Supporting Information

H_2O_2-stimulated Janus-Shaped Self-Propelled Nanomotors as an Active Treatment for Acute Renal Injury

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Methods

Materials

Chitosan was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (China). Melatonin was purchased from Sigma-Aldrich. L-serine was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (China). Reactive Oxygen Species Assay Kit was purchased from Biyuntian Biotechnology Co., Ltd. (China). MitoTracker® Green FM was purchased from Yisheng Biotechnology (Shanghai) Co., Ltd. (China). Lyso-Tracker Green was purchased from Biyuntian Biotechnology Co., Ltd. (China). Golgi-Tracker Green was purchased from Yisheng Biotechnology (Shanghai) Co., Ltd. (China). TUNEL kit was purchased from Abcam. H_2O_2 kit was purchased from Abcam. BUN, Scr, SOD and MDA were purchased from Nanjing Jiancheng Bioengineering Research Institute Co., Ltd. (China). ABTS and DPPH were purchased from Beijing Zhongsheng Ruitai Technology Co., Ltd. (China). Kim-1, Cx43 and HIF-1α antibodies were purchased from Sigma-Aldrich.

Synthesis of nanoparticles Pt@CS/Mel@S.

Pt@CS (35.6 mg) was dissolved in PBS (50 mL) by uniform vibration, and a certain amount of TPP (0.1%, 5 mL), pectin (0.08%, 2 mL) and Mel (10 mg/mL) were added under stirring for 24 h. Next, the EDC (24.3 mg) and NHS (41.6 mg) were added to stir and then the ‘S’ (10 mg) was added to stir for 24 h and Pt@CS/Mel@S was achieved. Then morphology and size of Pt@CS/Mel@S were observed by TEM and SEM.

Particle size measurement

The Pt@CS/Mel@S (PCMS) solution was first sonicated for 10 min, then 1 mL (100 μg/mL) PCMS was placed in a colorimetric dish and the particle size was detected by a Malvern Laser Particle Size Analyzer. And this particle size was repeated five times and the average value was calculated.

Loading rate and grafting

5 mL (1 mg/mL) Pt@CS/Mel@S was added to a dialysis bag and immersed in 80 mL PBS containing different pH (7.4/6.5/5.0). At fixed time points, Mel concentrations were measured by ultraviolet absorption spectrum, and the solution was replenished with equivoluminal volume fresh buffer. Then the loading of Mel were calculated by the contents of released Mel [Loading rate % =(coagulant dosage - the amount of medication in the external fluid of the dialysis bag)/the amount of Pt@CS/Mel@S]. And the grafting of 'S' was calculated by ultraviolet absorption spectrum. And the detailed method was as follows:

Firstly, 5 mg of L-serine was dissolved in 5 mL of ultrapure water to prepare a solution with a concentration of 1 mg/mL. 1mL of L-serine solution was selected and placed in a colorimetric dish for UV spectral analysis. The absorbance standard curve of L-serine was obtained at 210 nm. Similarly, 1mL (1 mg/mL) of PCMS was selected and placed in a colorimetric dish. The absorbance at 210 nm was measured, and the grafting rate of L-serine was ultimately calculated.

Mel release

5 mL (1 mg/mL) Pt@CS/Mel@S was added to a dialysis bag and immersed in 80 mL PBS containing different pH. At fixed time points, Mel concentrations were observed by ultraviolet absorption spectrum, and the solution was replenished with equivoluminal volume fresh buffer. Then the percentages of Mel release were calculated.

O_2 release

20 mL (250 μg/mL) Pt@CS/Mel@S solution was added to the beaker, and added N_2 for 10 minutes and subsequently added different concentration H_2O_2. Then to add 5×10^{-6} M Ru (dpp) $_3Cl_2$ reaction for 4 hours; At different time points, the emission of O_2 was detected using a fluorescence spectrophotometer at a wavelength of 455/610 nm.

