Supporting Information

Ultra-Small Natural Product Based Coordination Polymer Nanodots for Acute Kidney Injury Relief

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1. Experimental Procedures

1.1 Synthesis of CPNs

66 mg PVP was dissolved in 5 mL methanol, into which 20 mg $FeCl_3 \cdot 6H_2O$ in 1 mL methanol was added dropwisely under stirring. After 5 minutes, 10 mg of curcumin in 1 mL methanol was added dropwisely and kept under stirring for 3 hours. The resultant methanol solution was dialyzed against water overnight and collected for future test. Fe-Quer, Fe-GA, Fe-PC CPNs were synthesized following the same method. The concentration of Fe-Cur CPNs was calculated by Fe measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES).

1.2 Characterization

Transmission electron microscope (TEM) images were acquired by a FEI Tecnai F20 TEM at an acceleration voltage of 200 kV. X-ray photoelectron spectroscopy (XPS) measurement was conducted with a PHI Quantera SXM instrument equipped with an Al X-ray excitation source (1486.6 eV). UV-Vis-NIR absorption spectra were performed with a PerkinElmer UV-Vis-NIR spectrophotometer. The dynamic light scattering (DLS) of CPNs was recorded using MALVERN Nano ZS90. The concentration of Fe-Cur CPNs was calculated by Fe as measured by inductively coupled plasma atomic emission spectrometer (ICP-OES). IR spectra were carried out by BRUKER VERTX 70 (400-4000 cm^{-1}).

1.3 ROS scavenging assays

DPPH assay: Fe-Cur CPNs with different concentrations (0, 25, 50, 100, 200, 400 μg/mL) were prepared and mixed with 125 μM DPPH in ethanol with the same volume. The final Fe-Cur CPNs concentrations were 0, 12.5, 25, 50, 100, 200 μg/mL and the final DPPH concentration was 62.5 μM. 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 scavenging efficiency (%) = ((A_{DPPH}-A_{Sample})/A_{DPPH})* 100%. A_{DPPH} represented the absorbance of DPPH without additional processing. A_{Sample} represented the absorbance of DPPH with the addition of Fe-Cur CPNs. The DPPH scavenging abilities of Fe-Quer, Fe-GA, Fe-PC CPNs were calculated under the same method.

ABTS assay: Following a reported protocol[1], 7 mM ABTS solution was incubated with 2.45 mM potassium persulfate overnight for the activation of ABTS radicals. Fe-Cur CPNs with a final working concentration of 0, 12.5, 25, 50, 100, 200 μg/mL were mixed with ABTS radical solutions and incubated for 10 min. The absorption of ABTS radicals at 734 nm was measured. The scavenging efficiency of ABTS radicals was calculated by the following equation: ABTS scavenging efficiency (%) = ((A_{ABTS}-A_{Sample})/A_{ABTS})*100%. A_{ABTS} represented the absorbance of ABTS without additional processing. A_{Sample} represented the absorbance of ABTS with the addition of Fe-Cur CPNs.

Methylene blue assay: Since ·OH radicals generated by the Fenton reaction could beach MB, the absorbance of remained MB could reflect the ·OH radical scavenging ability of Fe-Cur CPNs. With a final working concentration of 75, 100, 150, 200 μg/mL, Fe-Cur CPNs were mixed MB and added with the Fenton reaction solution containing H_2O_2 and Fe^{2+}. After reaction for 15 min, the absorption of MB was measured. The scavenging efficiency of ·OH was calculation by the following equation: ·OH scavenging efficiency (%) = (A_{Sample}/A_{MB})*100%. A_{MB} represented the absorbance of MB without additional processing. A_{Sample} represented the absorbance of MB with the addition of Fe-Cur CPNs.

DMPO assay: In the ESR measurement, DMPO worked as the trapper of ·OH radicals generated from the Fenton reaction. In brief, Fe-Cur CPNs were added into a mixture of H_2O_2 solution, Fe^{2+} solution, and DMPO solution for 30 seconds. The ·OH characteristic peak signals were measured by the ESR spectrometer.

TEMPO assay: In the ESR measurement, TEMPO radicals showed a 1:1:1 characteristic peak signal which could be cleared off with the addition of scavenger. In brief, Fe-Cur CPNs were mixed with TEMPO solution for 1 minutes and then measured by the ESR spectrometer.

