed 3 times with PBS (pH 7.4) to remove the excess nanodots. Then, LPS (1 μg mL^{-1}) or H_2O_2 (400 uM) were used to stimulate the cells at 37 °C for 6 h. After that, DCFH-DA solutions were added into the RAW264.7 cells and incubated for another 2 h at 37 °C. For cells seeded in 6-well plates, flow cytometric analysis were carried out on BD FACS Aria. Cells seeded in 24-well plates were treated with the above procedure for the qualitative analysis of ROS by using confocal laser scanning microscope. Intracellular RNS levels were measured using total nitric oxide assay kit, and the protocol was provided by the supplier (Beyotime Biotechnology).

Evaluation of DNA damage: The Cells in 24-well plates were divided into four groups (control, LPS, LPS+CTMDs, CTMDs). Then, cells were rinsed with PBS and blocked with 0.05% BSA in 0.05% TritonX-100 for 30 min. Then, the plates were incubated with anti-γ-H_2AX antibody for 1 h after rinsing 3 times with PBS. Alexa488 - conjugated antibody was used to perform the secondary antibody detection. Pictures were obtained using confocal laser scanning microscope.

Lipid peroxidation: Cells were seeded in a 6-well plate and cultured for 24 h and then the adhesive cells were incubated with CTMDs (12.5 μg mL^{-1}) for 4 h at 37 °C. Next, the cells were cultured with LPS (1 μg mL^{-1}) at 37 °C for 2 h after washing 3 times with

PBS (pH 7.4) to remove the excess nanodots. Then, the samples were treated according to the lipid peroxidation MDA Assay Kit.

Animal experiments: All animal experiments were performed in accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication no. 85-23 Rev. 1985) and approved by the Jilin University Animal Care and Use Committee. Healthy Female Kunming mice (8–10 weeks, about 25 g) were purchased from the Laboratory Animal Center of Jilin University (Changchun, China), and all animal care and handling procedures were according to the guidelines approved by the ethics committee of Jilin University.

Biocompatibility evaluation: Hemolysis test of CTMDs was first carried out. 1 mL whole blood was collected in tubes containing Li-heparin from the Orbital venous of Kunming mice. Then, 1 mL blood was mixed with proper amount of 1×PBS, centrifuge 5 min at 2500 rpm and remove the supernatant, and repeated 3-4 times until the supernatant becoming colorless transparent. The precipitated erythrocytes were then dispersed in 1×PBS to get erythrocyte suspension. The CTMDs were added to the erythrocyte suspension to a final concentration of 20, 40, 60, 80, 100 μg mL^{-1} (in tubes numbered as 2-5). Tube 0 (negative control) was placed with erythrocyte suspension diluted with 1×PBS, tube 7 (positive control) was placed with erythrocyte suspension diluted with ultrapure water. The tubes were incubated for 8 h at room temperature, observe and record the hemolysis phenomenon. At the meanwhile, the specific 540 nm spectrophotometric absorptions of hemoglobin were analyzed. Calculation the hemolysis rate (HR %) by the following equation：

$$HR\% = (A_{CTMDs}-A_{NC})\times 100\%/(A_{PC}-A_{NC})$$

where A_{CTMDs}, A_{PC} and A_{NC} are the absorbance of the sample, the positive control and the negative control, respectively.

Subsequently, Kunming mice (n = 5) were divided into 3 groups at random, containing PBS (control), CTMDs (1 days post injection,2 mg kg^{-1} *i.v*), and CTMDs (28 days post injection 2 mg kg^{-1} *i.v*). After 1 and 28 days, the mice were sacrificed and the blood was used to carry out serum biochemistry assay and complete blood panel analysis at the Changchun Chain Medical Laboratories, Inc. The major organs containing kidney, lung, liver, spleen and heart were collected to stain with H&E.

In vivo biodistirbution, pharmacokinetic and renal-excretion of CTMDs: Firstly, CTMDs (2 mg kg^{-1}) were injected *i.v.* into Kunming mice (n = 3). The mice were euthanized at 24 h post administration. The main organs were collected and treated with aqua regia at 80 °C to obtain the amount of Cu^{2+} by ICP-MS. For pharmacokinetic and renal-excretion evaluation, female Kunming mice (n = 3) were injected with CTMDs (2 mg kg^{-1} *i.v.*). At different periods, 10 μL of blood were obtained by piercing the tail vein and dissolved with aqua regia. By using ICP-MS to quantify the concentrations of the remained CTMDs. A doublecompartment pharmacokinetic model were used to calculate *in vivo* circulating half-life ($\tau_{1/2}$) of CTMDs. Also, the feces and urine of mice

were collected at different periods. The content of CTMDs was quantified by the amount of Cu^{2+} by ICP-MS.

In vivo LPS-induced acute renal failure in an endotoxemia rat model: LPS-induced endotoxemia was induced in female Kunming mice (about 26g) by intravenous administration of 15 mg kg^{-1} of LPS *via* the intraperitoneal injection. Right after LPS injection, CTMDs (0.8 mg kg^{-1}) or saline was intravenously injected *via* the tail vein.