Cell viability

The *in vitro* cytotoxicity was observed by the CCK-8 assay. Briefly, human renal tubular epithelial (HKC) cells cells (5×10^6) were incubated on 96-well cell culture plates. The fresh DMEM containing different concentrations of Pt@CS/Mel@S were added and cultured for 24 h, respectively. In addition, the culture mediums were replaced with fresh DMEM including Pt@CS/Mel@S (200 μg/mL) and cultured for 24 h. CCK-8 solution (100 μL) was added for 2 h after washing twice with PBS. Then the absorbance was inspected at 450 nm to obtain the cell survival rates.

ROS observation

Human renal tubular epithelial (HKC) cells cells in logarithmic phase were harvested in

confocal special dishes, and each dish was 5×10^6 cells and incubated in an incubator for 12 h. Next Pt@CS/Mel@S (10 μL, 1 mg/mL) was added and incubated for 6 h. Then reactive oxygen species assay kit was added for 30 min and fluorescence image was acquired by fluorescence microscope.

Cell colocalization

Human renal tubular epithelial (HKC) cells cells in logarithmic phase were harvested in confocal special dishes, and each dish was 5×10^6 cells and incubated in an incubator for 12 h. Next Pt@CS/Mel@S (10 μL, 1 mg/mL) was added and incubated for 6 h. Then MitoTracker® Green FM or Lyso-Tracker Green or Golgi-Tracker Green was added for 30 min, and DAPI was added for 10 min and fluorescence image was observed by confocal microscopy.

Optical video recording

The motion of the Pt@CS/Mel@S motors was measured by an inverted fluorescence microscope. First, the diluted Pt@CS/Mel@S motor solution was placed on a hydrophilic glass slide in different media. To observe the O_2 release movement from the Pt@CS/Mel@S motor, videos were recorded with a camera. The velocity and diffusion coefficient of the Pt@CS/Mel@S motors were measured using ImageJ and Chemotaxis. The *MSD* was estimated and calculated using $MSD = 4D\Delta t + V^2\Delta t^2$, where D was the diffusion coefficient of Brownian motion, V the velocity of autonomous motion.

Cell H_2O_2

Human renal tubular epithelial (HKC) cells cells in logarithmic phase were harvested in confocal special dishes, and each dish was 5×10^6 cells and incubated in an incubator for 12 h. Next Pt@CS/Mel@S (10 μL, 1 mg/mL) was added and incubated for 6 h. H_2O_2 level was observed by H_2O_2 kit.

Animal model

All Procedures of Animals Experimental Works were consistent with the guidelines of institutional animal care and use committee of 'Jiaxing University'. All Experimental Works complied with the relevant 'Laws' or 'Guidelines'. All of Experimental Works followed the Institutional Guidelines. All Animal Procedures of Animals Experimental Works were performed in accordance with the 'Guide' for the care and use of laboratory animals of 'Jiaxing University', and the Animals Experimental Works were approved by the animal ethics committee of 'Jiaxing University'.

BALB/c mice (male, 25-30 g) were obtained from Zhejiang Experimental Animal Center and the mice were anesthetized with 1% pentobarbital sodium, and the abdominal cavity was opened and the renal arteries and veins were separated. The right renal artery and vein were clipped for 30 min, and then perfused for 48 h to create an AKI model.

***In vivo* biodistribution and therapeutic effects**

After intravenous injection of Pt@CS/Mel@S motors (15 mg/kg), the *in vivo* fluorescence distribution in AKI mouse was measured at fixed times using a multimode small-animal imaging system *in vivo*. Subsequently, the AKI region in the mice was measured by a ultrasound imaging.

35 BALB/c mice (male, 25-30 g) were divided into 7 groups: 1) control; 2) AKI; 3) Mel; 4) CM; 5) PCM; 6) CMS; 7) PCMS and each group was 5. After intravenous injection of Pt@CS/Mel@S motors (15 mg/kg), and the serum and tissues were separated. The BUN, Scr, SOD, MDA, H_2O_2 and scavenging of ROS ratio by kits, HE staining and TUNEL staining (TUNEL kit) were measured. And the Kim-1, Cx43 and HIF-1α expressions were observed by immunohistochemistry. (CM, CS/Mel; PCM, Pt@CS/Mel; CMS, CS/Mel@S; PCMS, Pt@CS/Mel@S; Mel, melatonin; CS, chitosan; S, L-serine).