1.4 In vitro cytotoxicity and ROS scavenging assays:

HEK293 cells were cultured under the standard condition. For cytotoxicity assay, different concentrations of Fe-Cur CPNs (50, 25, 12.5, 6.25 μg/mL of Fe) were cultured with HEK293 cells for 24 hours, and the relative cell viabilities were tested by the standard methyl thiazolyl tetrazolium (MTT) assay. For the protection of cells from H_2O_2 induced oxidative stress, different concentrations of H_2O_2 (200, 100, 50, 25 μM) were cultured with HEK293 cells in the absence or presence of Fe-Cur CPNs (50 μg/mL of Fe) for 12 hours, and the relative cell viabilities were tested by the MTT assay.

For confocal imaging and flow cytometry assays, HEK293 cells were cultured with Fe-Cur CPNs for 12 hours and then H_2O_2 was added. After 2 hours, 20 μM DCFH-DA was added to detect the intercellular ROS generation. After being washed with phosphate buffered saline (PBS) for three times, cells were observed under confocal microscopy (Leica SP5).

1.5 Biodistribution study

To perform ^{125}I labeling, 10 mCi ^{125}I was added into 200 μL Fe-Cur CPNs inside 1.5 mL tube coated with Iodogen[2]. After 0.5 h shaking at room temperature, free unlabeled ^{125}I was washed by centrifugation. The radio labeling stability was calculated at different time points (0.5, 3, 6, 12, and 24 h). After i.v. injection with ^{125}I labeled Fe-Cur CPNs at the radio-activity dose of 100 μCi, mice were sacrificed at different time points (0.5, 8, and 24 h) and the main organs were weighted and measured by the gamma counter (Zonkia G1500) to determine their radio-activities (n=3). For Prussian blue staining of kidney slices, kidneys of mice with or without Fe-Cur CPNs injection were taken out at 0.5 h post injection for slicing and the standard Prussian blue staining. The stained kidney slices were imaged under an optical microscope.

1.6 AKI mouse model and its treatments

Balb/c female mice (~8 weeks) used in this work were treated in accordance with the Ethics Committee Guidelines of Soochow University, and all animal experiments were carried out under the permission by Soochow University Laboratory Animal Center. Balb/c mice were dehydrated for 15 hours but free to access food. After the dehydration, the two hind limbs of mice were intramuscularly injected with 50% of glycerol at the dose of 8 mL/kg. Water was then free to access. The symptom of AKI would gradually appear in the next hours.

After different treatments, the blood of mice (n=3) was taken out to collect the serum. BUN and Cr levels in the serum samples were then tested. The H&E staining and DCFH-DA staining of kidney slices were conducted following the standard protocols. The H&E stained slices were observed under an optical microscope and the DCFH-DA stained slices were observed under a confocal fluorescence microscope.

To evaluate the kidney functions after different treatments (Blank, Fe-Cur, AKI 24 h+PBS, and AKI 24 h+Fe-Cur), mice (n=3) were injected with FITC-sinistrin. The fluorescence intensity of FITC-sinistrin in the body was recorded by the optical device (Transdermal GFR Monitor, MediBeacon™, Biotimestech.lnc) tied in the back skin of the hair-removed mice following the standard protocol[3, 4].

1/7 Toxicity of Fe-Cur CPNs

After i.v. injection of Fe-Cur CPNs for 1, 14, and 30 days, mice (n=3) were sacrificed and their main organs (liver, spleen, kidney, heart, and lung) and blood samples were collected and stored under appropriate conditions for further tests. The blood biochemistry assay, complete blood panel analysis, and H&E staining of organ slices were carried out by Wuhan Servicebio Technology Co., Ltd.

