## *In vitro* measurement of superoxide dismutase-like nanozyme activity:

## A comparative study

Yufeng Liu<sup>1</sup>, Yihong Zhang<sup>1</sup>, Quanyi Liu<sup>2</sup>, Quan Wang<sup>1</sup>, Anqi Lin<sup>1</sup>, Jie Luo<sup>5</sup>, Yan Du<sup>2</sup>, Ying-Wu Lin<sup>5</sup>, Hui Wei<sup>\*,1, 3, 4</sup>

<sup>1</sup>Department of Biomedical Engineering, College of Engineering and Applied Sciences, Nanjing University, Nanjing, Jiangsu 210023, China.

<sup>2</sup>State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Science, Changchun, Jilin 130022, China.

<sup>3</sup>Nanjing National Laboratory of Microstructures, Jiangsu Key Laboratory of Artificial Functional Materials, Nanjing University, Nanjing, Jiangsu 210023, China.

<sup>4</sup>State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Chemistry and Biomedicine Innovation Center (ChemBIC), Nanjing University, Nanjing, Jiangsu 210023, China.

<sup>5</sup>Laboratory of Protein Structure and Function, School of Chemistry and Chemical Engineering, University of South China, Hengyang, Hunan 421001, China.

Email: weihui@nju.edu.cn (H.W.)



**Figure S1.** PXRD patterns of (A)  $CeO_2$ , (B)  $Mn_3O_4$ , (C) PB, (D) PCN222-Mn, and (E) Pt NPs. (The red lines at the bottom mark the reference patterns of the JCPDS database.)



**Figure S2.** UV-vis absorption spectra of (A)  $CeO_2$ , (B)  $Mn_3O_4$ , (C) PB, (D) PCN222-Mn, and (E) Pt NPs.



**Figure S3.** Fluorescent spectra of (A) CeO<sub>2</sub>, (B) Mn<sub>3</sub>O<sub>4</sub>, (C) PB, (D) PCN222-Mn, and (E) Pt NPs.  $\lambda_{ex} = 470$  nm.



**Figure S4.** Absorption spectra of the mixture of X, XO, CAT, and cytochrome *c* in the absence and presence of different concentrations of (A) CeO<sub>2</sub>, (B) Mn<sub>3</sub>O<sub>4</sub>, (C) PB, (D) PCN222-Mn, and (E) Pt NPs. (F) Corresponding dependence between the elimination efficiency of  $\cdot$ O<sub>2</sub><sup>-</sup> and concentrations of nanozymes. The data are shown as means ± SD (n = 3).



Figure S5. Natural SOD activity assays with different methods. (A) Fluorescent spectra of the mixture of X, XO, and HE in the absence and presence of different concentrations of natural SODs. (B) Absorption spectra of the mixture of X, XO, and NBT in the absence and presence of different concentrations of natural SODs. (C) Absorption spectra of the mixture of X, XO, and INT in the absence and presence of different concentrations of natural SODs. (D) Absorption spectra of the mixture of X, XO, and INT in the absence and presence of different concentrations of natural SODs. (D) Absorption spectra of the mixture of X, XO, and WST-8 in the absence and presence of different concentrations of natural SODs. (E) Absorption spectra of the mixture of X, XO, CAT, and cytochrome *c* in the absence and presence of different concentrations of natural SODs. (F) Dependence between the elimination efficiency of  $O_2^-$  and concentrations of natural SODs, data analyzing from panels A-E.



**Figure S6.** (A) Fluorescent spectra of the mixture of HE and riboflavin under illumination for 5 min in the absence and presence of different concentrations of natural SODs. (B) Absorption spectra of the mixture of NBT and riboflavin under illumination for 5 min in the absence and presence of different concentrations of natural SODs. (C) Dependence between the elimination efficiency of  $O_2^-$  and concentrations of natural SODs, data analyzing from panels A and B.



Figure S7. EPR spectra of the samples of X, XO, DMPO, and DTPA in different mixing time.

Probes	Methods of <sup>•</sup> O₂ <sup>−</sup> generation	Methods	Pros	Cons
HE	X+XO	Fluorimetry	High sensitivity	Moderate specificity; Slow
HE	Irradiation of riboflavin	Fluorimetry	Fast	Low specificity; Low sensitivity
NBT	X+XO	Colorimetry	High specificity	Moderate sensitivity
NBT	Irradiation of riboflavin	Colorimetry	High specificity; Fast; High sensitivity	Moderate specificity
INT	X+XO	Colorimetry	High specificity	Moderate specificity
Cyt. c	X+XO	Colorimetry	/	Low specificity; Low sensitivity
WST-8	X+XO	Colorimetry	High specificity; High sensitivity	Expensive probe; Slow
DMPO	X+XO	EPR	High specificity; High sensitivity; Very fast	Expensive probe; Expensive detection instrument

Table S1. The pros and cons of detection methods in this work.

## Note:

Cyt. *c*, Cytochrome *c* 

EPR, Electron paramagnetic resonance