Electronic Supplementary Information

## Intrinsic peroxidase-like activity and catalase-like activity of Co<sub>3</sub>O<sub>4</sub> nanoparticles

Jianshuai Mu<sup>a</sup>, Yan Wang<sup>\*a</sup>, Min Zhao<sup>b</sup> and Li Zhang<sup>a</sup>

<sup>*a</sup></sup>Academy of Fundamental and Interdisciplinary Sciences, Harbin Institute of Technology, Harbin 150001, China. Email: <u>wangy\_msn@hotmail.com</u>. <sup><i>b*</sup>College of Life Science, Northeast Forestry University, Harbin 150040, China</sup>

## **Experimental details**

**Materials:** All chemicals used in this work were of analytical grade and used as received without further purification. 3,3',5,5'-tetramethylbenzidine (TMB), horseradish peroxidase (EC1.11.1.17, 250-330 U/mg using pyrogallol, type VI-A) and glucose oxidase (GOx, from Aspergillus niger,  $\geq$ 100 U/mg) were purchased from Sigma-Aldrich (St. Louis, American). H<sub>2</sub>O<sub>2</sub>, Co(COOH)<sub>2</sub>·4H<sub>2</sub>O, 25% ammonia solution, ethanol and other regents were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Glucose, fructose, lactose and maltose were from Beijing Chemical Reagent Company (Beijing, China). Fe<sub>3</sub>O<sub>4</sub> nanoparticles (spherical, 20 nm) were purchased from Aladdin Regent Company (Shanghai, China).

**Preparation of Co<sub>3</sub>O<sub>4</sub> nanoparticles (NPs):** The Co<sub>3</sub>O<sub>4</sub> NPs were prepared according to the method reported by Dong *et al.*<sup>1</sup> Briefly, 0.50 g of Co(CH<sub>3</sub>COO)<sub>2</sub>·4H<sub>2</sub>O was dissolved in solution of 10 ml water and 15 ml ethanol, and 2.5 ml of 25% ammonia was added under vigorous stirring. The mixture was stirred in air for about 10 min to form a homogeneous fuscous slurry. Then the suspension was transferred into a 48 ml autoclave, sealed and maintained at 150 for 3 h. After this, the autoclave was cooled to room temperature naturally. The resulting black solid products were separated by centrifugation and washed with water three times, dried at 60 under vacuum for 4 h, and collected for characterization.

The composition and phase of the product were identified by powder x-ray diffraction (XRD) on an D/max-rB x-ray diffractometer (Rigaku, Japan) using Cu K $\alpha$  radiation ( $\lambda$ =1.5418 Å). The morphology and size of the product were examined by transmission electron microscopy (TEM) with a Hitachi H-800 transmission electron microscope (Hitachi, Japan) at an accelerating voltage of 200 kV.

**Kinetic analysis:** Kinetic measurements were carried out in time-drive mode by monitoring the absorbance change at 652 nm on a Lambda 750 UV-Vis-NIR spectrophotometer (Perkin Elmer, American). Experiments were carried out using 10 µg mL<sup>-1</sup>Co<sub>3</sub>O<sub>4</sub> NPs in a reaction volume of 3 ml buffer solution (100 mM acetate buffer, pH 5.0) with 0.3 mM TMB as substrate, and H<sub>2</sub>O<sub>2</sub> concentration was 100 mM, unless otherwise stated. 30 µg Co<sub>3</sub>O<sub>4</sub> NPs contain ~6.2×10<sup>11</sup> nanoparticles.<sup>2</sup> The Michaelis–Menten constant was calculated using Lineweaver–Burk plots of the double reciprocal of the Michaelis-Menten equation,  $1/v=(K_m/V_{max})\cdot(1/[S])+1/V_{max}$ .