2. Supporting Figures

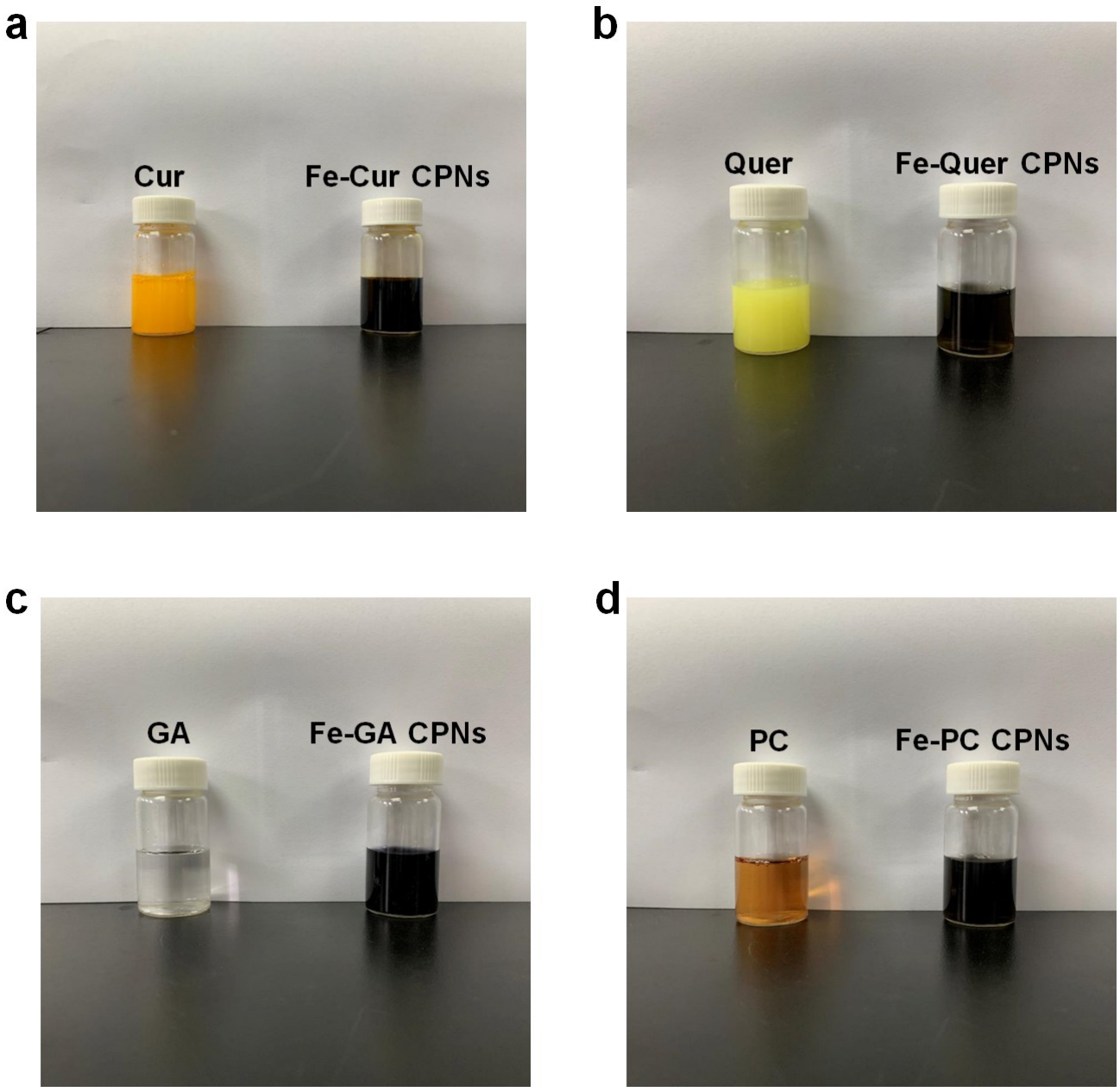

Figure S1. Photographs of Curcumin (Cur) and Fe-Cur CPNs (**a**), quercetin (Quer) and Fe-Quer CPNs (**b**), gallic acid (GA) and Fe-GA CPNs (**c**), and Proanthocyanidin (PC) and Fe-PC CPNs (**d**).