Statistical analysis

The statistical significances in different groups were estimated through one-way ANOVA with post hoc test. Values of $P<0.01$ were considered to display statistical significance in different groups.

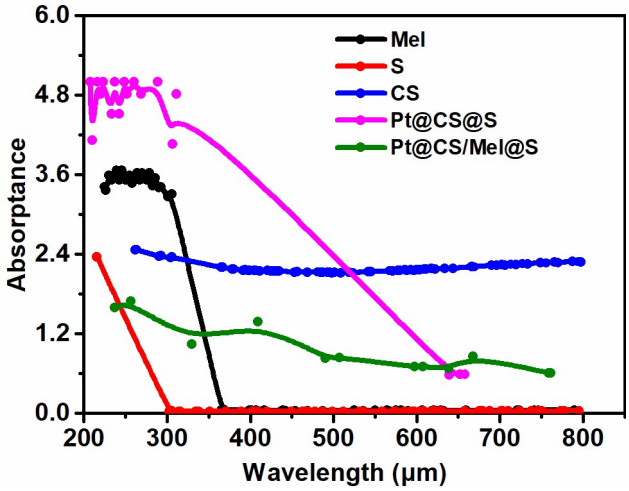

Figure S1 I Ultraviolet absorption spectrum of Mel, S, CS, Pt@CS@S and Pt@CS/Mel@S.

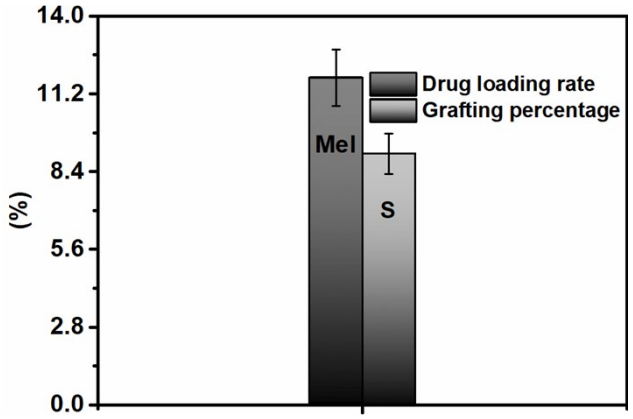

Figure S2 I Loading rate of Mel and grafting percentage of S. n=5.

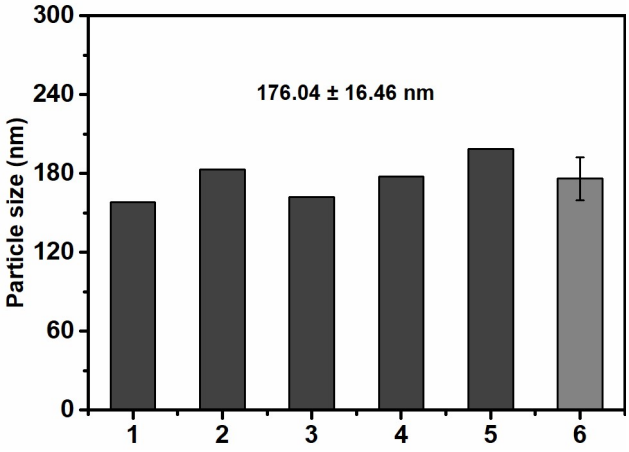

Figure S3 I Particle size of PCMS (1-5 was when we repeated the data five times and 6 was the average of the first five replicates). n=5