Anti-inflammation effect, blood test, histopathological investigation and mortality assessment of the LPS-induced endotoxemia rat model: Female Kunming mice (about 26g) were randomly divided into 3 groups: control group (group 1, only treated with saline), LPS-treated group (group 2, LPS 15 mg kg^{-1} *i.p.*) and LPS-CTMDs-treated group (group 3, LPS 15 mg kg^{-1} *i.p.*, CTMDs 0.8 mg kg^{-1} *i.v.*). LPS could induce oxidative stress and inflammatory response in the model mice. 24 h later, the mice were euthanized, and the kidney tissue was collected for the detection of reactive oxygen and nitrogen species level. For the detection of ROS, the kidney tissues from control and treated animals harvested, weighted, and frozen quickly in PBS (pH 7.4) using liquid nitrogen for future use. The kidney tissues were homogenized in PBS (pH 7.4) after thawing, and the supernatant was collected from the kidney homogenate through centrifugation at a speed of 2000-3000 rpm for 20 min at 4 ^{0}C. The level of ROS in kidneys was then detected by using the ROS assay kit (Beyotime Biotechnology). The RNS levels in the kidney tissue were measured using total nitric oxide assay kit and the protocol was provided by the supplier (Beyotime Biotechnology). At the meantime, the blood and serum were collected for the detection of blood biochemical levels and hematological parameters at the Changchun Chain Medical Laboratories, Inc. The TNF-α level of the kidney tissue was also measured using immunofluorescence staining assays. Serum TNF-α and interleukin (IL)-6 were determined by the ELISA assay (at 3 h post the treatment with LPS). To investigate the histopathology, the main organs including kidney, liver, lung, stomach and intestines were prepared for staining with H&E (at 24 h post the various treatment) and then examined by an Olympus BX-51 microscope. For mortality assessment, the LPS-induced endotoxemia rat model was assigned to two groups: the PBS-treated control group and the CTMDs -treated group (n = 6 for each group). After intravenous LPS injection and subsequent PBS or CTMDs injection (with the dose of 0.8 mg kg^{-1} every other day in the first week), the animals were observed for 14 days and their survival rate was checked every 24 h.

Statistics. In this article, all data were presented as mean result ± standard deviation (SD). The statistical analysis was performed by using Origin 8.0 software. All figures illustrated were obtained from several independent experiments with similar results. Asterisks indicate significant differences (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

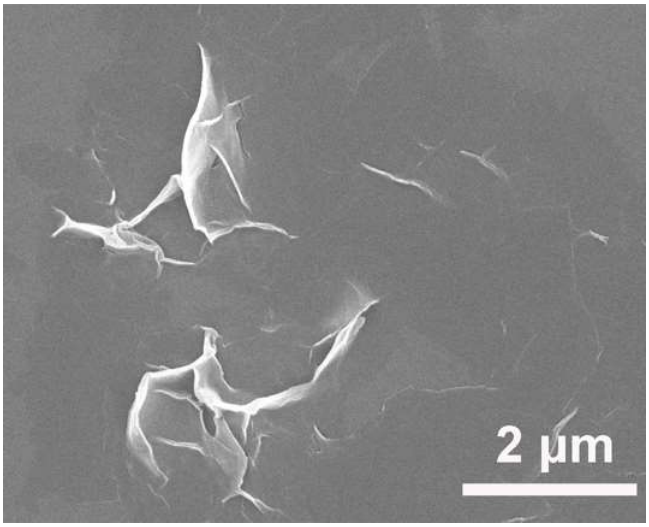

Fig. S1 The scanning electron microscopy image of Cu-TCPP nanosheets.

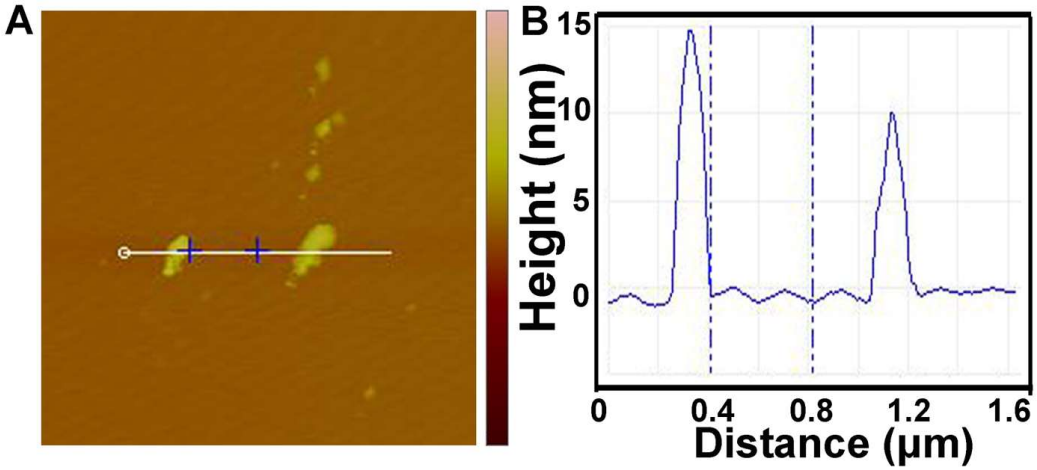

Fig. S2 (A) AFM image and (B) thickness of Cu-TCPP nanosheets.

