Supporting Information

Clinically translatable gold nanozymes with broad spectrum antioxidant and anti-inflammatory activity for alleviating acute kidney injury

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Methods

**POD-like activity of Au NCs-NAC**
The POD-like activity of Au NCs-NAC was evaluated by 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB) assay in the presence H2O2 in phosphatic buffer solution (pH 7.4) at room temperature. The absorbance of solution containing TMB (2 mM), H2O2 (1 M) and Au NCs-NAC (30 μg/mL) was recorded every 1 min till 5 min.

The kinetic experiments of Au NCs-NAC for POD-like activity was conducted at room temperature in phosphatic buffer solution (pH 7.4) with Au NCs-NAC (30 μg/mL) in the presence of H2O2 and TMB. The kinetic analysis of Au NCs-NAC with TMB as a substrate was performed by varying the concentrations of TMB at a fixed H2O2 concentration and vice versa. Finally, the catalytic parameters were calculated by fitting the Michaelis-Menten equation.

**Hydroxyl radical (·OH) scavenging activity of Au NCs-NAC**
The ·OH scavenging activity of Au NCs-NAC was evaluated using a radical antioxidant capacity (HORAC) assay kit (Cell Biolabs, Inc., USA). Experiments with different concentrations of Au NCs-NAC were carried out according to the instructions provided by the manufacturer. Further, 5 mM H2O2, 50 μM FeCl2, and 100 μg/mL of Au NCs-NAC or free NAC were mixed in PBS (pH 7.4), and ESI-MS spectra were recorded.

**ABTS radical (ABTS·) scavenging activity of Au NCs-NAC**
The ABTS radicals (ABTS·) were first obtained by mixing 7 mM ABTS with 2.45 mM potassium persulfide for 12 h. Next, the absorbance of ABTS· solution with 12.5-100 μg/mL Au NCs-NAC (A₅) and pure ABTS· solution (A₆) was determined at 734 nm. The scavenging efficiency of Au NCs-NAC was calculated by the following formula: [(A₆ - A₅)/A₆] * 100. All measurements were made in triplicate.

**Superoxide anion radical (O2·−) scavenging activity of Au NCs-NAC**
The O2·− scavenging capacity of various concentrations of Au NCs-NAC was assessed by a SOD assay kit (Sigma-Aldrich, USA). The experiment was conducted in full accordance with the instructions provided by the supplier. The ESR signals of sample containing 100 mM DMPO, 100 μg/mL of Au NCs-NAC and 10 mM KO2 were recorded.

**Cytochrome c (Cyt c) electron transfer experiment**
Au NCs-NAC (100 μg/mL) were mixed with Cyt c (400 μM) in PBS (pH = 7.4) in dark for 1 h. Then, UV-vis spectrophotometer was used to record the absorption of the resultant solution. To determine whether Cyt c was oxidized by the dissolved oxygen, N2 gas was pumped for 1 h in the stock solutions of Cyt c, Au NCs-NAC solution, and PBS, simultaneously.
Subsequently, the experiment was repeated following the above-described process.

**Hemolysis assay**

For hemolysis assay, the whole blood was drawn from all mice and washed with PBS to obtain a suspension of red blood cells (RBC). Then, RBC suspension (0.2 mL) was mixed with different concentrations (0.2 mg/mL, 0.8 mL) of Au NCs-NAC for 6 h, followed by the centrifugation. Finally, the absorbance of a supernatant was measured at 541 nm. The hemolysis rate of Au NCs-NAC was calculated as follows: Hemolysis (%) = (As - APBS)/(A_{water} - APBS) × 100%, where As, APBS, and A_{water} are the absorbance of the sample groups, the PBS group, and the water group, respectively.
Figure S1. The size distribution of Au NCs-NAC was statistically analyzed by HR-TEM image.

Figure S2. (A) TEM image of Au NCs. The scale bar is 50 nm. (B) Hydrophilic diameter of Au NCs as determined by DLS.

Figure S3. The zeta potential of Au NCs-NAC.
Figure S4. Absorption spectra of Au NCs-NAC and NAC.

Figure S5. Fluorescence spectra of Au NCs-NAC (1 mg/mL) and NAC (1 mg/mL).

Figure S6. Au 4f XPS spectrum of Au NCs-NAC.
Figure S7. TGA curve of Au NCs-NAC.

Figure S8. (A) Chemical structure of NAC and reduced NAC. ESI-MS spectrum of (B) NAC, (C) Au NCs-NAC, (D) NAC+·OH, and (E) Au NCs-NAC+·OH.
Figure S9. Investigation of electron transfer between Au NCs-NAC and cytochrome C (Cyt c) by Cyt c assay.

Figure S10. The intracellular uptake of Au NCs-NAC at different time points.

Figure S11. Cell viability of HEK293T cells after treated with various concentrations of Au NCs-NAC.
Figure S12. Fluorescence images of HEK293T cells by mitochondrial staining after different treatments. The scale bar is 10 μm.

Figure S13. Percentage of cells stained with PI/Annexin V-FITC under different conditions.

Figure S14. Cell viability of HEK293T cells incubated with LPS when pre-treated with NAC or Au NCs-NAC.
Figure S15. Fluorescence intensity of IR800 labeled Au NCs-NAC.

Figure S16. H&E stained images of kidney after treated with PBS or Au NCs-NAC. The scale bar is 100 μm.

Figure S17. H&E stained images of organs (heart, spleen, liver and lung) after treated with PBS or Au NCs-NAC. The scale bar is 100 μm.
Figure S18. The levels of (A) BUN/CRE and (B) ALT/AST in the serum of normal mice after treated with PBS or Au NCs-NAC.

Figure S19. (A-D) Blood biochemical analysis of normal mice after treated with PBS or Au NCs-NAC.
Figure S20. Body weight of normal mice after treated with PBS or Au NCs-NAC.

Figure S21. The hemolysis rate of Au NCs-NAC on red blood cells.

Figure S22. Body weight of AKI mice after treated with PBS or Au NCs-NAC.
Figure S23. BUN and CRE levels in serum of normal/AKI mice after treated with PBS, free NAC or Au NCs-NAC.

Figure S24. The percentage of macrophages in renal tissues after different treatments.