Supporting Information

Mesoporous Encapsulated Nanozyme Decontaminating Two Kinds of Wastewater and Avoiding Secondary Pollution

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S1. General Information

Reagents and materials:

Tetraethylorthosilicate (TEOS), cetyltrimethylammonium tosylate (CTATos) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich. Hydrogen tetrachloropalladate (II) ($H_2PtCl_6•6H_2O$), Hydrogen tetrachloroaurate (III) ($HAuCl_4•3H_2O$) and Cobalt chloride hexahydrate ($CoCl_2•6H_2O$) were obtained from Sinopharm Chemica1 Reagent Co. (Shanghai, China). Aminopropyltriethoxysilane (APTES) and sodium borohydride ($NaBH_4$) were from Aladdin. NaH_2PO_4, Na_2HP_4, HCl and H_2O_2 were obtained from Beijing Chemicals (Beijing, China). MTT was from Sangon Biotechnology Inc. (Shanghai, P.R. China). All other reagents were of analytical reagent grade and used as received. Ultrapure water (18.2 MΩ; Millipore Co. USA) was used throughout the experiment.

Apparatus and characterization:

UV-Vis absorbance measurement was carried out on a JASCO V-550 UV-Vis spectrophotometer. Fluorescence spectra were detected by JASCO F-6000 fluorescence spectrometer with a Peltier temperature control accessory. FT-IR spectra were carried out on a BRUKE Vertex 70 FT-IR spectrometer. Scanning electron microscopic (SEM) images were recorded using a Hitachi S-4800 Instrument (Japan). Transmission electron microscopic (TEM) images of cells were captured with a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV. N_2 adsorption-desorption isotherms were recorded on a Micromeritics ASAP 2020M automated sorption analyzer. The pore size was determined following the BJH method. The crystalline structures of the as prepared samples were evaluated by X-ray diffraction (XRD) analysis on a Rigaku-Dmax 2500 diffractometer by using CuKα radiation. X-ray photoelectron Spectroscopy (XPS) spectra were analyzed by Thermo Fisher Scientific ESCALAB 250Xi Spectrometer Electron Spectroscopy (America). ICP-MS measurements were performed on a TheroScientific Xseries Ⅱ inductively coupled plasma mass spectrometer. The flow cytometry data were obtained by BD LSRFortessa™ Cell Analyzer. The TOC value of the withdrawn products was determined by a total organic carbon analyzer (TOC-L CPH, Shimadzu Corporation, Kyoto, Japan).

S2. Synthesis and characterization of DMSN@AuPtCo

Synthesis of dendrimer-like macroporous silica nanoparticles (DMSN):

The DMSN was prepared according to the literature with little modification.[1] In brief, a mixture of cetyltrimethylammonium tosylate (CTATos, 1.92 g), triethanolamine (0.31 g) and water (100 mL) was stirred at 80 °C for 1 h, then 15.6 mL TEOS was added and the mixture was stirred at 80 °C for another 2 h. The synthesized DMSN was filtered, washed with water and ethanol and dried in oven at 60 °C for 12 h.

To modify the DMSN inner and outer surfaces with amino groups, the surfactant template CTATos in the pores of DMSN were removed first. The as-synthesized DMSN were dispersed in a solution of hydrochloric acid in ethanol (10 % V/V) and extracted at 78°C for 24 h. This process was repeated for three times. Afterward, 500 mg of the template-remove DMSN were dispersed in 50 mL toluene by sonication and 200 µL (3-aminopropyl) triethoxysilane were added to the suspension prior to refluxing at 113°C under N_2 for 12 h. Thus, DMSN was aminated and then collected after centrifugation, washing with ethanol and drying under vacuum.

Deposition of AuPtCo tri-metal in the pores of the DMSN (DMSN@AuPtCo):

For the synthesis of DMSN@AuPtCo, AuPtCo tri-metal with 2-3 nm diameter was formed and simultaneously attached to the inner surface. Typically, the aminated DMSN nanoparticles (100 mg) were dispersed in 10 mL distilled water by sonication for 30 min, followed by the addition of the $HAuCl_4 \cdot 4H_2O$ (0.3 mL, 20 mM), $H_2PtCl_6 \cdot 6H_2O$ (0.3 mL, 20 mM), $CoCl_2 \cdot 6H_2O$ (0.3 mL, 20 mM) diluted in 2 mL distilled water. After 1 h, a freshly prepared $NaBH_4$ (36 mg in 4 mL cold water) was added into the above aqueous solution under vigorous stirring. After mixture, the resulting suspension was stirred for another 3 h. Finally, the suspension was centrifuged at 10000 rpm for 10 min to separate the DMSN@AuPtCo. Then, DMSN@AuPtCo was washed by water 3 times and dried under vacuum.

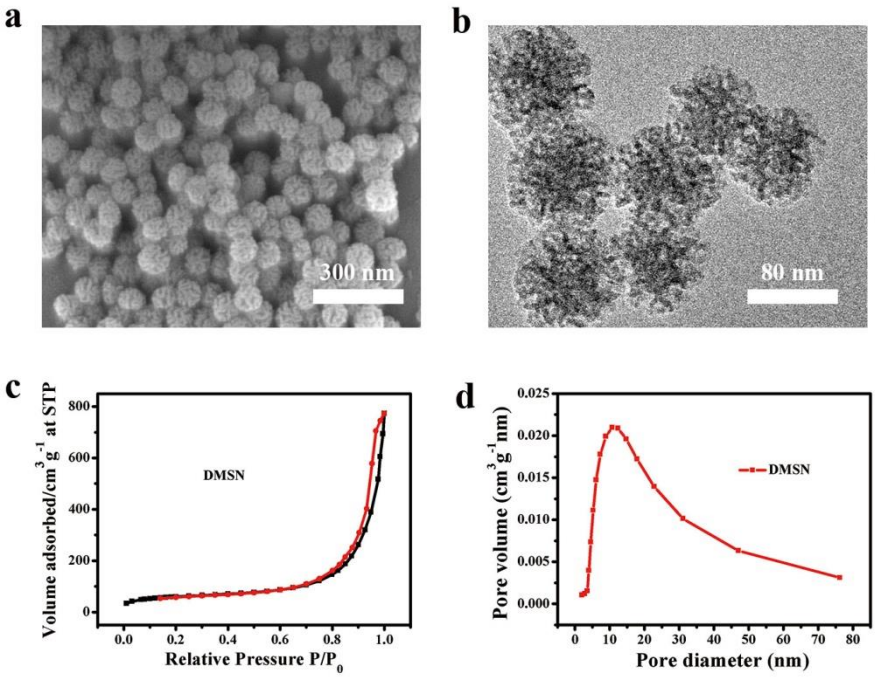

Fig. S1. a) The SEM image of the obtained DMSN (scale bar = 300 nm). b) The TEM image of the obtained DMSN (scale bar = 80 nm). The N_2 adsorption-desorption isotherms (c) and the corresponding pore-size distribution curve (d) indicate the mean pore size of ESNM is 11.5 nm.