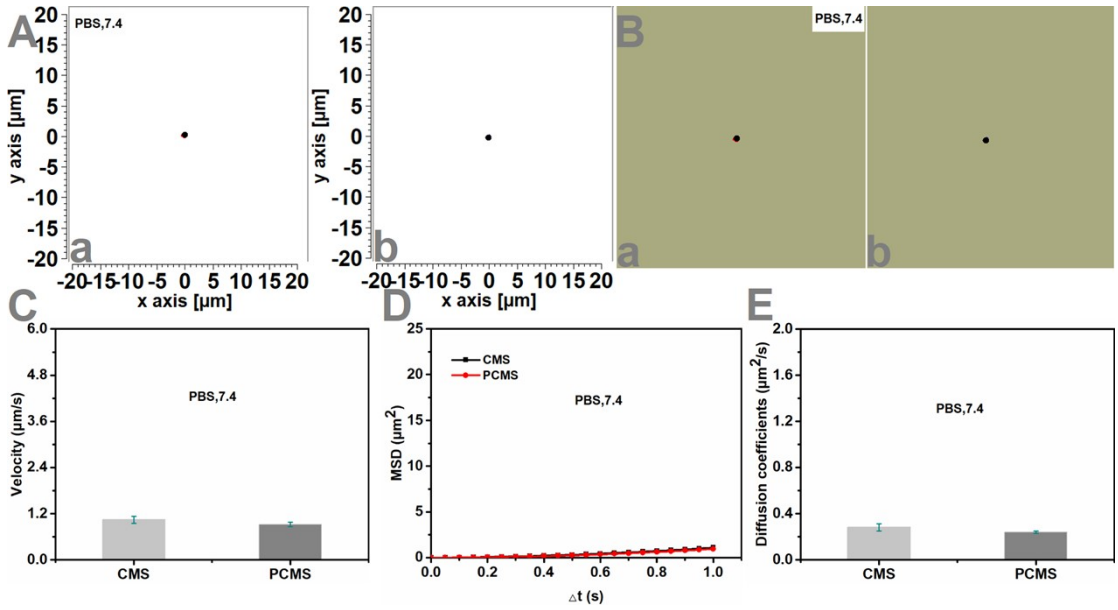

Figure S4 I Motion of Nanomotors. (**A**) Motion trajectories (10 s) of PCMS nanomotors at PBS, 7.4 (a, CMS; b, PCMS). (**B**) Time-lapse images of PCMS nanomotors at PBS, 7.4 (a, CMS; b, PCMS). (**C**) The velocity of PCMS nanomotors at PBS, 7.4. (**D**) *MSD* of PCMS nanomotors at PBS, 7.4. The directional motion was fitted to the Equation $(4D)\Delta t + (V^2)(\Delta t^2)$, and the Brownian motion was fitted to the Equation $(4D)\Delta t$. (**E**) The diffusion coefficients of PCMS nanomotors at PBS, 7.4. (CMS, CS/Mel@S; PCMS, Pt@CS/Mel@S; Mel, melatonin; CS, chitosan; S, L-serine). n=20

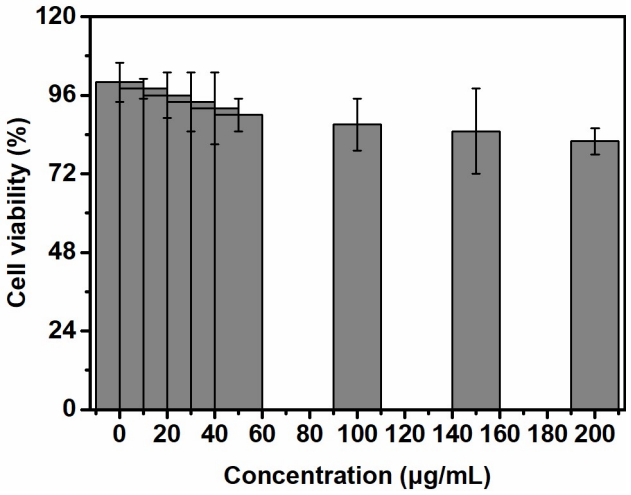

Figure S5 I Cell viability of PCMS. n=5.

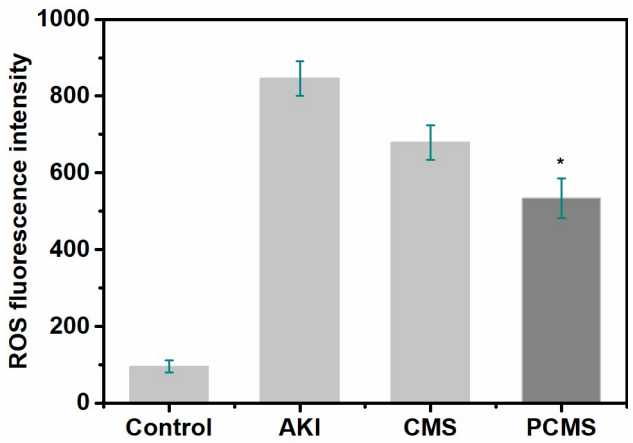

Figure S6 I ROS fluorescence intensity *in vitro* in AKI. ROS fluorescence intensity after after different treatments. (CMS, CS/Mel@S; PCMS, Pt@CS/Mel@S; Mel, melatonin; CS, chitosan; S, L-serine). n=5

