Electronic Supplementary Information

Phage capsid protein-directed MnO_2 nanosheets with peroxidase-like activity for spectrometric biosensing and evaluation of antioxidant behaviour

Lei Han,[ab] Pei. Liu,[bc] Haijiao Zhang,[a] Feng Li*[a] and Aihua Liu*[cde]

[a] *College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University, 700 Changcheng Road, Qingdao, Shandong, China.*

[b] *University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing, China.*

[c] *Institute for Biosensing, and College of Chemistry and Chemical Engineering, Qingdao University, 308 Ningxia Road, Qingdao, China.*

[d] *College of Medicine, Qingdao University, 38 Dengzhou Road, Qingdao, China.*

[e] *Joint Key Laboratory for Biosensors of Shandong Province, Qingdao University, 308 Ningxia Road, Qingdao, China.*

* Corresponding authors.

E-mail addresses: liuah@qdu.edu.cn (A. Liu); lifeng@qust.edu.cn (F. Li).

1. MATERIALS AND METHODS

1.1. Chemicals and Materials. Manganese acetate ($MnAc_2$), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2, 30%), glucose, lactose, maltose, sucrose, fructose, xylose, galactose, ascorbic acid (AA) and gallic acid (GA) were obtained from Sinopharm Chemical Reagent Co. Ltd. (China). Glucose oxidase (GOx) and 3,3', 5,5'-tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich (USA). Ultrapure water (18 MΩ cm) was prepared by using a Millipore Milli-Q system and used throughout. All other reagents were of the analytical grade.

1.2. Instrumentation. Transmission electron microscopic (TEM) images were obtained using an H-7650 transmission electron microscope (Hitachi, Japan). Samples for TEM analysis were prepared by evaporating a drop of aqueous product on a copper grid. The ultraviolet visible (UV–Vis) absorption spectra were performed with UV-vis spectrophotometer TU-1901 (Persee General Instrument Co. Ltd., Beijing, China).

1.3. Isolation and purification of M13 major capsid proteins pVIII

The phage solution was obtained according to the previous report.[1] M13 major capsid proteins pVIII were extracted by saturated phenol according to the previous reports with minor modification.[2, 3] Briefly, phenol-saturated Tris–HCl solution (pH 8.0) was added into equal volume of phage solution. The mixed solution was vigorously shaked for 10 min and then centrifuged at 3000×*g* for 10 min. Subsequently, the aqueous supernatant containing phage DNA was removed and the phenol phase containing hydrophobic capsid proteins was re-extracted with Tris-HCl (10 mM, pH 8.0) thrice to completely remove the phage DNA. The phenol phase containing capsid proteins pVIII was diluted with twofold volume of methanol and dialyzed against a mixture of methanol and Tris-HCl buffer, Tris-HCl buffer and water in turn for each 12 h. Finally, the pure capsid proteins was freeze-dried into powder for subsequent experiments. Since 2700 copies of pVIII accounted for more than 99% of capsid proteins, the obtained proteins was regarded as major capsid proteins pVIII.

1.4. Synthesis of protein-MnO_2 NSs. MnO_2 NSs were synthesized by chemical precipitation at alkaline solution.[1] Briefly, 100 μg and 1 mg of capsid proteins pVIII

were added into 10 mL of $MnAc_2$ aqueous solution (1 mM). After stirring for 1 h at room temperature, diluted NaOH solution was added into the mixture solution to adjust the pH to about 10. After stirring for 6 h, the MnO_2 NSs were filtered by 0.02 μm membrane to remove unreacted $MnAc_2$ and NaOH.

1.5. Analysis of peroxidase-like activity. As for the standard assay of peroxidase-like activity, H_2O_2 and TMB were selected as two substrates, in which H_2O_2 could oxidize TMB to a colored oxidative product (TMB^{*+}) in the catalysis of the MnO_2 (H_2O_2 + TMB → H_2O + TMB^{*+}). In a representative test, the reaction system (200 μL) included MnO_2 NSs (25 μg/ml), TMB (0.5 mM) and H_2O_2 (1 mM) in acetate buffer (100 mM, pH 3.5) and the absorbance of TMB^{*+} at 652 nm was recorded by using the spectrophotometer.