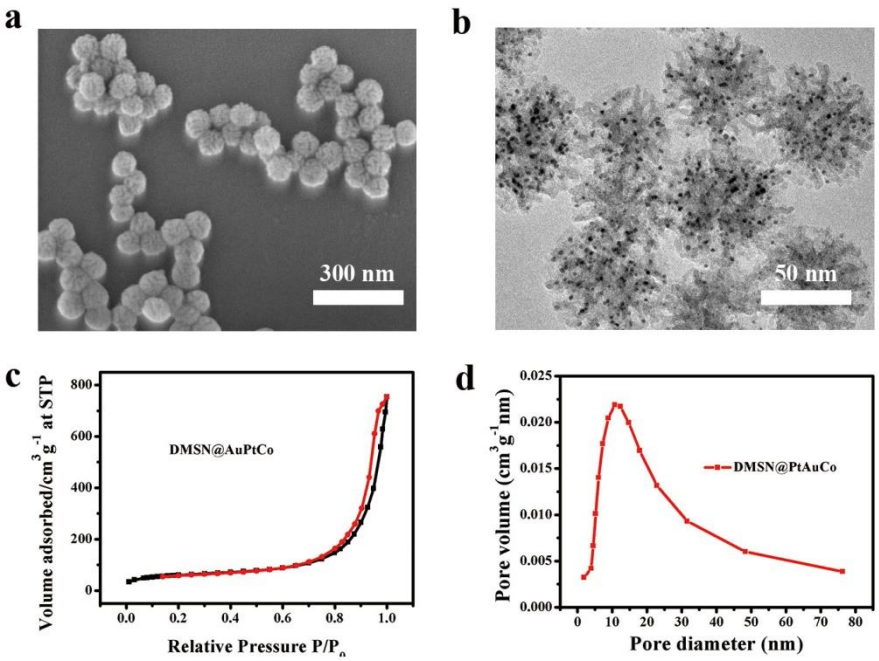

Fig. S2. a) The SEM image of the obtained DMSN@AuPtCo (scale bar = 300 nm). b) The TEM image of the obtained DMSN@AuPtCo, indicated that the AuPtCo nanoparticles were formed in the DMSN successfully (scale bar = 50 nm). The N_2 adsorption-desorption isotherms (c) and the corresponding pore-size distribution curve (d) indicated the metal reduction didn't destroy the structure of DMSN.

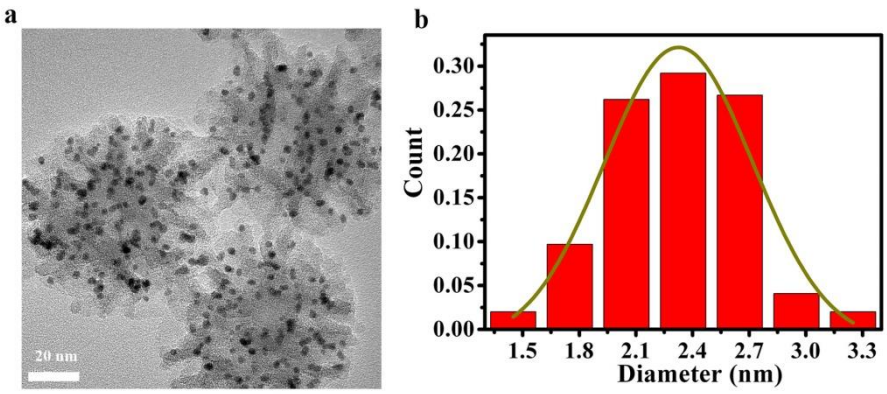

Fig. S3. a) High-resolution TEM images of DMSN@AuPtCo (scale bar = 20 nm). (b) Size distribution histogram of AuPtCo clusters in DMSN@AuPtCo. The total number of clusters counted for the histogram was 170. The average diameter of the AuPtCo on the silica was 2.2 nm.

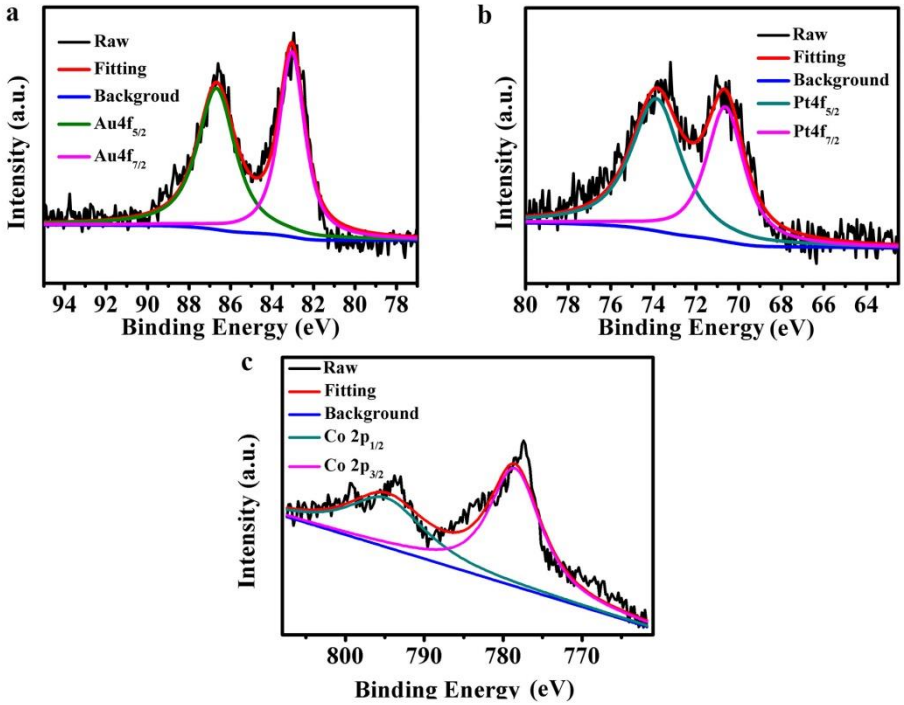

Fig. S4. X-Ray Photoelectron Spectroscopy (XPS) spectra of DMSN@AuPtCo. a) Au 4f core level, b) Pt 4f core level and c) Co 2p core level spectra. The XPS spectrum indicated that the AuPtCo tri-metal nanoparticles actually existed in obtained DMSN@AuPtCo.

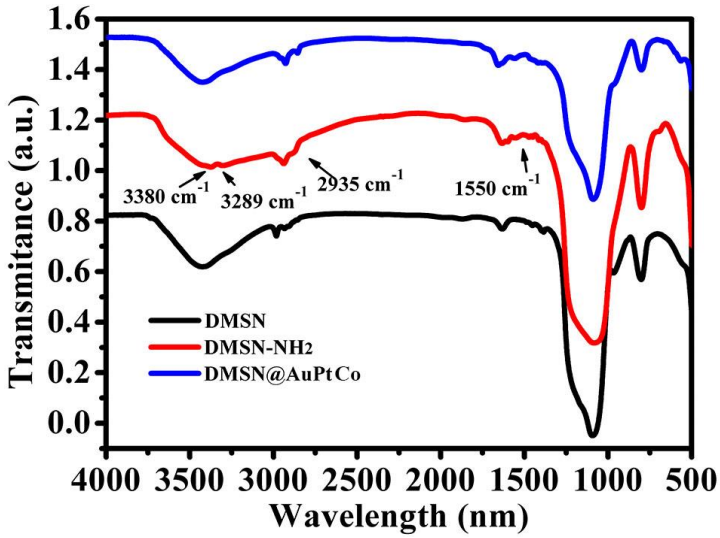

Fig. S5. The FTIR spectra of the DMSN (black), DMSN-NH_2 (red) and DMSN@AuPtCo (blue). As compared with DMSN, the bands at 3390, 3289 cm^{-1} were the ascribed to asymmetric and symmetric stretching vibration of the N-H respectively, a weak band at 2935 cm^{-1} was ascribed to symmetric vibration of the C-H groups and a band at 1550 cm^{-1} was the bending vibration N-H, indicating the presence of NH_2; for DMSN@AuPtCo (blue), no obvious changes were observed in FTIR spectra.