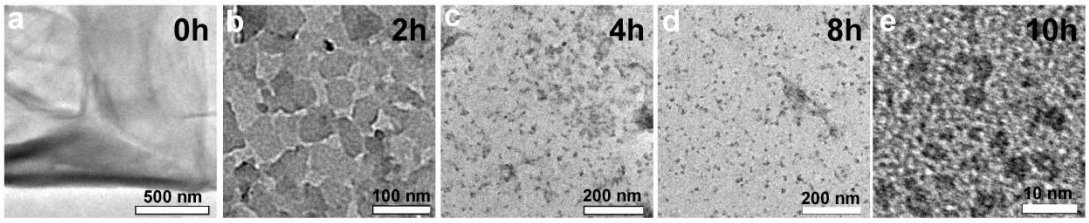

Fig. S3 The TEM image of (a) Cu-TCPP sheets, (b-e) Cu-TCPP sheets with various ultrasonic time.

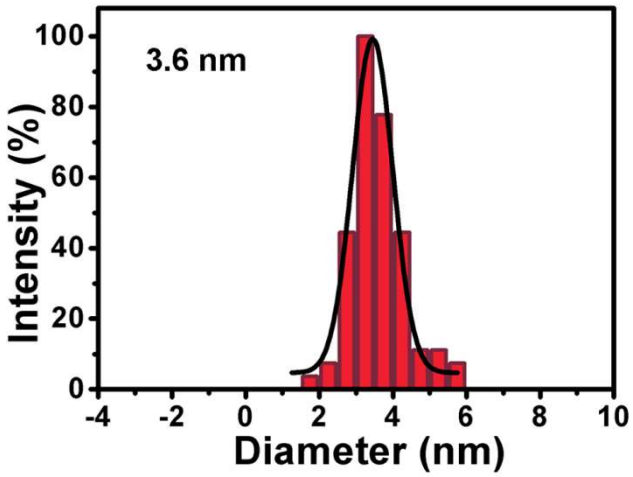

Fig. S4 Diameter distribution measured from TEM image of prepared CTMDs.

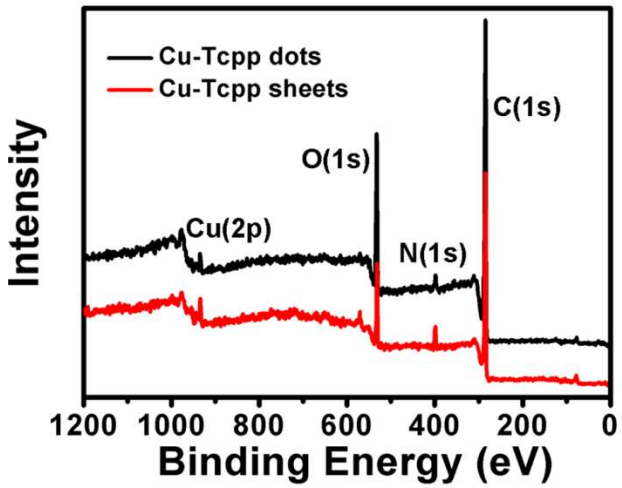

Fig. S5 XPS spectra of the obtained Cu-TCPP nanosheets and CTMDs.

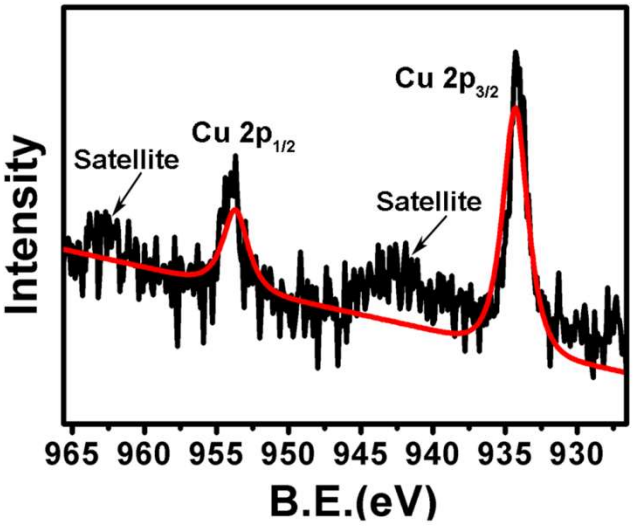

Fig. S6 The XPS Cu2p analysis of CTMDs.