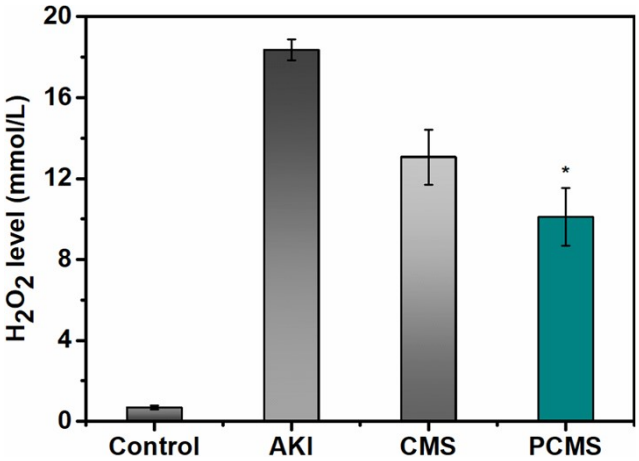

Figure S7 I H_2O_2 level *in vitro* in AKI. H_2O_2 level after after different treatments. (CMS, CS/Mel@S; PCMS, Pt@CS/Mel@S; Mel, melatonin; CS, chitosan; S, L-serine). n=5

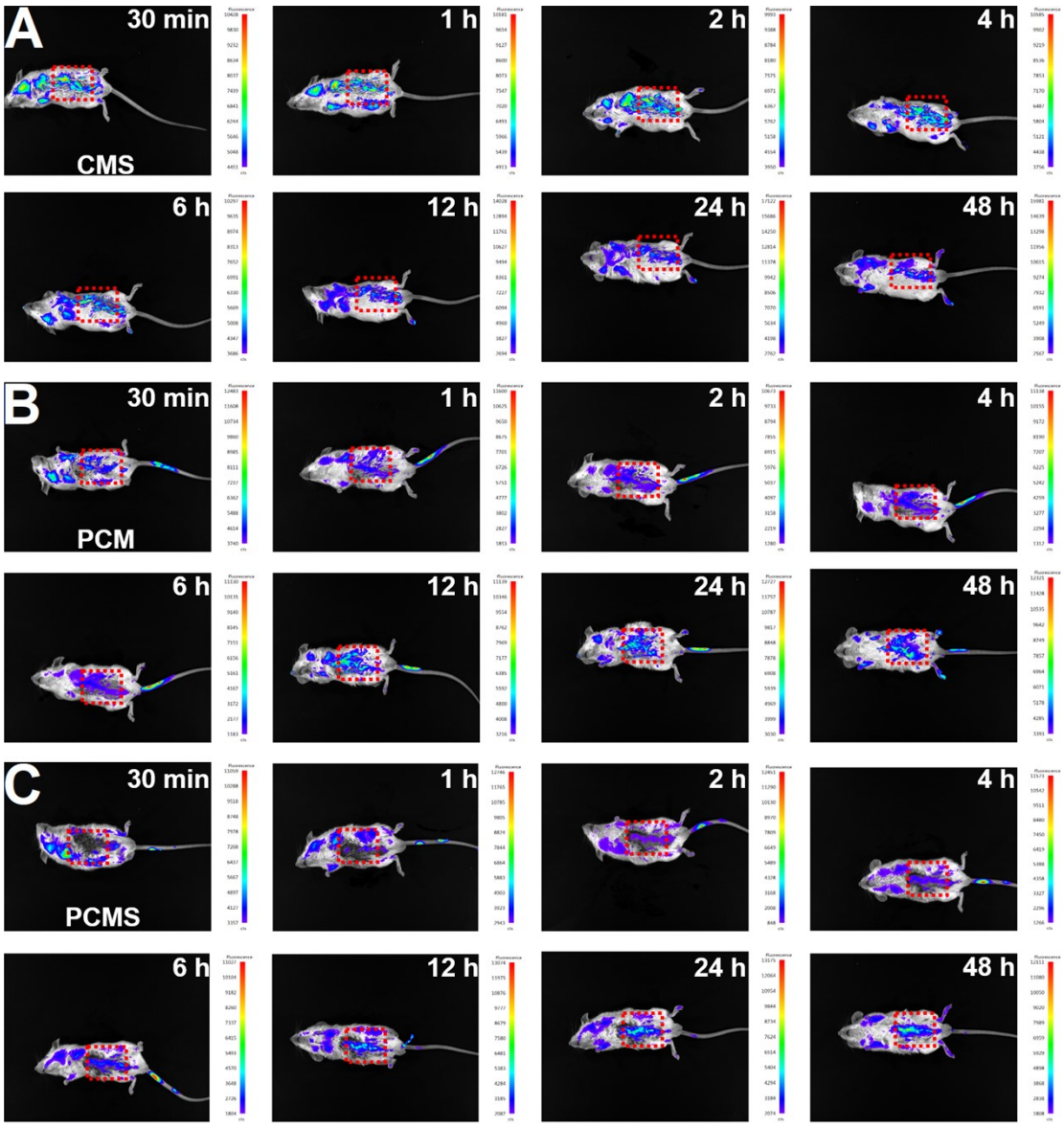

Figure S8 I *In vivo* biodistribution of PCMS nanomotors. (**A**) *In vivo* distribution of FITC-conjugated CMS nanomotors at different time points. (**B**) *In vivo* distribution of FITC-conjugated PCM nanomotors at different time points. (**C**) *In