Electrochemistry experiments of the Co<sub>3</sub>O<sub>4</sub> NPs modified electrodes: The Co<sub>3</sub>O<sub>4</sub> NPs (30 mg) was dispersed into distilled water (10 mL) to obtain a suspension of Co<sub>3</sub>O<sub>4</sub> NPs (3 mg mL<sup>-1</sup>). The glassy carbon electrodes (GCE, 3.0 mm in diameter) were firstly polished with 0.3 and 0.05 mm alumina slurry followed by thoroughly rinsing with water. The colloidal solution (5  $\mu$ L) of the Co<sub>3</sub>O<sub>4</sub> NPs was then dropped on the pretreated GCE surface and allowed to dry under ambient conditions for 3 h to obtain Co<sub>3</sub>O<sub>4</sub> NPs modified electrodes. Cyclic voltammetric and amperometric measurements were performed on CHI 660D (Chenhua, China). A three-electrode system comprising a platinum wire as auxiliary, a saturated calomel electrochemical experiments. All experimental solutions were deoxygenated by bubbling highly pure nitrogen for at least 20 min and maintained under nitrogen atmosphere during the measurements. In amperometric experiments, the current–time data were recorded after a constant residual current had been established and successive additions of H<sub>2</sub>O<sub>2</sub> solution into the buffer were done. It was carried out by applying a potential of -0.6 V on a stirred cell at room temperature.

•**OH radical measurement:** 10 mM  $H_2O_2$ , 0.5 mM terephthalic acid and different concentrations of the  $Co_3O_4$  NPs were first incubated in 100 mM acetate buffer (pH 5.0) exposed to UV light at 365 nm for 20 min. After centrifugation, the solutions were used for fluorometric measurement by a LS55 fluorescence spectrometer (Perkin Elmer, American).

 $H_2O_2$  detection using the Co<sub>3</sub>O<sub>4</sub> NPs as peroxidase mimetics: A typical colorimetric analysis was realized as follows. Firstly, 48 µL of 25 mM TMB, 100 µL of 0.45 mg mL<sup>-1</sup> Co<sub>3</sub>O<sub>4</sub> NPs, and 200 µL of  $H_2O_2$  with different concentrations were added into 2652 µL of 100 mM acetate buffer (pH 5.0). Secondly, the mixed solution was used for adsorption spectroscopy measurement with the time-drive method.

**Glucose detection using Co<sub>3</sub>O<sub>4</sub> NPs and GOx:** Glucose detection was realized as follows: (1) 20  $\mu$ L of 5.0 mg mL<sup>-1</sup> GOx and 180  $\mu$ L of glucose with different concentrations in 10 mM acetate buffer solution (pH 5.5) were incubated at 37 °C for 30 min; (2) 20  $\mu$ L of 25 mM TMB, 100  $\mu$ L of 0.45 mg·mL<sup>-1</sup> Co<sub>3</sub>O<sub>4</sub> NPs, and 180  $\mu$ L of 200 mM acetate buffer (pH 5.0) were added into the above 200  $\mu$ L glucose reaction; (3) the mixed solution was incubated at room temperature for 30 min, and used for adsorption spectroscopy measurement.

In control experiments, 5 mM maltose, 5 mM lactose, and 5 mM fructose were used instead of glucose in a similar way.



Scheme S1 Schematic illustration of colorimetric determination of glucose by using glucose oxidase (GOx) and  $Co_3O_4$  NPs-catalyzed reactions.



Fig. S1 XRD patterns of Co<sub>3</sub>O<sub>4</sub> nanoparticles before (a) and after (b) the catalytic reaction



Fig. S2 TEM image of Co<sub>3</sub>O<sub>4</sub> nanoparticles



**Fig. S3** Dependency of the Co<sub>3</sub>O<sub>4</sub> NPs peroxidase-like activity on Co<sub>3</sub>O<sub>4</sub> NPs concentration (A), pH (B), temperature (C) and H<sub>2</sub>O<sub>2</sub> concentration (D). (A) The Co<sub>3</sub>O<sub>4</sub> NPs oxidation activity was determined in 100 mM acetate buffer (pH 5.0) using the Co<sub>3</sub>O<sub>4</sub> NPs concentration ranging from 0 up to 30  $\mu$ g mL<sup>-1</sup>, TMB (0.5 mM) and H<sub>2</sub>O<sub>2</sub> (6.7 mM) were added to initiate the reaction. (B)-(D) Experiments were carried out using 10  $\mu$ g mL<sup>-1</sup> Co<sub>3</sub>O<sub>4</sub> NPs in 100 mM acetate buffer (pH 5.0) with 0.5 mM TMB as substrate. The H<sub>2</sub>O<sub>2</sub> concentration was 50 mM at 25 °C unless otherwise stated. The maximum point in each curve was set as 100 %.