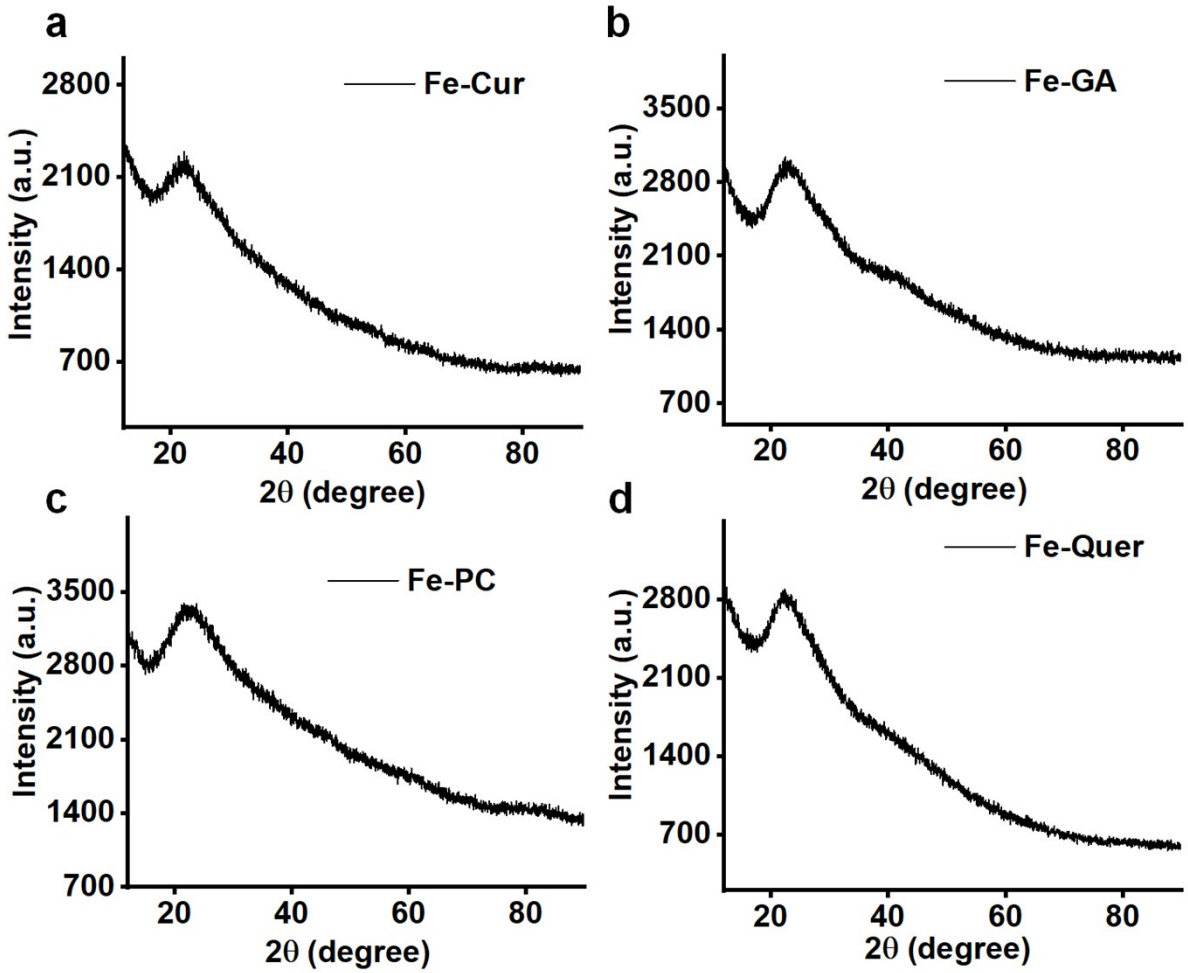

Figure S2. XRD data of Fe-Cur, Fe-GA, Fe-PC and Fe-Quer CPNs indicated that all of those samples were amorphous.