vivo* distribution of FITC-conjugated PCMS nanomotors at different time points. (PCMS, Pt@CS/Mel@S; PCM, Pt@CS/Mel; CMS, CS/Mel@S).

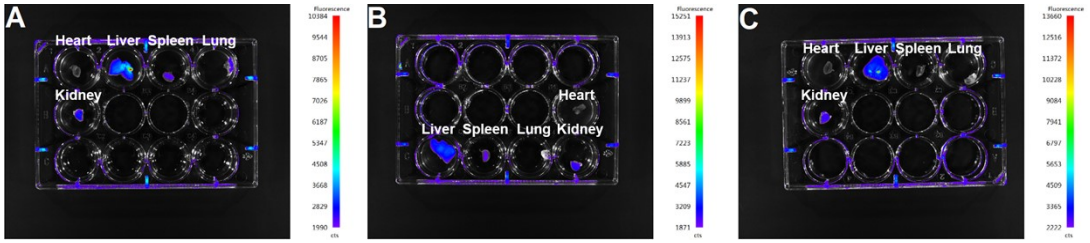

Figure S9 I (**A**) Live imaging of the *in vivo* tissue after FITC-CMS administration. (**B**) Live imaging of the *in vivo* tissue after FITC-PCM administration. (**C**) Live imaging of the *in vivo* tissue after FITC-PCM administration. (PCMS, Pt@CS/Mel@S; PCM, Pt@CS/Mel@; CMS, CS/Mel@; Mel, melatonin; CS, chitosan; S, L-serine).