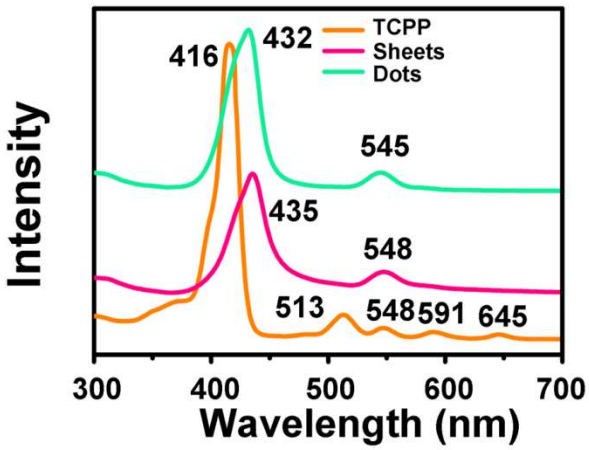

Fig. S7 The UV-Vis spectra of CTMDs, Cu-TCPP nanosheets and TCPP ligands.

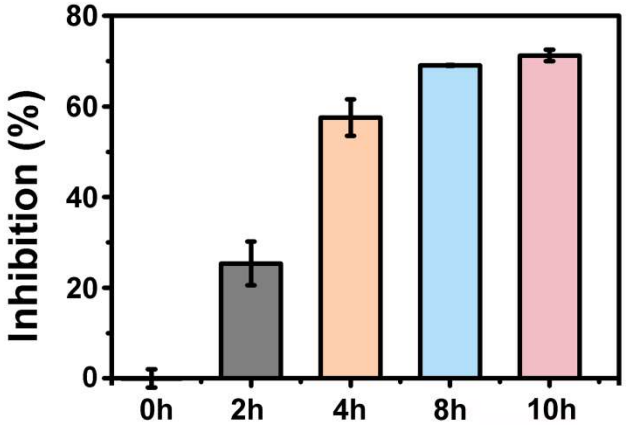

Fig. S8 Percent inhibition of NBT oxidation with Cu-TCPP sheets under various ultrasonic time.

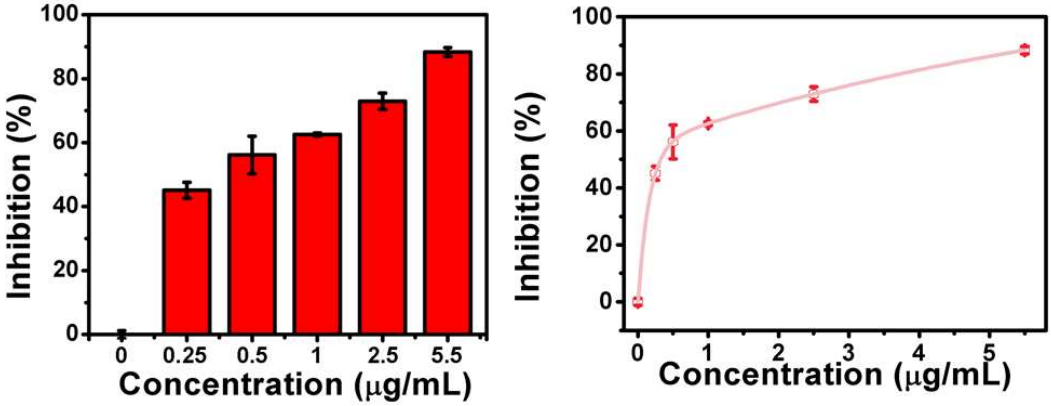

Fig. S9 Percent inhibition of NBT oxidation with different concentrations of CTMDs.

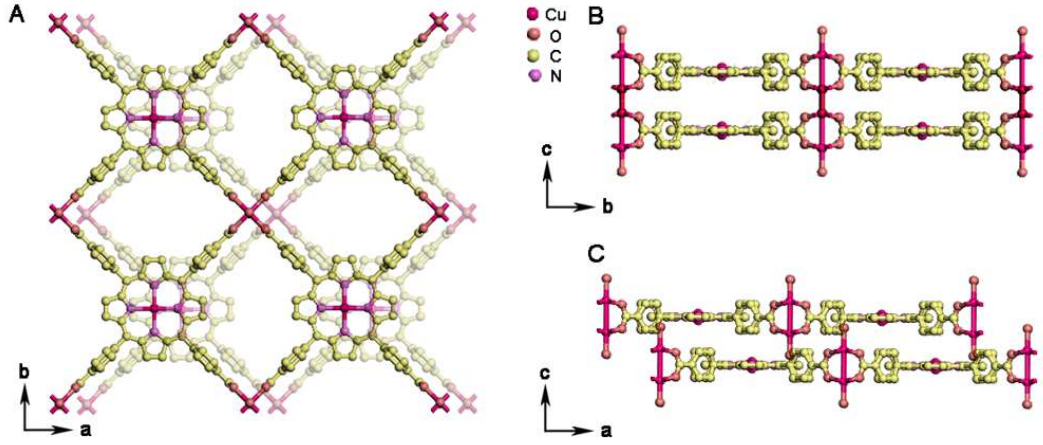

Fig. S10 projections of the proposed crystal structure of the Cu-TCPP MOF unites on the (A) ab plane, (B) bc plane, and (C) ac plane. H atoms have been omitted for clarity.

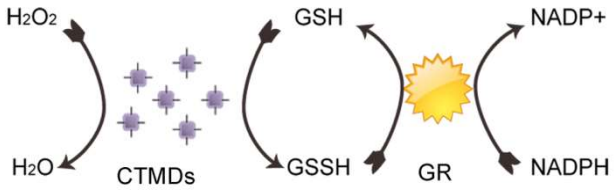

Fig. S11 The schematic illustration for the GPx-like activity of CTMDs.