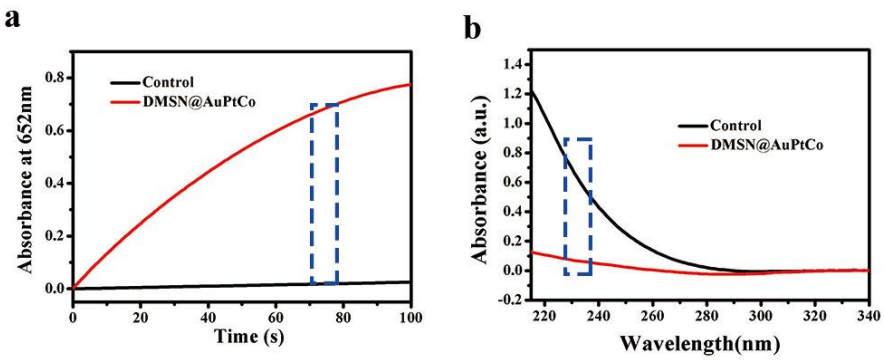

Fig. S6. The preliminary 3,3',5,5'-Tetramethylbenzidine (TMB) oxidation experiment a) and H_2O_2 decomposition experiment b) proved our nanozyme meanwhile possessed the peroxidase mimic activity and catalase mimic activity at pH 5.5.

Synthesis of DMSN@AuPtCo with different metal ratio:

To further optimize the relationship between catalytic activity and metal ratio, we synthesized DMSN@AuPtCo$_{123}$, DMSN@AuPtCo$_{222}$, DMSN@AuPtCo$_{321}$ three nanozymes with different metal ratio.*

DMSN@AuPtCo$_{123}$: Typically, the aminated DMSN nanoparticles (100 mg) were dispersed in 10 mL distilled water by sonication for 30 min, followed by the addition of the $HAuCl_4 \cdot 4H_2O$ (0.15 mL, 20 mM), $H_2PtCl_6 \cdot 6H_2O$ (0.3 mL, 20 mM), $CoCl_2 \cdot 6H_2O$ (0.45 mL, 20 mM) diluted in 2 mL distilled water. After 1 h, a freshly prepared $NaBH_4$ (36 mg in 4 mL cold water) was added into the above aqueous solution under vigorous stirring. After mixture, the resulting suspension was stirred for another 3 h. Finally, the suspension was centrifuged at 10000 rpm for 10 min to separate the DMSN@AuPtCo$_{123}$. Then, DMSN@AuPtCo$_{123}$ was washed by water 3 times and dried under vacuum.

DMSN@AuPtCo$_{222}$: Typically, the aminated DMSN nanoparticles (100 mg) were dispersed in 10 mL distilled water by sonication for 30 min, followed by the addition of the $HAuCl_4 \cdot 4H_2O$ (0.3 mL, 20 mM), $H_2PtCl_6 \cdot 6H_2O$ (0.3 mL, 20 mM), $CoCl_2 \cdot 6H_2O$ (0.3 mL, 20 mM) diluted in 2 mL distilled water. After 1 h, a freshly prepared $NaBH_4$ (36 mg in 4 mL cold water) was added into the above aqueous solution under vigorous stirring. After mixture, the resulting suspension was stirred for another 3 h. Finally, the suspension was centrifuged at 10000 rpm for 10 min to separate the DMSN@AuPtCo$_{222}$. Then, DMSN@AuPtCo$_{222}$ was washed by water 3 times and dried under vacuum.

DMSN@AuPtCo$_{321}$: Typically, the aminated DMSN nanoparticles (100 mg) were dispersed in 10 mL distilled water by sonication for 30 min, followed by the addition of the $HAuCl_4 \cdot 4H_2O$ (0.45 mL, 20 mM), $H_2PtCl_6 \cdot 6H_2O$ (0.3 mL, 20 mM), $CoCl_2 \cdot 6H_2O$ (0.15 mL, 20 mM) diluted in 2 mL distilled water. After 1 h, a freshly prepared $NaBH_4$ (36 mg in 4 mL cold water) was added into the above aqueous solution under vigorous stirring. After mixture, the resulting suspension was stirred for another 3 h. Finally, the suspension was centrifuged at 10000 rpm for 10 min to separate the DMSN@AuPtCo$_{321}$. Then, DMSN@AuPtCo$_{321}$ was washed by water 3 times and dried under vacuum.

* Compared with three nanozymes, we chose DMSN@AuPtCo$_{222}$ as the model nanozyme in our following experiments at last. So, with no special instructions, the abbreviation " DMSN@AuPtCo" means DMSN@AuPtCo$_{222}$ in our paper.

Inductively coupled plasma mass spectrometry (ICP-MS) instrumentation for quantification of DMSN@AuPtCo

ICP-MS measurements were performed on a TheroScientific Xseries Ⅱ inductively coupled plasma mass spectrometer. 0.5 mL of fresh aqua regia was added to the 10 μL sample solution and then the sample was diluted to 10 mL with distilled water. The amount of ^{197}Au, ^{195}Pt and ^{59}Co were tracked by ICP-MS.

Table S1: Au Pt Co amount in nanozyme calculated by ICP-MS

	Au (ppb)	Pt (ppb)	Co (ppb)	Au/Pt/Co
DMSN@AuPtCo$_{123}$	232	413	259	1.1 /2.0 /3.1
DMSN@AuPtCo$_{222}$	401	390	140	2.05 /2.0 /2.1
DMSN@AuPtCo$_{321}$	617	407	70	3.01 /2.0 /1.04

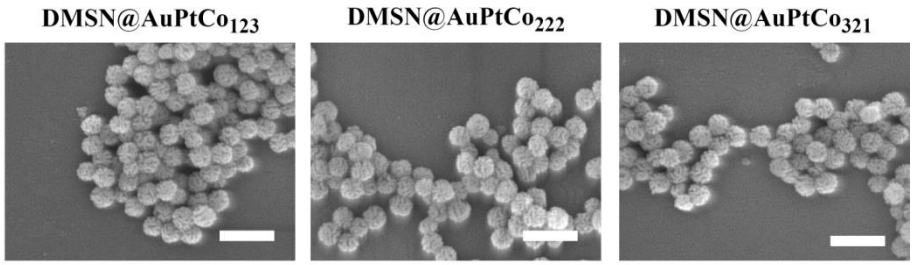

Fig. S7. The SEM images of the DMSN@AuPtCo$_{123}$, DMSN@AuPtCo$_{222}$ and DMSN@AuPtCo$_{321}$ (scale bar = 200 nm).

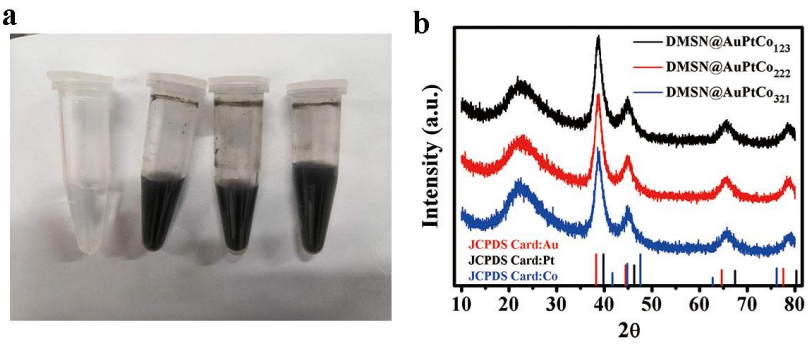

Fig. S8. a) The tubes from left to right in the photograph were the DMSN, DMSN@AuPtCo$_{123}$, DMSN@AuPtCo$_{222}$ and DMSN@AuPtCo$_{321}$ respectively. b) The XRD patterns of DMSN@AuPtCo$_{123}$, DMSN@AuPtCo$_{222}$ and DMSN@AuPtCo$_{321}$ also proved the stable structure.