1.6. Colorimetric detection of glucose. For standard glucose assay (Scheme 1), the reaction solutions containing a fixed concentration of GOx (500 μg/ml) and various concentrations of glucose (1 − 12 mM) were incubated for 30 min, and then added into the detection systems containing TMB (0.5 mM) and MnO_2 NSs (25 μg/ml) for the spectrophotometric measurement at 652 nm. Further, the selectivity of the proposed method was researched by using 5 mM lactose, maltose, sucrose, fructose, xylose and galactose to instead glucose.

1.7. Evaluation of antioxidant behavior. The capabilities of antioxidants (AA and GA) were evaluated by continuously monitoring the reaction solutions containing MnO_2 (25 μg/ml), TMB (0.5 mM), H_2O_2 (1 mM) and varying concentrations of antioxidants in acetate buffer (100 mM, pH 3.5) over time. Further, to study the electron transfer patterns between antioxidants and the MnO_2 NSs, the reactions between antioxidants and the MnO_2 NSs were continuously monitored in the absence and presence of H_2O_2 over time at corresponding maximum absorption wavelength of antioxidants.

2. Figure

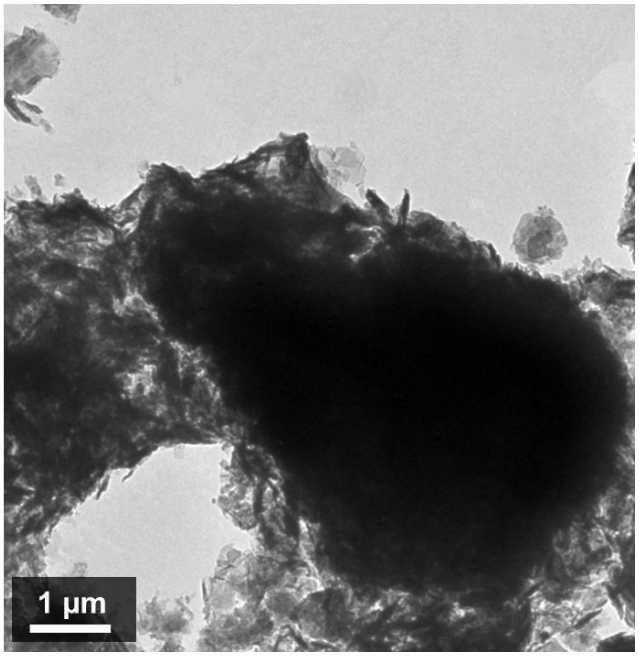

Fig. S1 TEM images of non-templated MnO_2 NSs.

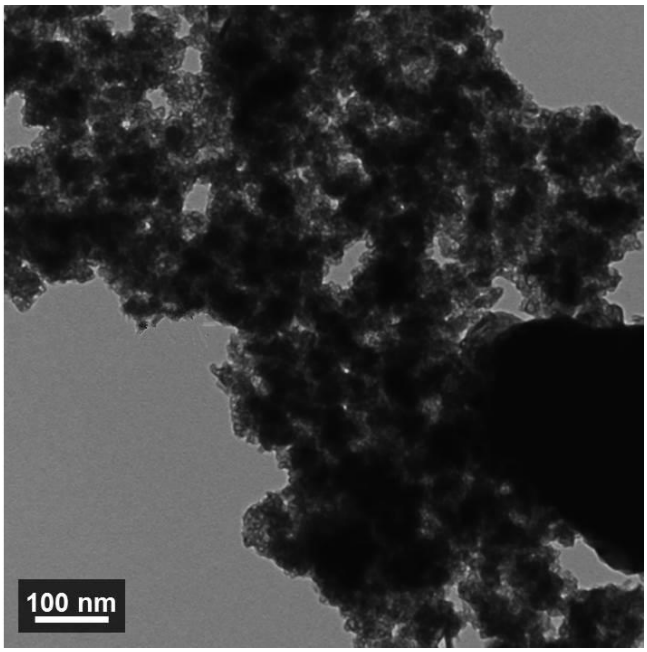

Fig. S2 TEM image of BSA-templated MnO_2 NSs at the same dosage (100 μg) of BSA with pVIII-templated MnO_2 NPs.