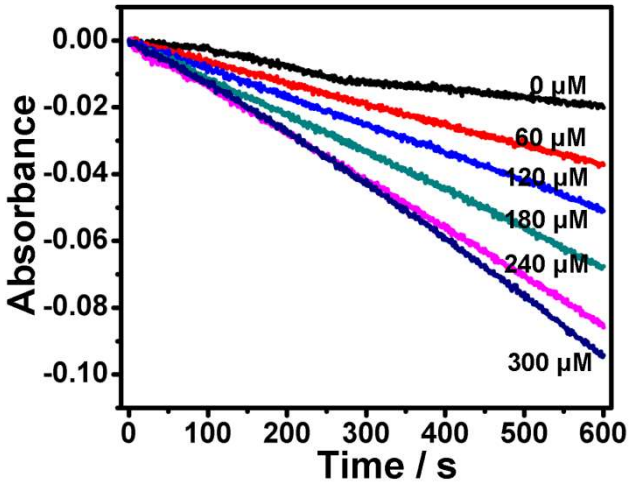

Fig. S12 Time-dependent absorbance changes of NADPH in presence of different concentrations of H_2O_2.

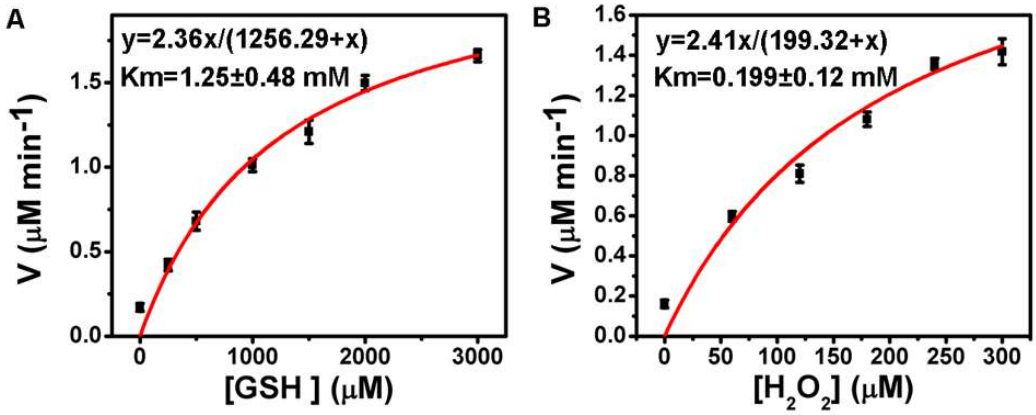

Fig. S13 Saturation curves corresponding to the reduction rate of NADPH at variable concentrations of (A) GSH and (B) H_2O_2.

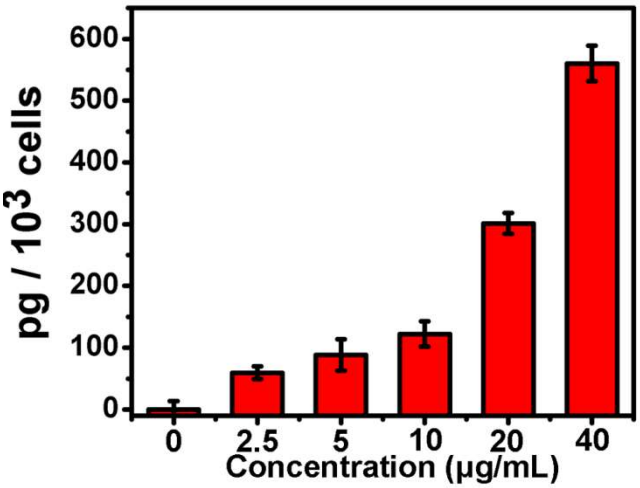

Fig. S14 Cellular uptake of CTMDs quantified by ICP-MS. Cells were incubated with CTMDs for 12 h (RAW264.7) and copper level was measured by ICP-MS. Bars represent the mean ± standard error of the mean (s.e.m.). (n = 3).

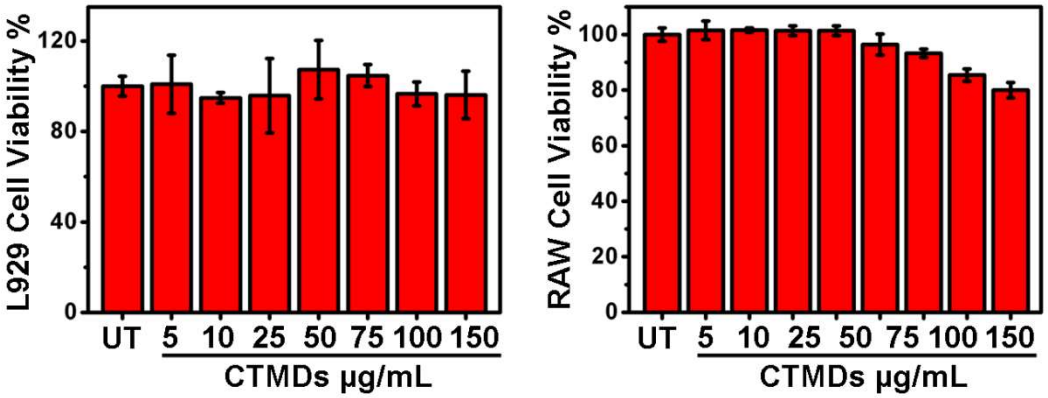

Fig. S15 Effect of variable concentrations of CTMDs on viability of L929 cells and RAW264.7 cells.