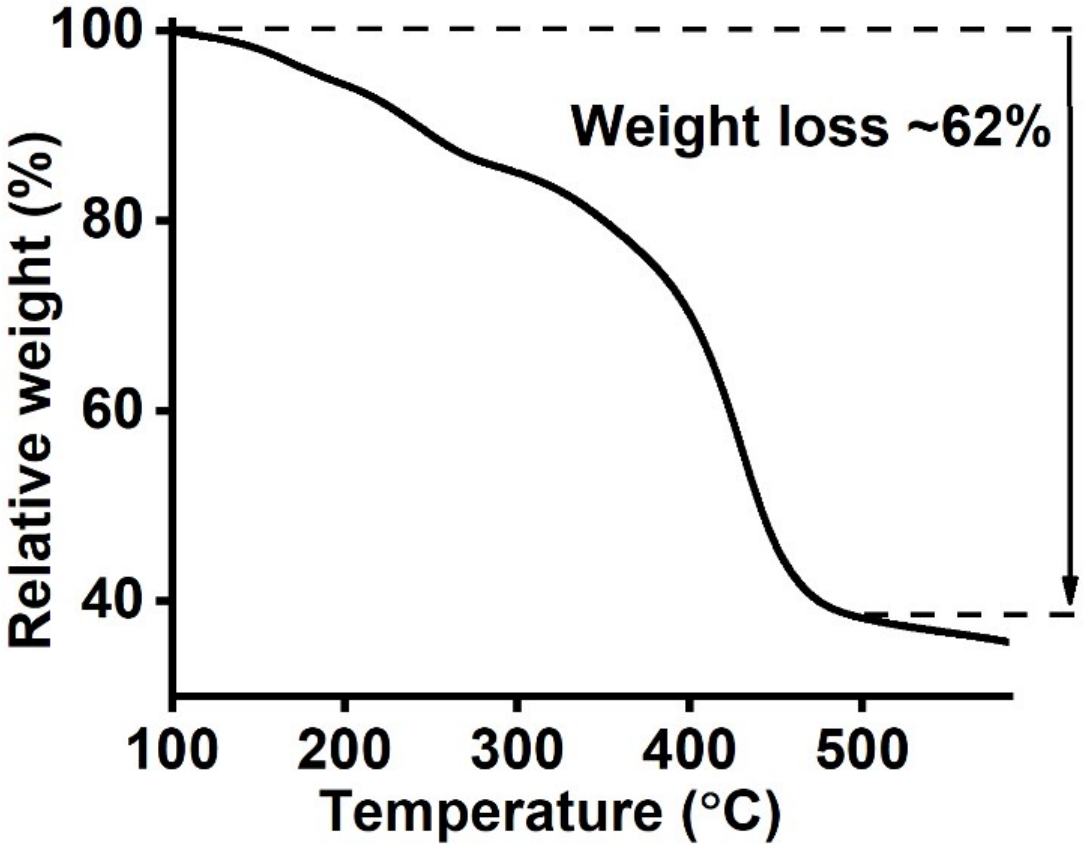

Figure S3. TGA curve of as-made Fe-Cur CPN sample.

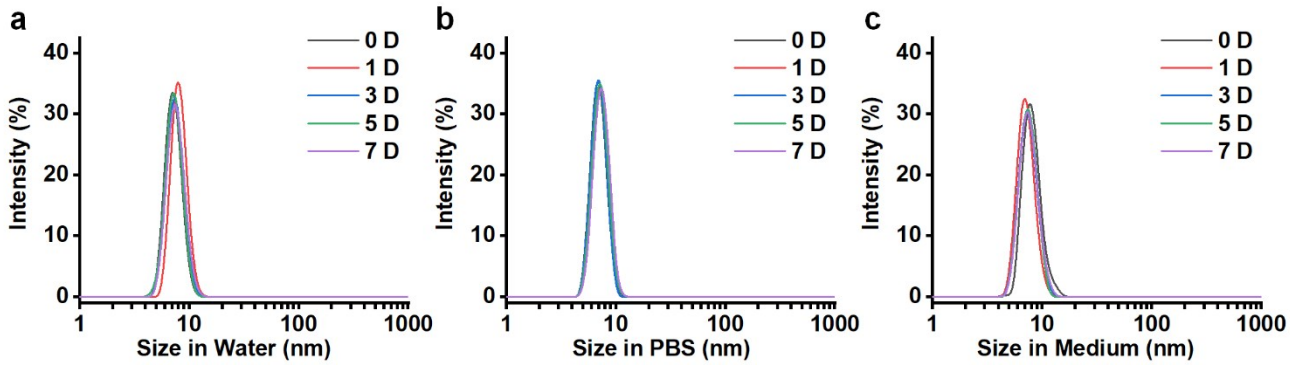

Figure S4. DLS data of Fe-Cur CPNs in water (**a**), PBS (**b**) and cell medium (**c**).