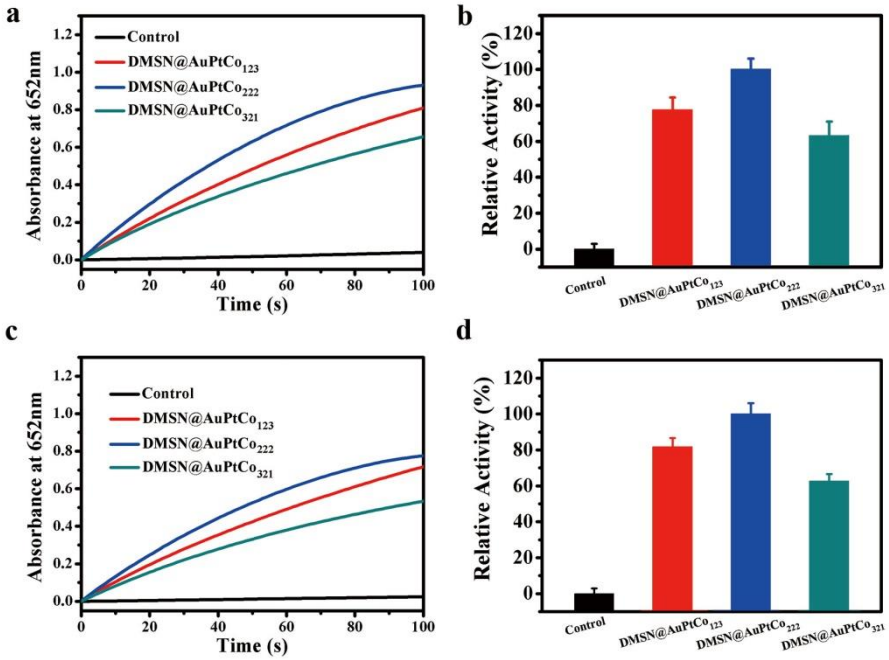

Fig. S9. a) The study of HRP-mimic activity of DMSN@AuPtCo$_{123}$ (red), DMSN@AuPtCo$_{222}$ (blue) and DMSN@AuPtCo$_{321}$ (green) at pH= 4.0; b) The histogram of relative HRP-mimic activity. c) The study of HRP-mimic activity of DMSN@AuPtCo$_{123}$ (red), DMSN@AuPtCo$_{222}$ (blue) and DMSN@AuPtCo$_{321}$ (green) at pH= 5.5; d) The histogram of relative HRP-mimic activity. Data were presented as mean ± s.d. (n = 3)

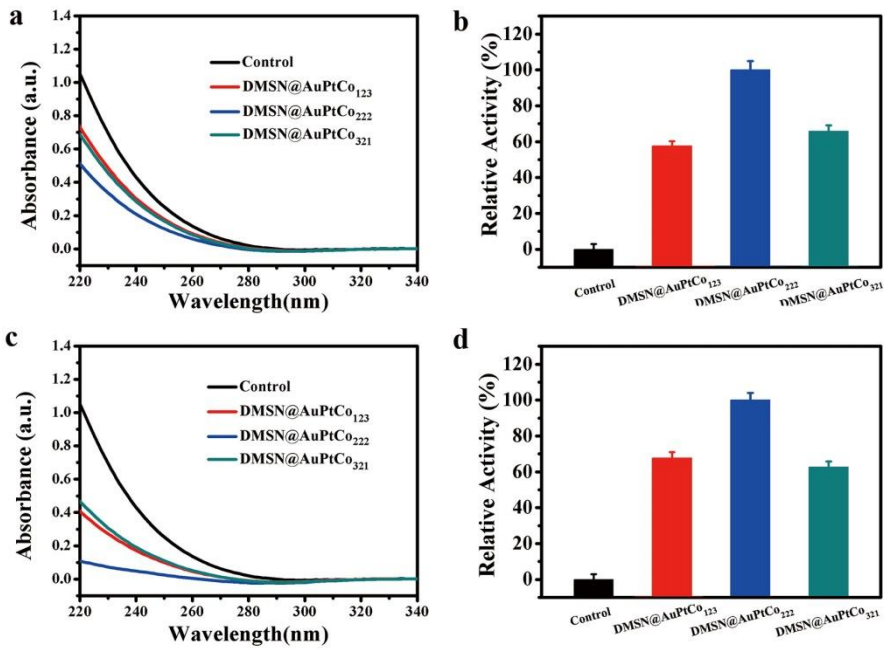

Fig. S10. a) The UV-Vis spectra of H_2O_2 after reacted with DMSN@AuPtCo$_{123}$ (red), DMSN@AuPtCo$_{222}$ (blue) and DMSN@AuPtCo$_{321}$ (green) for 30 min; b) The histogram of relative CAT-mimic activity. c) The UV-Vis spectra of H_2O_2 after reacted with DMSN@AuPtCo$_{123}$ (red), DMSN@AuPtCo$_{222}$ (blue) and DMSN@AuPtCo$_{321}$ (green) for 60 min; d) The histogram of relative CAT-mimic activity. Data were presented as mean ± s.d. (n = 3)

Assessment of the HRP-mimic activity of DMSN@AuPtCo complex nanozyme:

The HRP-mimic catalytic activity of DMSN@AuPtCo was studied through the oxidation of TMB in the presence of H_2O_2 by following the increase absorbance at 652 nm on JASCO V550 UV-Vis spectrophotometer. In a typical assay, the experiments were carried out using 20 μg mL^{-1} DMSN@AuPtCo in a reaction volume of 0.5 mL PBS solution (25 mM pH=5.5) with 1 mM TMB as substrate at 37 °C unless otherwise stated. The maximum point in each curve was set as 100%.

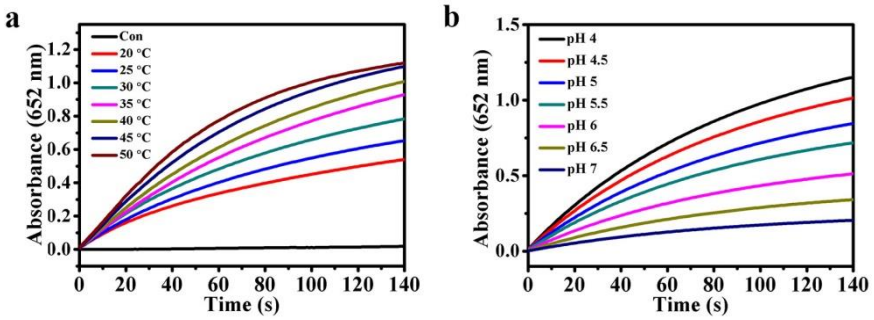

Fig. S11. a) The increasing absorbance at 652 nm along with time, indicated the changes of HRP-mimic activity of DMSN@AuPtCo under different temperatures. b) The increasing absorbance at 652 nm along with time, indicated the changes of HRP-mimic activity of DMSN@AuPtCo under different pH.