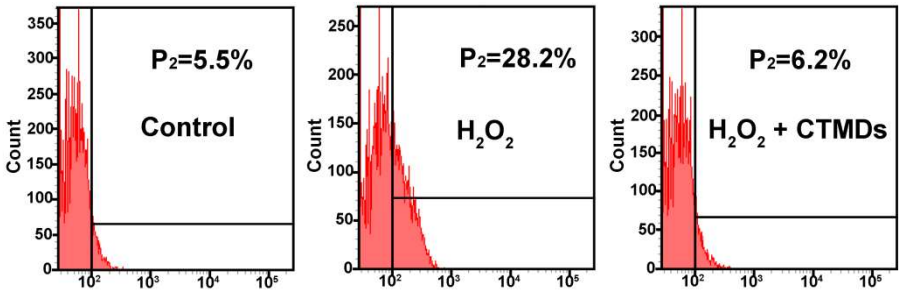

Fig. S16 Flow cytometry analysis used to monitor the changes of intracellular ROS.

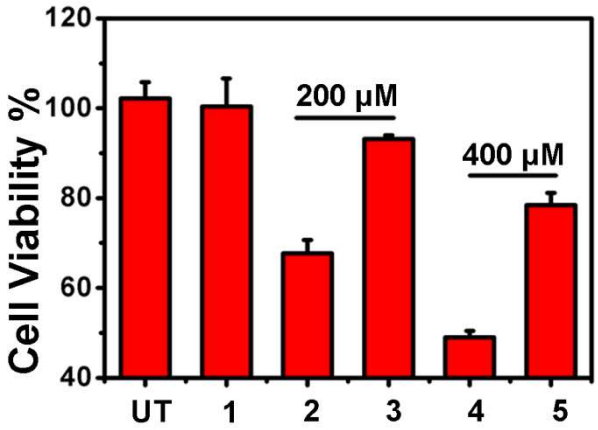

Fig. S17 The cell viability of H_2O_2-treated cells, 1 CTMDs 12.5 μg mL^{-1}, 2 H_2O_2 200 μM, 3 CTMDs 12.5 μg mL^{-1} + H_2O_2 200 μM, 4 H_2O_2 400 μM, 5 CTMDs 12.5μg mL^{-1} + H_2O_2 400 μM.

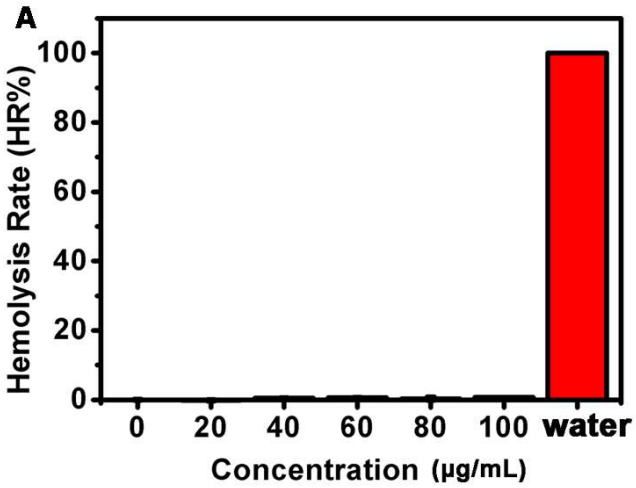

Fig. S18 Hemolysis rate (HR%) of CTMDs.