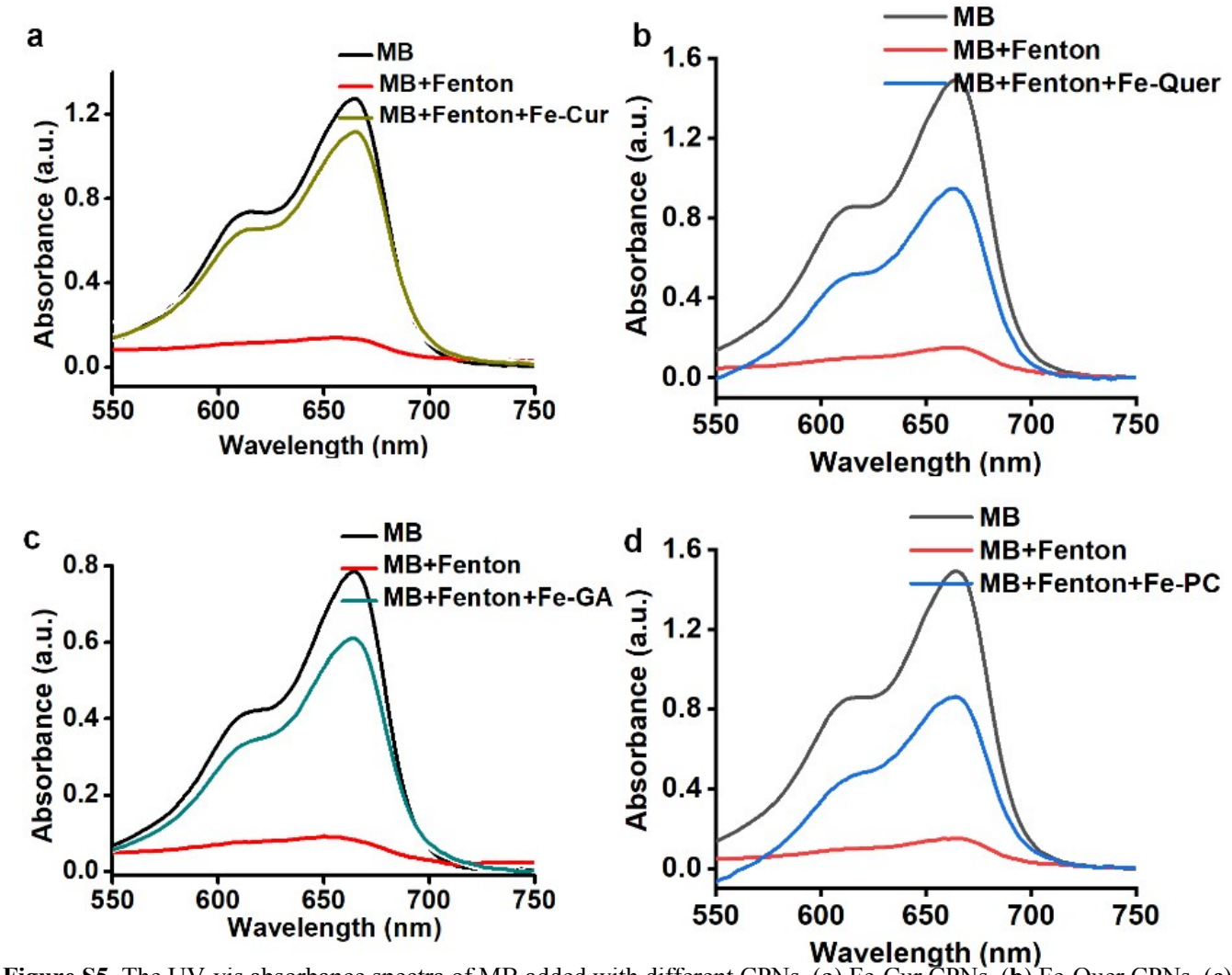

Figure S5. The UV-vis absorbance spectra of MB added with different CPNs. (**a**) Fe-Cur CPNs. (**b**) Fe-Quer CPNs. (**c**) Fe-GA CPNs. (**d**) Fe-PC CPNs.

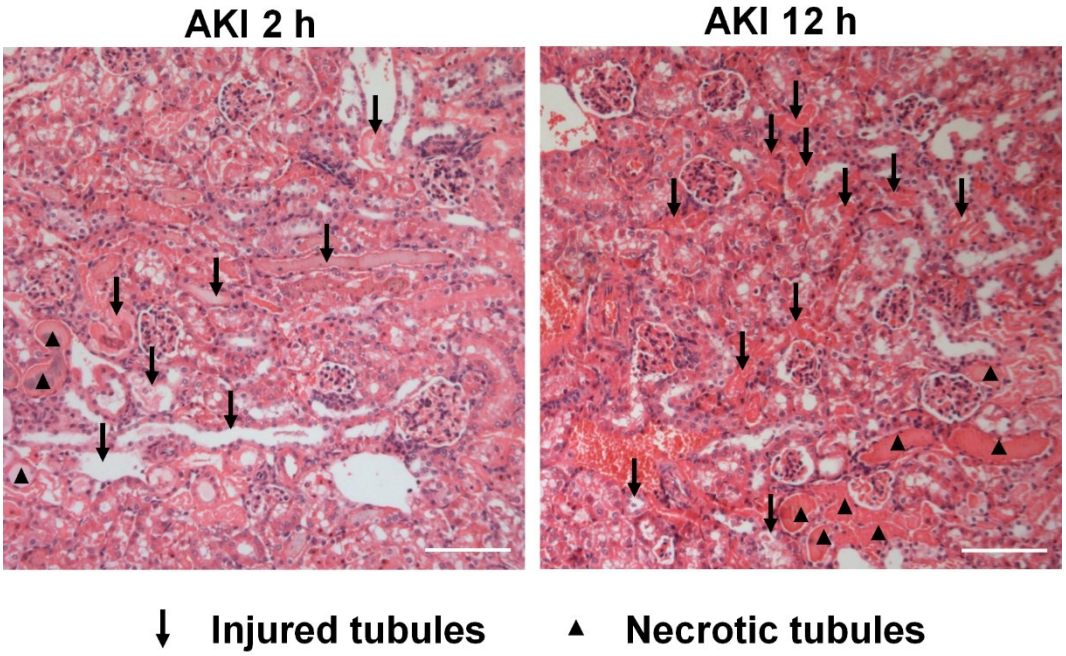

Figure S6. The H&E stained kidney slices of mice under different treatments. Scale bar = 50 μm.