Steady-state kinetic assay of HRP-mimic activity:

Kinetic measurements were carried out in time course mode by monitoring the absorbance change of TMB at 652 nm. We mixed 20 μg mL^{-1} DMSN@AuPtCo, H_2O_2, and TMB in PBS buffer. The velocity (v) of the reaction was measured at different concentration of TMB (0.4, 0.6, 0.8 mM) or H_2O_2 (10, 15, 25 mM). The Michaelis- Menten constant was calculated by the Lineweaver-Burk plot: $1/v= (K_m/V_{max})*(1/C) + 1/V_{max}$, where v is the initial velocity, V_{max} is the maximal reaction velocity, and C is the concentration of substrate.

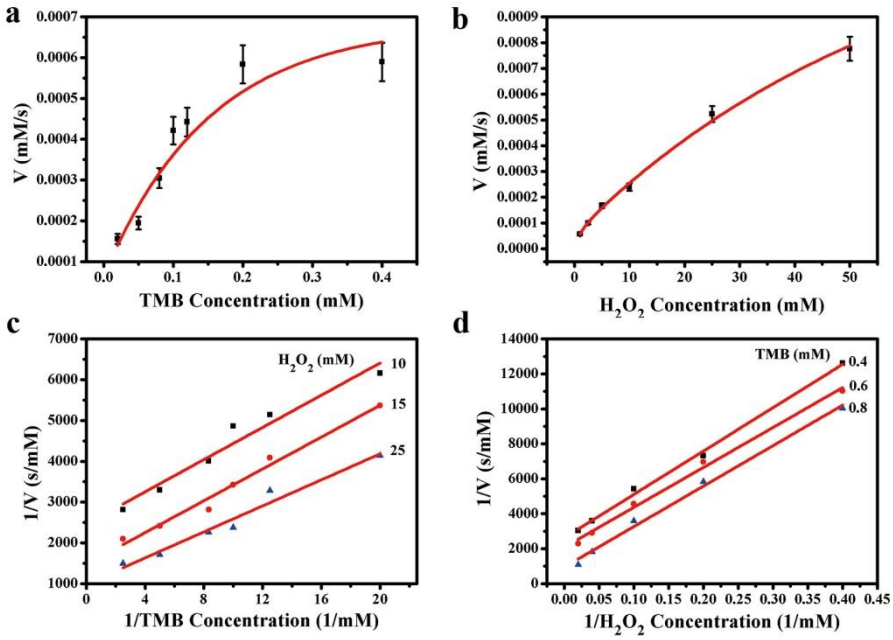

Fig. S12. Steady-state kinetic assay and catalytic mechanism of DMSN@AuPtCo. a) The velocity (v) of the reaction was measured using 20 μg mL^{-1} DMSN@AuPtCo and 10 mM H_2O_2 at different concentrations of TMB. b) The velocity (v) of the reaction was measured using 20 μg mL^{-1} DMSN@AuPtCo and 0.6 mM TMB at different concentrations of H_2O_2. c) The double-reciprocal plots of activity of DMSN@AuPtCo at a fixed concentration of 10 mM, 15 mM and 25mM H_2O_2. d) The double-reciprocal plots of activity of DMSN@AuPtCo at a fixed concentration of 0.4 mM, 0.6 mM and 0.8mM TMB. Data were presented as mean ± s.d. (n = 3)

Table S2. Comparison of the apparent Michaelis-Menten constant (Km) and maximum reaction rate (Vm) between DMSN@AuPtCo and HRP.

Catalyst	Substance	Km (mM)	Vm (10^{-8} mM s^{-1})
DMSN@AuPtCo	TMB	0.0905	6.843
DMSN@AuPtCo	H_2O_2	5.8	3.663
HRP*	TMB	0.275	1.24
HRP*	H_2O_2	0.214	2.46

The detection of hydroxyl radical (·OH)

To evidence the presence of hydroxyl radical (·OH) in our system, the Terephthalic acid (TA) was used as a fluorescence probe for tracking of ·OH. Because terephthalic acid easily reacted with ·OH to form highly fluorescent 2-hydroxy terephthalic acid. The procedure of experiment was carried out using 1 mM H_2O_2, 0.5 mM terephthalic acid, and 20 μg/mL DMSN@AuPtCo at 37 °C for 12 h. It was clearly shown that gradual increase of the fluorescence intensity was observed in TA+H_2O_2+DMSN@AuPtCo group. However, there was no fluorescence intensity in the absence of DMSN@AuPtCo.

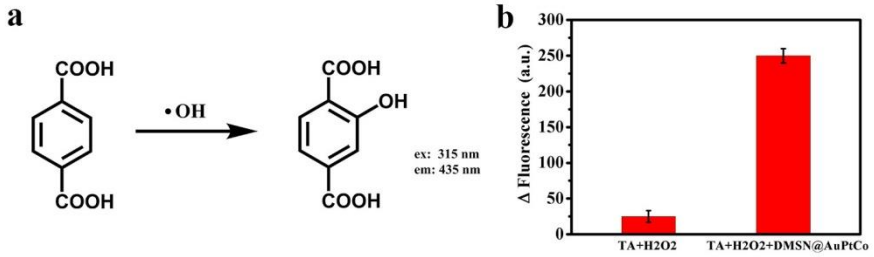

Fig. S13. a) The reaction between hydroxyl radical (·OH) and terephthalic acid (TA). b) Histograms of ΔFL intensity of the solution include TA and H_2O_2; TA, H_2O_2 and DMSN@AuPtCo after 12 h reaction. The concentrations of TA, H_2O_2 and DMSN@AuPtCo were 0.5 mM, 1 mM and 20 μg/mL, respectively. Data were presented as mean ± s.d. (n = 3)

Assessment of the CAT-mimic activity of DMSN@AuPtCo complex nanozyme:

The calibration curve of absorbance and the concentration of H_2O_2 (0-12 mM) were recorded by monitoring the absorbance at 240 nm on a Jasco V550 UV-Vis spectrophotometer with different concentration of H_2O_2. Besides, the H_2O_2 scavenging experiment was carried out by adding DMSN@AuPtCo (20 μg mL^{-1}) in PBS solution (25 mM pH=5.5) containing H_2O_2 (about 12 mM) at 37 °C. After a period of time, DMSN@AuPtCo was centrifuged and the UV-Vis spectra of the remaining H_2O_2 were recorded. The concentration of the comsuming H_2O_2 was calculated according to the decrease of absorbance at 240 nm.

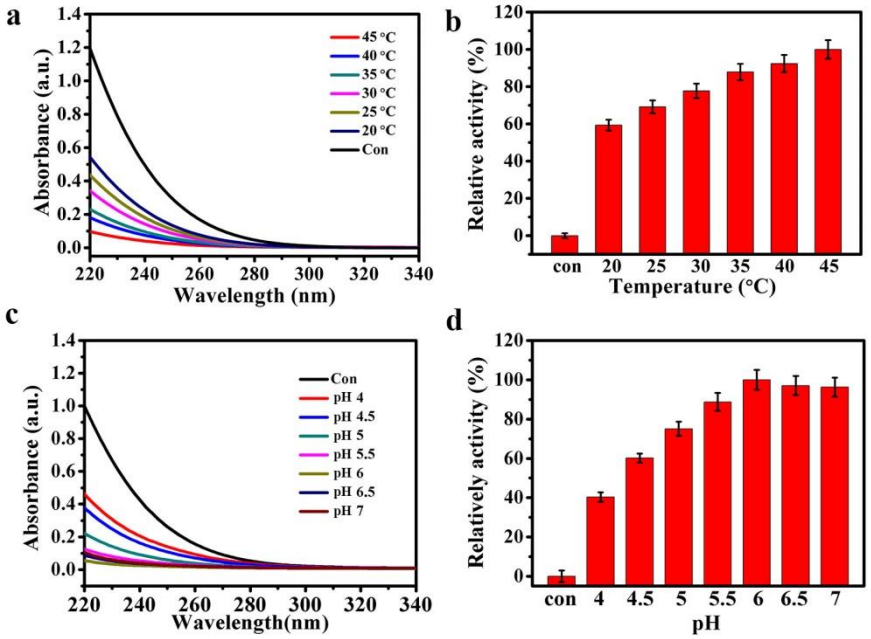

Fig. S14. a) The changes of the UV-Vis spectra of H_2O_2 indicated the changes of CAT-mimic activity of DMSN@AuPtCo under different temperatures. b) The histogram of relative CAT-mimic activity under different temperatures. c) The changes of UV-Vis spectra of H_2O_2 indicated the changes of CAT-mimic activity of DMSN@AuPtCo under different pH. d) The histogram of relative CAT-mimic activity under different pH. Data were presented as mean ± s.d. (n = 3)