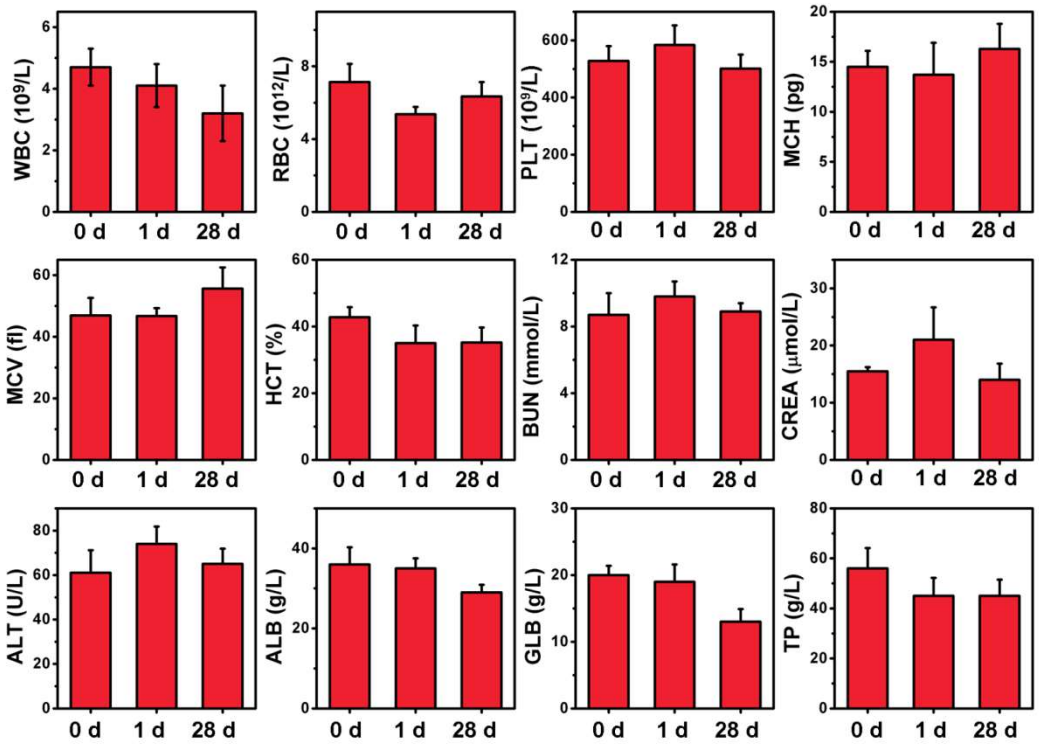

Fig. S19 Blood biochemical levels and hematological parameters of the mice after treatment with CTMDs for 0, 1and 28 days.

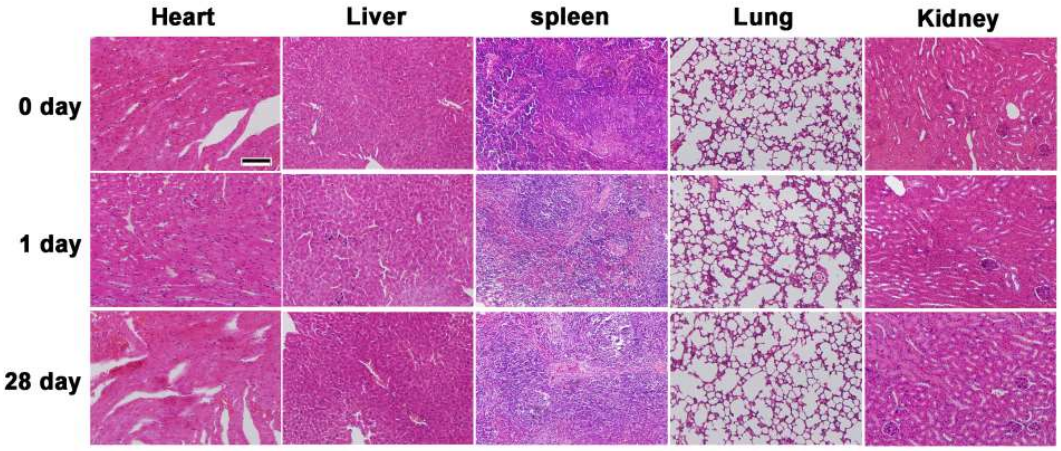

Fig. S20 Haematoxylin and eosin staining of the main tissues of CTMDs at 0, 1, 28 days post injection. The scale bar is 100 μm.

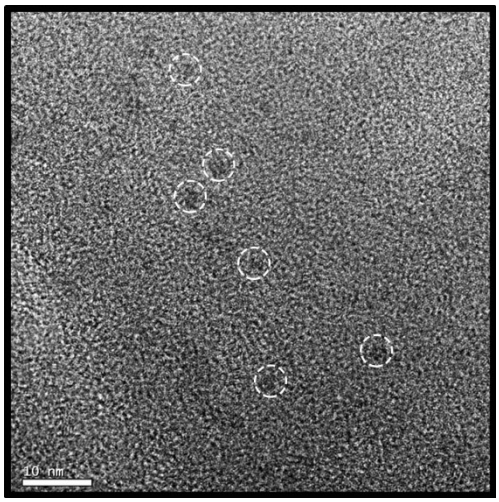

Fig. S21 TEM image of CTMDs in the urine at 4 h post injection.

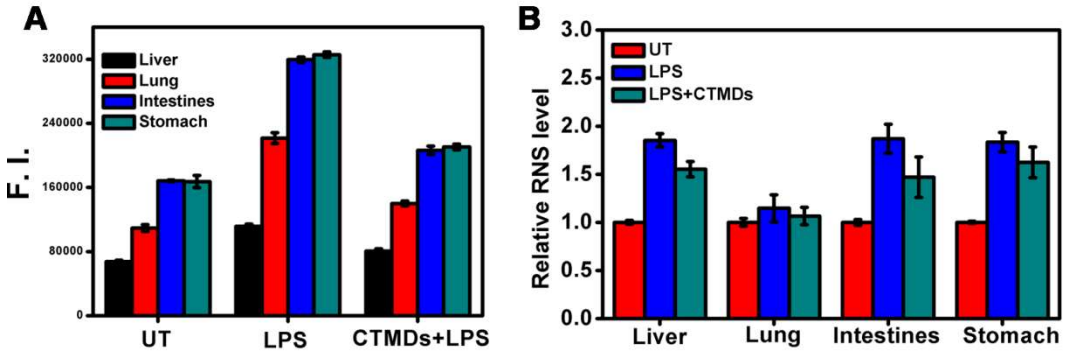

Fig. S22 (A) ROS intensities and (B) reactive nitrogen species (RNS) production in different tissues.