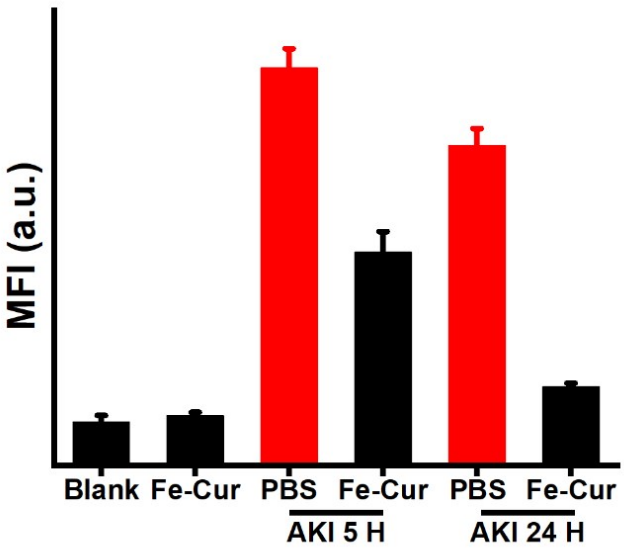

Figure S7. The mean fluorescence intensity (MFI) of ROS stained kidney slices measured from Image J.

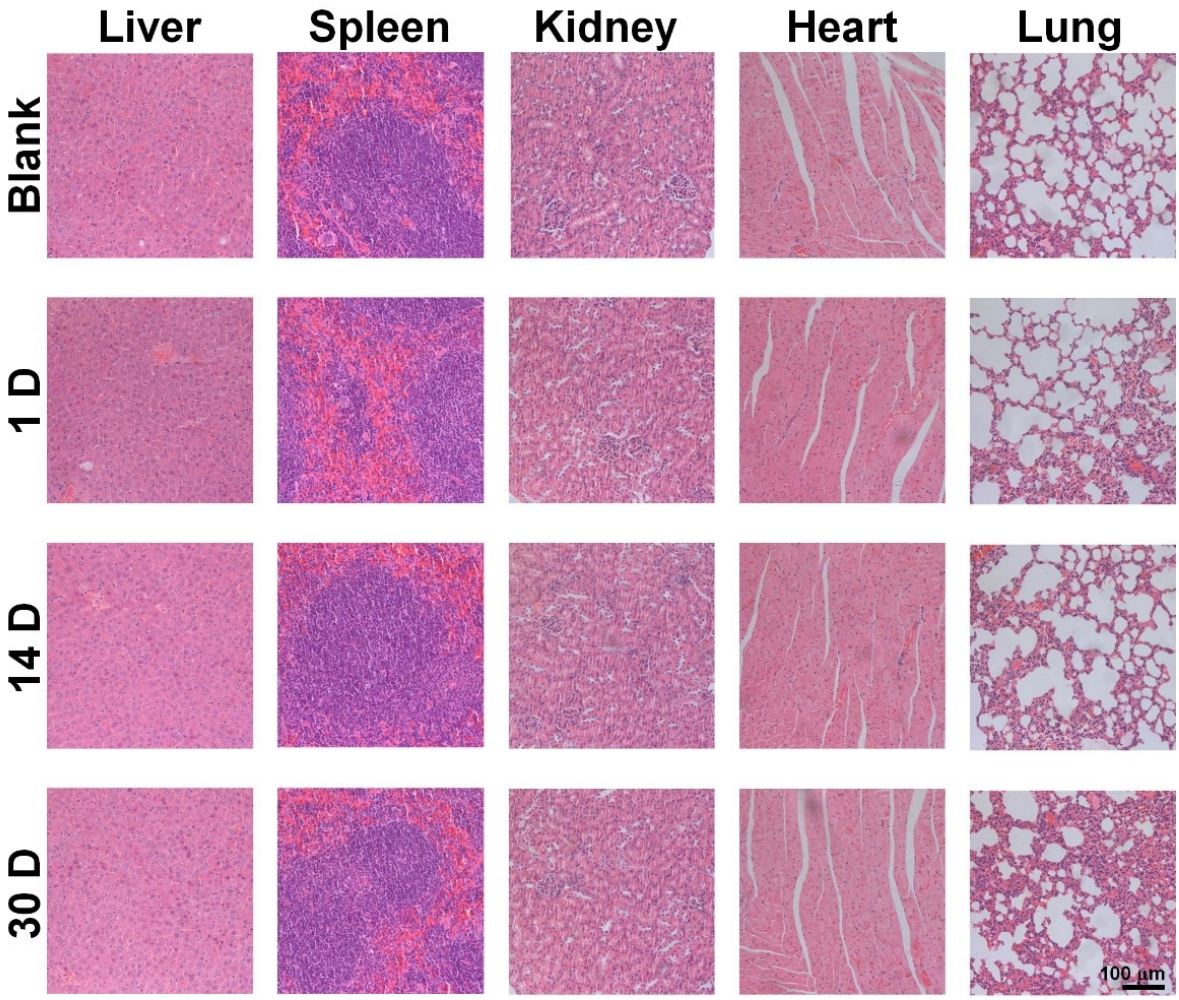

Figure S8. H&E stained slices of main organs of normal mice (blank) and mice post Fe-Cur CPNs injection at different time points (1, 14, and 30 days).