Steady-state kinetic assay of CAT-mimic activity

Kinetic measurement was carried out by monitoring the absorbance changes at 240 nm on a Jasco V550 UV_Vis spectrophotometer. Experiments were carried out using 20 μg mL^{-1} DMSN@AuPtCo in a reaction volume of 500 μL buffer solution and in the presence of H_2O_2 (the concentration was titrated by $KMnO_4$). Lineweaver-Burk plot: $1/v= (K_m/V_{max})*(1/C) + 1/V_{max}$, where v is the initial velocity, V_{max} is the maximal reaction velocity, and C is the concentration of substrate.

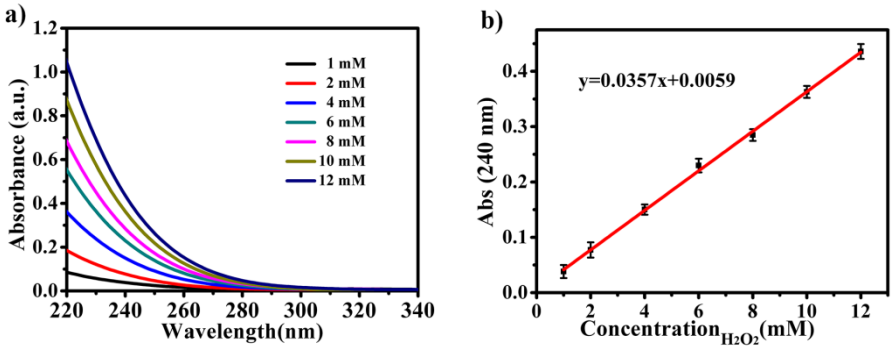

Fig. S15. a) The UV-Vis spectra of H_2O_2 along with different concentrations (1-12 mM). b) The corresponding concentration calibration curve at 240 nm. Data were presented as mean ± s.d. (n = 3)

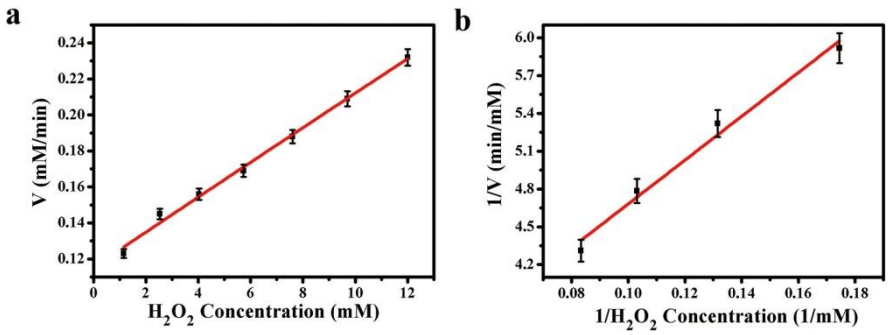

Fig. S16. Steady-state kinetic assay of DMSN@AuPtCo. a) The reaction rate was measured using 20 μg mL^{-1} DMSN@AuPtCo at different H_2O_2 concentration. b) The double-reciprocal plots of activity of DMSN@AuPtCo. Data were presented as mean ± s.d. (n = 3)

Table S3. Comparison of the apparent Michaelis-Menten constant (Km) and maximum reaction rate (Vm) between DMSN@AuPtCo and CAT.

Catalyst	Substance	Km (mM)	Vm (mM min^{-1})
DMSN@AuPtCo	H_2O_2	5.9	0.34
CAT	H_2O_2	25	0.6

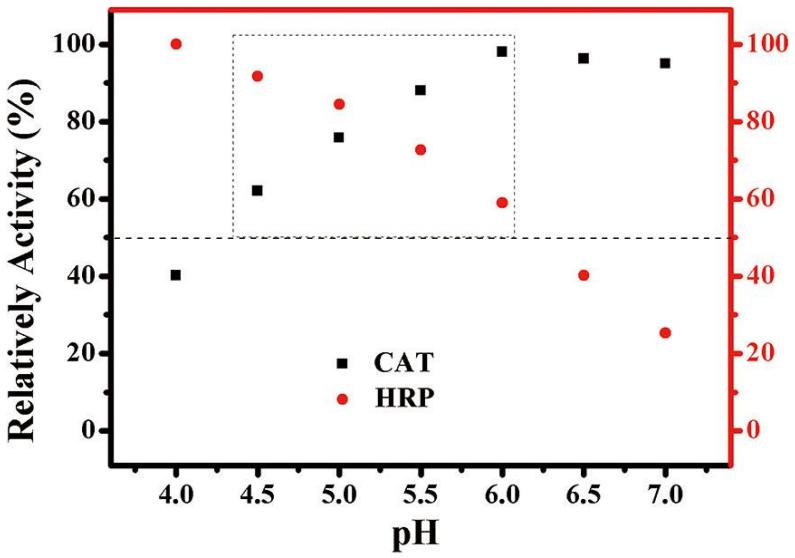

Fig. S17. The scatter plot of the relative HRP-mimic activity (red) and CAT-mimic activity (black) of DMSN@AuPtCo under different pH. The dashed box indicated both the relative HRP-mimic activity and CAT-mimic activity of DMSN@AuPtCo were above 50%.

Decontamination of wastewater by our nanozyme:

Phenol degradation was conducted in a plastic tube at room temperature. In each tube, the phenol solution was mixed with 1M H_2O_2 and then added DMSN@AuPtCo$_{222}$ for decontamination study. For the study the changes of phenol concentration, the mix solution was diluted and then catalase was added to decompose excessive H_2O_2. After that, the solution was centrifuged at 11000g.

The phenol concentration was determined based on Emerson's method. A series of 200 μL aliquots of the above mentioned supernatants were added to wells of a 96-well plate. Then, 1 μL of ammonia was added to each well and mixed. Finally, 1 μL of 4-aminoantipyrine solution (2%) and 1 μL of potassium ferricyanide (8%) were added to the wells and mixed. The 96-well plate was incubated in the dark for 10 min. The absorbance at 490 nm of each well was recorded. The "removal efficiency" is defined as the percentage of phenol removed from solution under a given set of conditions.

The detailed H_2O_2 concentration was estimated by ammonium molybdate method according to the absorbance at 405 nm directly. At different reaction times, the solution was diluted and get 200 μL the diluted solution. Then, the 200 μL freshly prepared ammonium molybdate solution (32.4 mM) was added and the solution was analyzed by UV-Vis light spectrum at 405 nm.