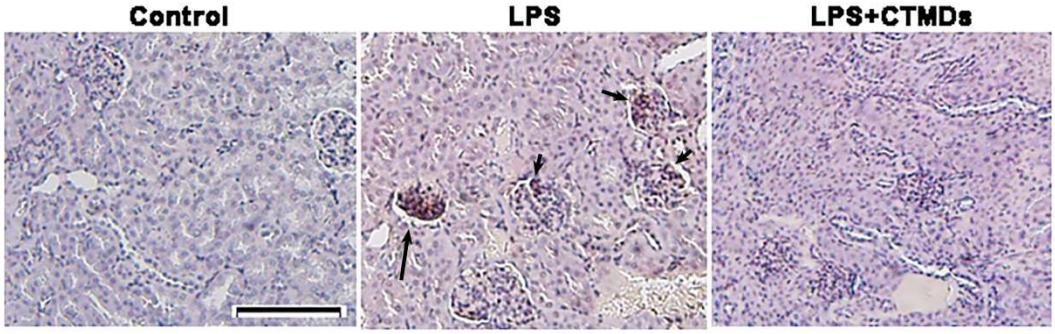

Fig. S23 The TNF-α immunohistochemical staining assays of the kidney tissues after different treatments. The scale bar is 100 μm.

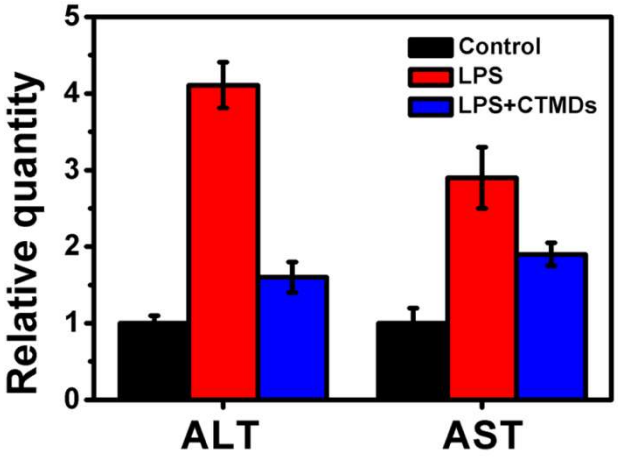

Fig. S24 Blood biomedical indexes for serum ALT, AST of control mice, LPS-treated mice and LPS-treated mice with injection of CTMDs.

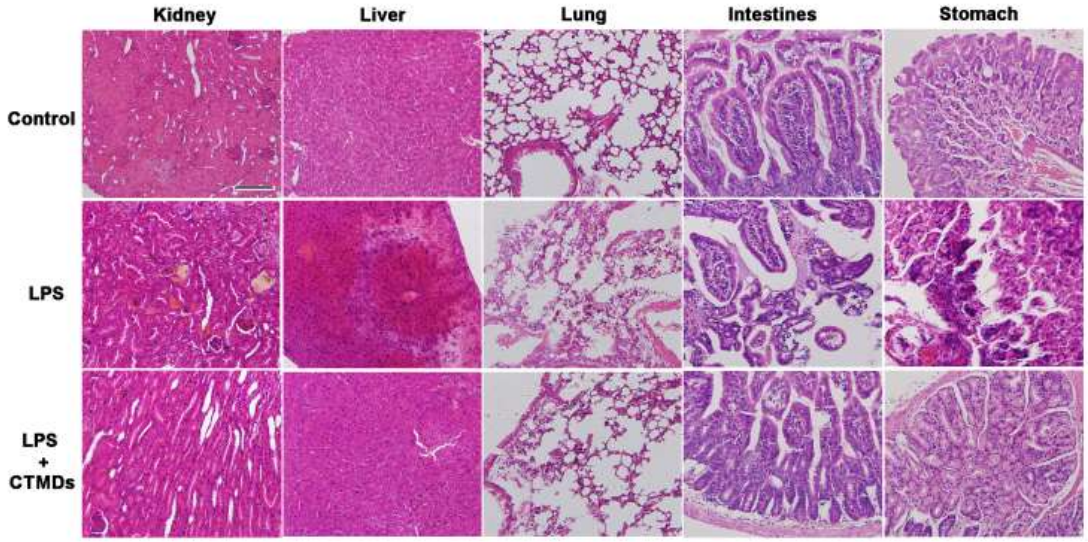

Fig. S25 Haematoxylin and eosin staining of different tissues after treatment with various groups. The scale bar is 100 μm.