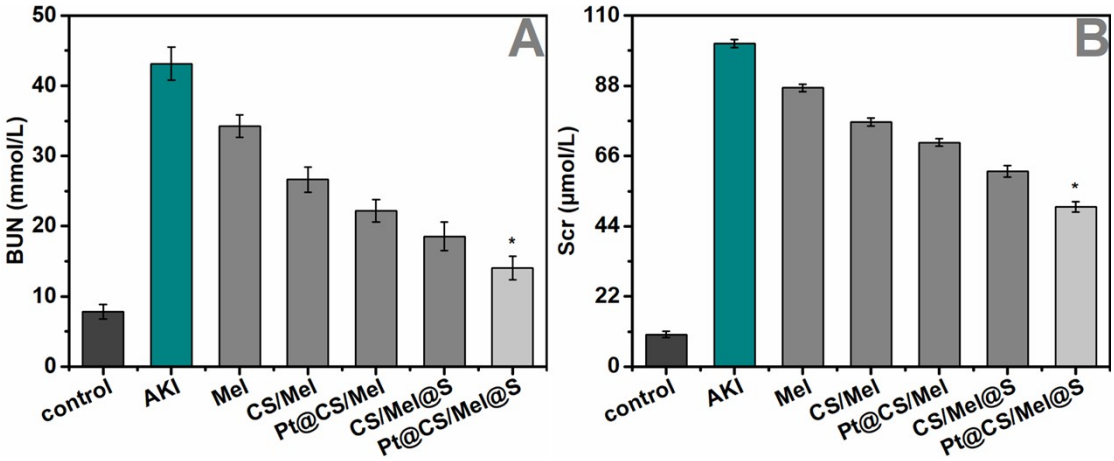

Figure S10 I The BUN (**A**) and Scr (**B**) after different treatments by intravenous administration. n=5

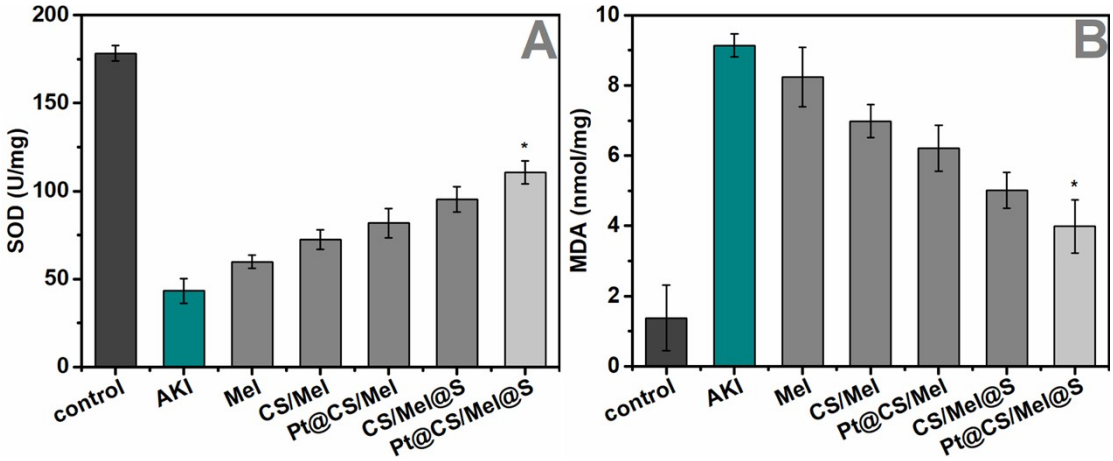

Figure S11 I SOD (**A**) and MDA (**B**) contents in renal tissues after different treatments by intravenous administration. n=5

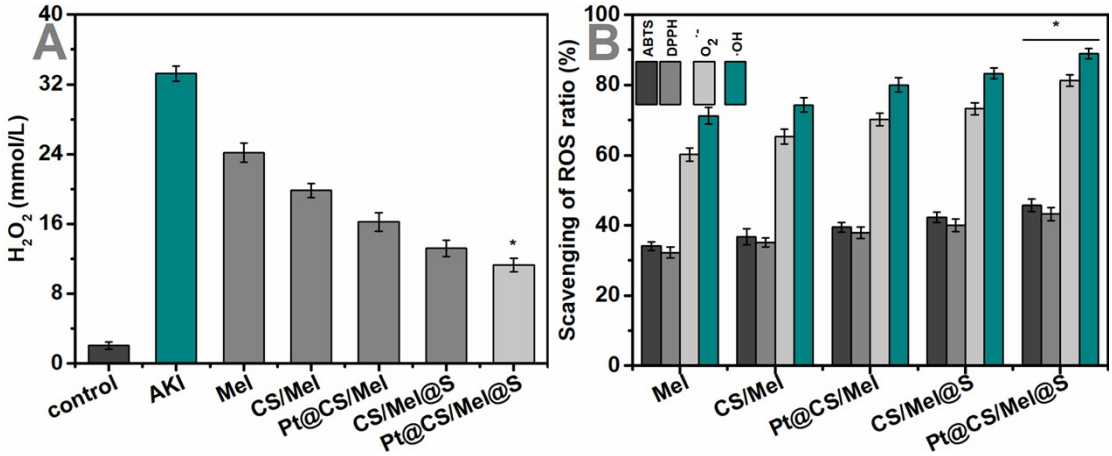

Figure S12 I H_2O_2 (**A**) and Scavenging of ROS ratio (**B**) after different treatments by intravenous administration. n=5