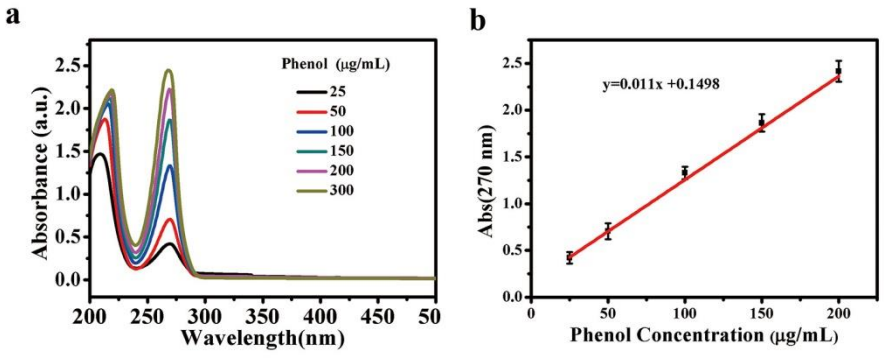

Fig. S18. a) The UV-Vis spectra of phenol solution along with different concentration. b) The corresponding concentration calibration curve. Data were presented as mean ± s.d. (n = 3)

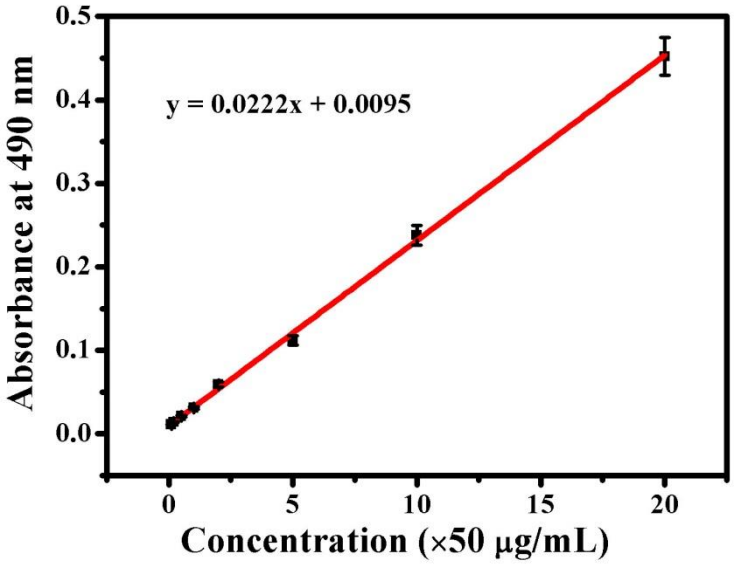

Fig. S19. The calibration curve of different phenol concentrations in reaction analyzed by 4-AAP method at 490 nm.

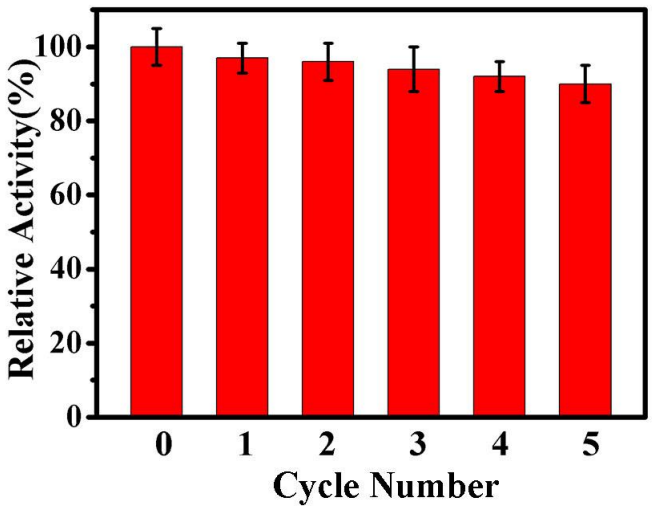

Fig. S20. The relative activity of recycled DMSN@AuPtCo. The results indicated that the DMSN@AuPtCo contained high catalytic activity at least in 5 cycles. Data were presented as mean ± s.d. (n = 3)

Inductively coupled plasma mass spectrometry (ICP-MS) instrumentation for quantification of DMSN@AuPtCo

ICP-MS measurements were performed on a TheroScientific Xseries Ⅱ inductively coupled plasma mass spectrometer. The amount of ^{197}Au, ^{195}Pt and ^{59}Co were tracked by ICP-MS.

We performed the ICP-MS experiment of the water substrate. We used our nanozyme to do the waste water treatment for 1 to 3 days. After that, we removed the nanozyme and got the water substrate. According to the results of ICP-MS, our nanozyme did not show obviously metal leaching in the reaction.

Table S4. Au Pt Co in supernatant of waste water treatment process calculated by ICP-MS

	Au (ppb)	**Pt (ppb)**	**Co (ppb)**
Day 0	1.036	2.741	0.951
Day 1	1.439	3.232	1.064
Day 2	1.063	3.463	1.346
Day 3	1.515	3.053	1.301

We performed the ICP-MS experiment of our nanozyme after different cycles. We used our nanozyme to do the waste water treatment for 5 cycles. After that, the nanozyme was collected and treated by fresh aqua regia. According to the results of ICP-MS, our nanozyme showed well stability at least in 5 cycles. The percentage of loss of the metals was less than 5 %, (3.9 % for Au, 3.7 % for Pt, 4.8 % for Co).

Table S5. Au Pt Co amount of DMSN@AuPtCo with different cycles calculated by ICP-MS

	Au (ppb)	**Pt (ppb)**	**Co (ppb)**
Cycle 0	546.750	511.502	140.688
Cycle 1	531.663	510.111	139.256
Cycle 2	526.610	507.743	138.768
Cycle 3	529.254	479.520	135.208
Cycle 4	532.281	530.496	138.208
Cycle 5	525.520	492.998	133.512

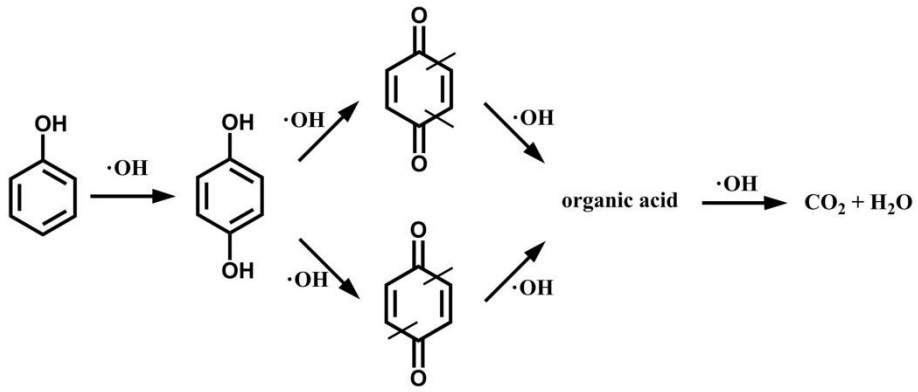

Fig. S21. Possible pathway for phenol degradation.

Cell culture:

Human cervical cancer cells (HeLa) or leukemia cells of mouse mononuclear macrophage cells (RAW 264.7) 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. Cell numbers in the solution were estimated by using a hemacytometer.