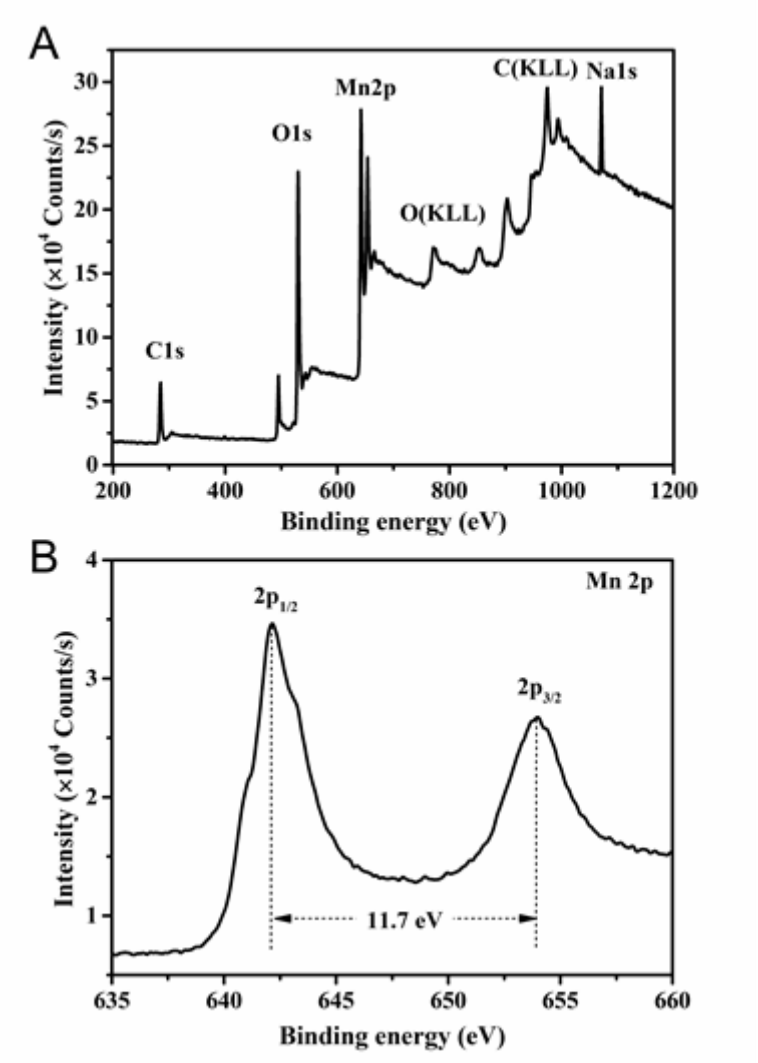

Fig. S3 (A) XPS pattern of protein-MnO_2 NSs and (B) high-resolution XPS pattern of Mn 2p.