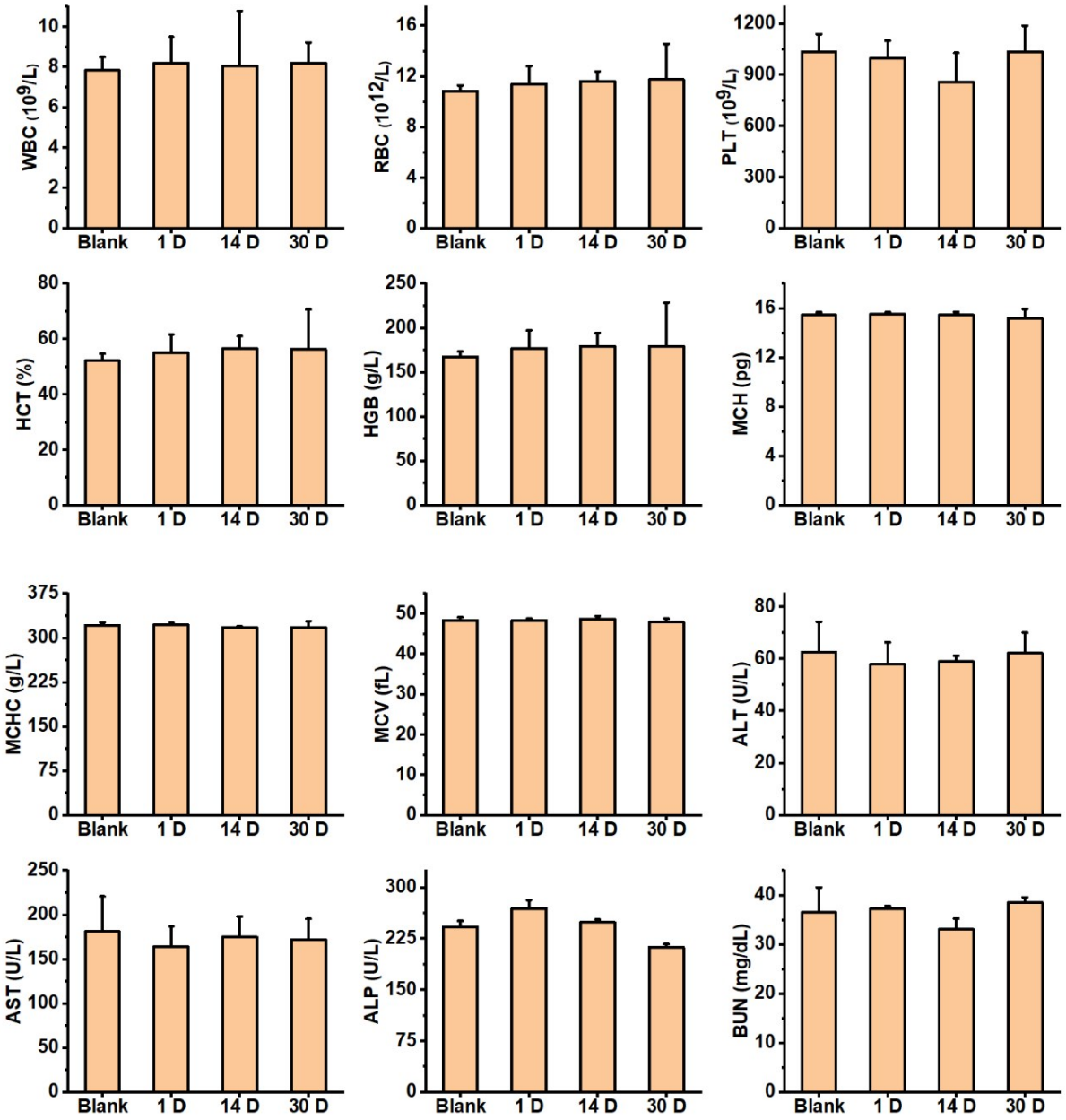

Figure S9. Serum biochemistry assay and complete blood panel data of normal mice (blank) and mice post Fe-Cur CPNs injection at different time points (1, 14, and 30 days).