Fig. S4 Demonstration that the peroxidase-like activity of  $Co_3O_4$  NPs does not result from cobalt ion leaching.  $Co_3O_4$  NPs were incubated in the pH 5.0 reaction buffer for 30 min, and then removed by centrifugation. The activity of the leaching solution was then compared to that of the intact  $Co_3O_4$  NPs. Reaction conditions: 0.25 mM TMB, 10 µg mL<sup>-1</sup>  $Co_3O_4$  NPs, 50 mM H<sub>2</sub>O<sub>2</sub> in a 100 mM NaAc buffer (pH 5.0) at 25 .



**Fig. S5** Steady-state kinetic assay of  $Co_3O_4$  NPs. The velocity of the reaction was measured using 10  $\mu$ g mL<sup>-1</sup>Co<sub>3</sub>O<sub>4</sub> NPs in 100 mM acetate buffer (pH 5.0) at 25 °C. (A) The concentration of TMB was 0.3 mM and the H<sub>2</sub>O<sub>2</sub> concentration was varied. (B) The concentration of H<sub>2</sub>O<sub>2</sub> was 100 mM and the TMB concentration was varied. (C), (D) Double-reciprocal plots of activity of Co<sub>3</sub>O<sub>4</sub> NPs at a fixed concentration of one substrate versus varying concentration of the second substrate for H<sub>2</sub>O<sub>2</sub> and TMB.

**Table S1** Comparison of the kinetic parameters between  $Co_3O_4$  and HRP. [*E*] is the  $Co_3O_4$  NPs (or HRP) concentration,  $K_m$  is the Michaelis-Menten constant,  $V_{max}$  is the maximal reaction rate and  $K_{cat}$  is the catalytic constant, where  $K_{cat} = V_{max} / [E]$ .

Catalyst	[ <i>E</i> ]/M	Substrate	$K_{\rm m}$ / mM	$V_{\rm max}$ / M s <sup>-1</sup>	$K_{\rm cat}/{\rm s}^{-1}$	Reference
Co <sub>3</sub> O <sub>4</sub>	3.43×10 <sup>-10</sup>	TMB	0.037	6.27×10 <sup>-8</sup>	$1.83 \times 10^{2}$	the present work
$Co_3O_4$	3.43×10 <sup>-10</sup>	$H_2O_2$	140.07	1.21×10 <sup>-7</sup>	3.53×10 <sup>2</sup>	the present work
HRP	2.5×10 <sup>-11</sup>	TMB	0.434	1.00×10 <sup>-7</sup>	4.00×10 <sup>3</sup>	2
HRP	2.5×10 <sup>-11</sup>	$H_2O_2$	3.70	8.71×10 <sup>-8</sup>	3.48×10 <sup>3</sup>	2



**Fig. S6** Comparison of the stability of  $Co_3O_4$  NPs and HRP. (A)  $Co_3O_4$  NPs and HRP were first incubated at a range of values of pH from 2 to 12 for 2 h and the peroxidase activity was measured under standard conditions. (B)  $Co_3O_4$  NPs and HRP were first incubated at a range of temperatures between 4 and 70 for 2 h and then the peroxidase activity was measured under standard conditions.



Fig. S7 The linear calibration plot for  $H_2O_2$  (A) and glucose (B) determination.



**Fig. S8** The selectivity of glucose determination with 5 mM fructose, 5 mM maltose, 5 mM lactose and 1 mM glucose. Insert: the color change of different solutions (form left to right: blank, fructose, maltose, lactose and glucose).

## References

- 1 Y. Dong, K. He, L. Yin and A. Zhang, Nanotechnology, 2007, 18, 435602.
- 2 L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang, S. Perrett and X. Yan, *Nat. Nanotechnol.*, 2007, **2**, 577.