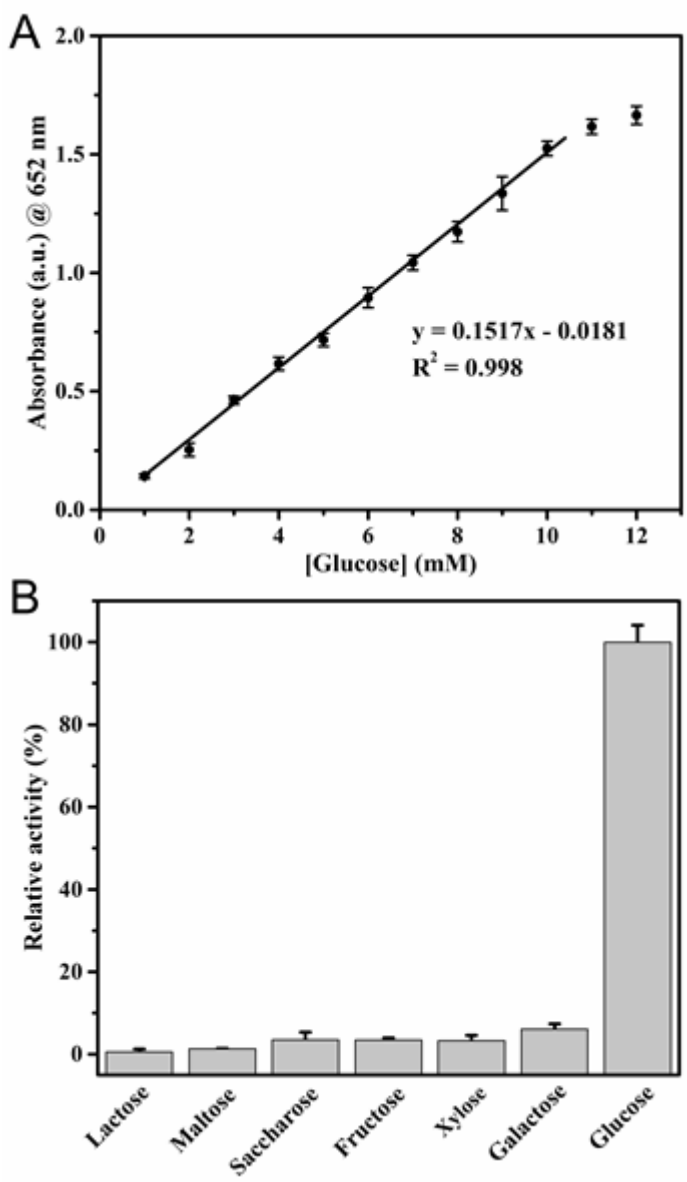

Fig. S4 (A) Working curve for glucose detection. (B) Selectivity of the proposed glucose detection method.

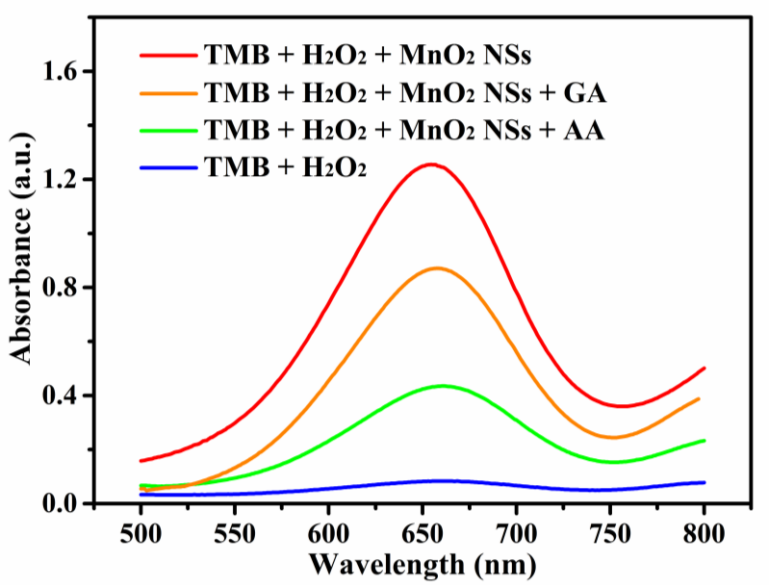

Fig. S5 The absorption curves of typical catalytic oxidation of TMB by MnO_2 NSs with the same concentration (10 μM) of antioxidants (AA and GA).

3. References

1. L. Han, C. Shao, B. Liang and A. Liu, *ACS Appl. Mater. Inter.*, 2016, **8**, 13768-13776.
2. P. Liu, L. Han, F. Wang, V. A. Petrenko and A. Liu, *Biosens. Bioelectron.*, 2016, **82**, 195-203.
3. F. Wang, P. Liu, L. Sun, C. Li, V. A. Petrenko and A. Liu, *Sci. Rep,*, 2014, **4**, 6808.