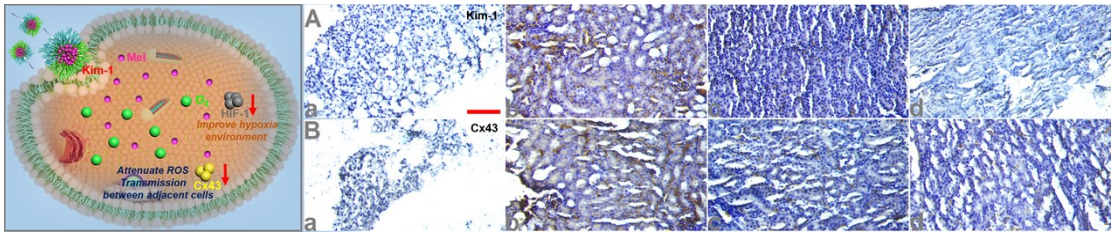

Figure S13 I *In vivo* immunohistochemical analysis. Kim-1 (**A**) and Cx43 (**B**) expressions in renal tissues after different treatments. (a, control; b, AKI; c, CS/Mel@S; d, Pt@CS/Mel@S). Scale bar: 50 μm. (Mel, melatonin; CS, chitosan; S, L-serine).

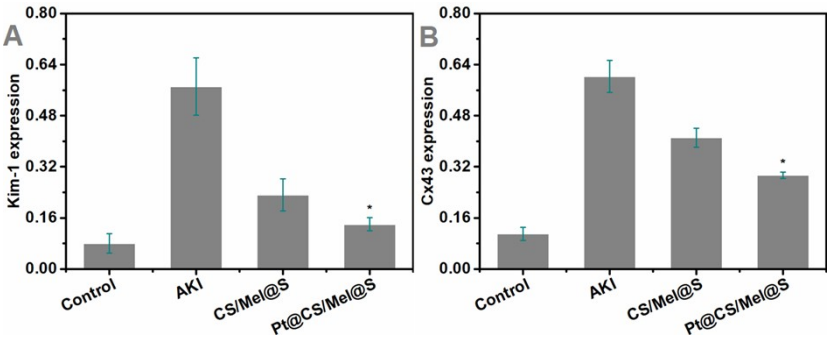

Figure S14 I The quantitative analysis (%) of Kim-1 (**C**) and Cx43 (**D**) expressions in renal tissues after different treatments. (a, control; b, AKI; c, CS/Mel@S; d, Pt@CS/Mel@S). Scale bar: 50 μm. (Mel, melatonin; CS, chitosan; S, L-serine).

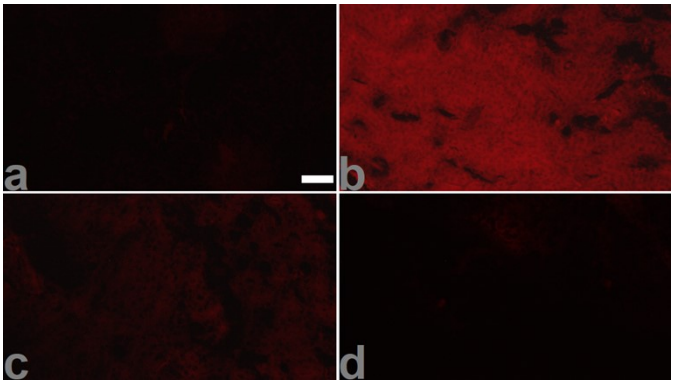

Figure S15 I *In vivo* immunofluorescence analysis of AF-594-HIF-1α in renal tissues after different treatments (a, control; b, AKI; c, CS/Mel@S; d, Pt@CS/Mel@S). Scale bar: 50 μm. (Mel, melatonin; CS, chitosan; S, L-serine).