The biocompatibility of the treated wastewater:

Briefly, the HeLa cells or RAW 264.7 cells were plated in DMEM, supplemented with 10% (v/v) heat-inactivated fetal bovine serum in a 96-well plate at a density of 10000 cells per well and grown for 24 h at 37 °C and 5% CO_2. Thereafter, the wastewater after treated with the nanozyme DMSN@AuPtCo mixed with fresh DMEM with different ratios (0, 10, 25, 50%) were added and incubated with the cells for 24 h (the water treated by HRP enzyme was used as a control). After incubation, MTT was added into the wells and further incubated for an additional 4 h. Subsequently, the supernatant was discarded, followed by the addition of 100 mL DMSO into each well and incubation in the shaking incubator with gentle shakes. Then the optical density (OD) was read at a wavelength of 490 nm.

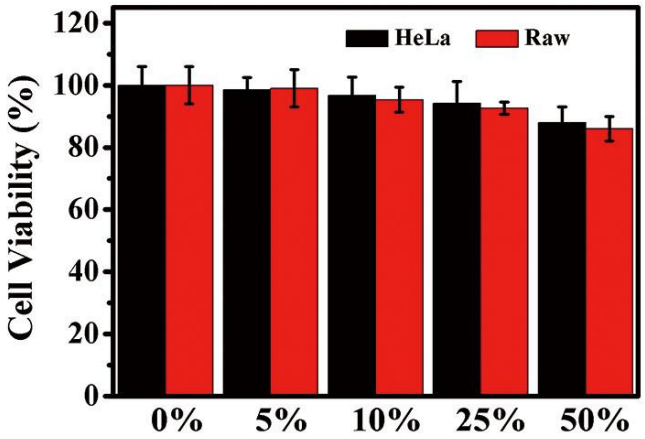

Fig. S22. The biocompatibility of the treated wastewater mixed with different concentrations of DMEM on HeLa cells and RAW 264.7 cells tested by MTT assay.

Total organic carbon of the products:

To analyze the TOC changes of the products, the phenol sewage was treated by our nanozyme. The TOC value of the withdrawn products was determined by a Total organic carbon analyzer (TOC-L CPH, Shimadzu Corporation, Kyoto, Japan).

Table S6. TOC abatement efficiency of waste water treatment process by nanozyme

	TOC (mg/L)	*TOC(mg/L)	Remove efficiency
No.1	363.5	47.61	86.9%
No.2	370.4	52.32	85.8%
No.3	377.1	55.64	85.2%

*TOC means the TOC value of the group of the products treated by nanozyme.

From the results, the TOC remove efficiency is above 85% in our system. Besides, The sewage purified by our nanozyme showed good security in our cellular and mice experiments.

The biocompatibility of the treated wastewater toward mice

In our experiments, we used the Kunming mouse as the model to investigate the effect of the treated water on animals. We fed the mice with the treated sewage instead of normal drinking water for five weeks. the weights of the mice were recorded respectively at 2:00 pm every day.

For histology, major organs (heart, liver, spleen, lung, kidney, stomach and intestine) were harvested after a period of time. The collected and organs were fixed in 10 % paraformaldehyde, embedded in paraffin, sectioned into ~4 μm, and stained with H&E.

Besides, in order to observe the acute effects, another group of experiments was carried out. In this group, the treated water was given to the mice via gavage directly. We fed the mice with 1 mL treated sewage by gavage every day for one week. The weights of the mice were also recorded respectively at 2:00 pm every day.

For histology, the four organs (lung, kidney, stomach and intestine) were harvested after 7 days. The collected and organs were fixed in 10 % paraformaldehyde, embedded in paraffin, sectioned into ~4 μm, and stained with H&E.

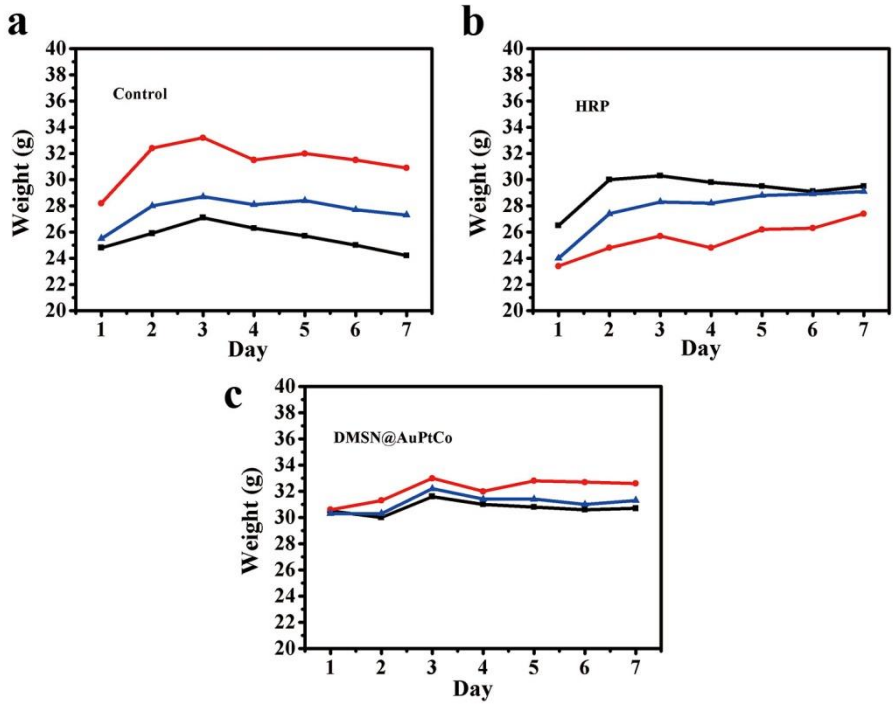

Fig. S23. Time-dependent body-weight curves of mice after different treatments for 7 days through gavage. a) Control. b) HRP. c) DMSN@AuPtCo. Data were presented as mean ± s.d. (n = 3)

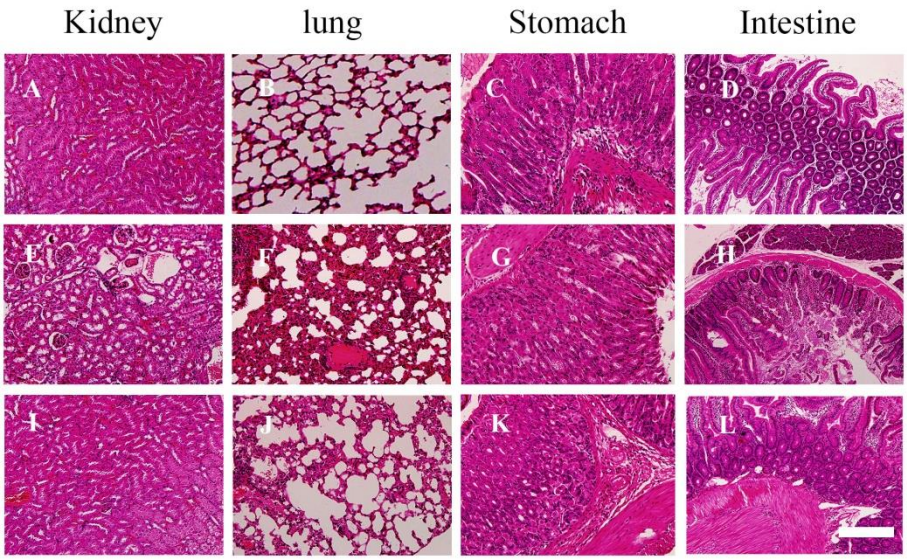

Fig. S24. Histopathological examinations via H&E staining of major organs (kidney, lung, stomach and intestine) after 7 days of treatment with various treated water by gavage, Control (A-D), HRP (E-H), DMSN@AuPtCo (I-L). Scale bars are 100 μm.