

## Supporting Information

### **Colorimetric immunoassay of carcinoembryonic antigen based on glucose oxidase/MnO<sub>2</sub> nanosheet cascade reaction with self-supplying oxygen**

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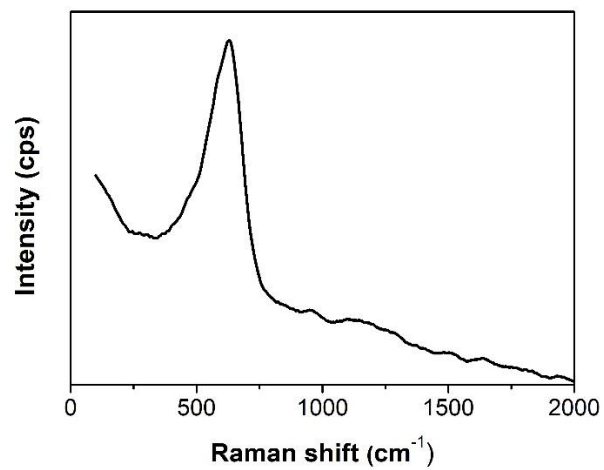


Figure S1. Raman spectrum of MnO<sub>2</sub> nanosheet.

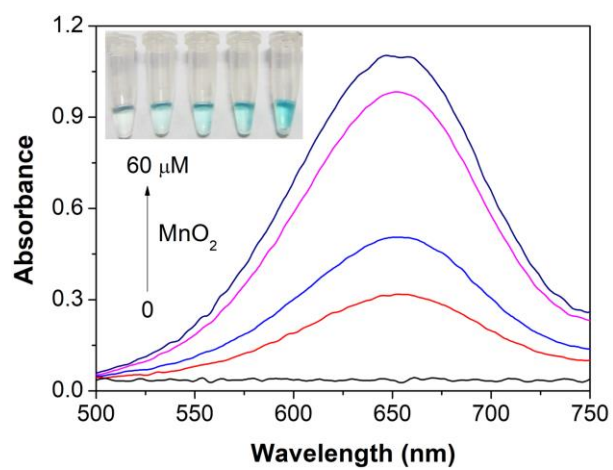


Figure S2. Absorption spectra of TMB in the presence of MnO<sub>2</sub> nanosheet with different concentration.

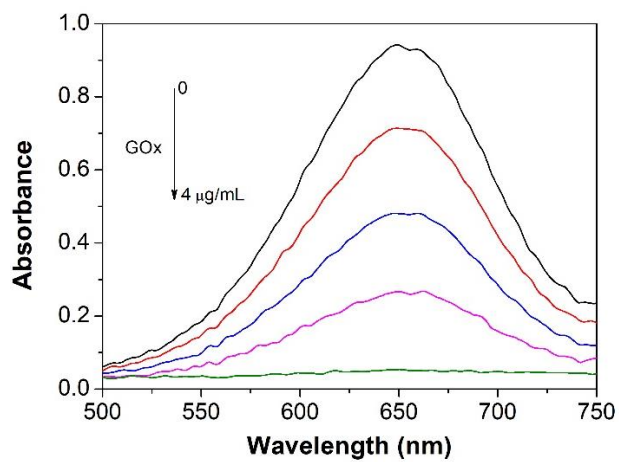


Figure S3. Absorption spectra of TMB in the presence of the mixture of MnO<sub>2</sub> nanosheet and GOx with different concentration.

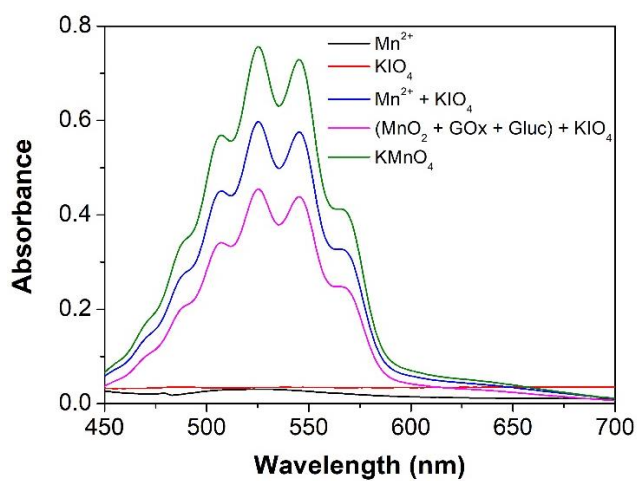


Figure S4. Absorption spectra of Mn<sup>2+</sup>, KIO<sub>4</sub>, Mn<sup>2+</sup> + KIO<sub>4</sub>, MnO<sub>2</sub> nanosheet + GOx + glucose + KIO<sub>4</sub>, and KMnO<sub>4</sub>.

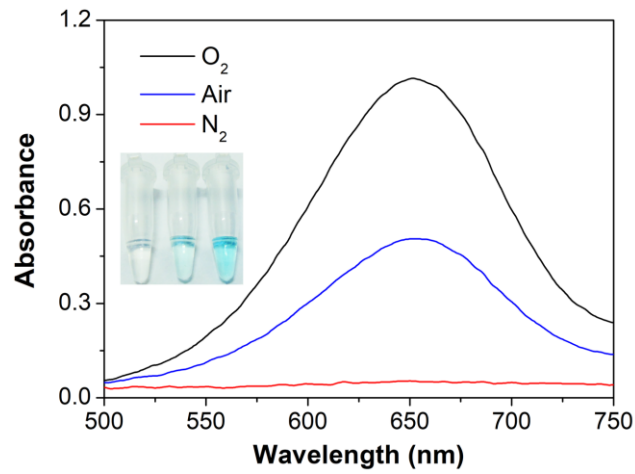


Figure S5. Absorption spectra of TMB produced from the cascade reaction of GOx and HRP at different conditions.

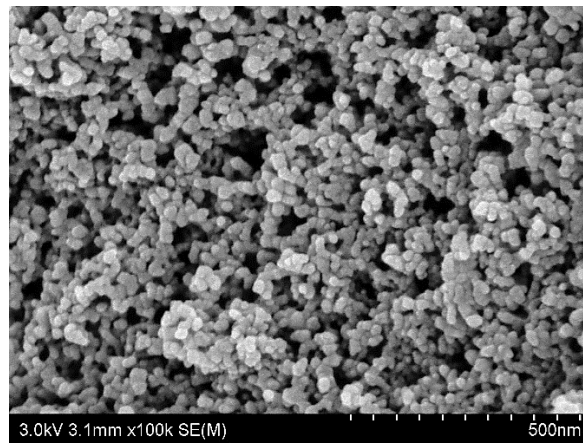


Figure S6. SEM image of ZnCPs.

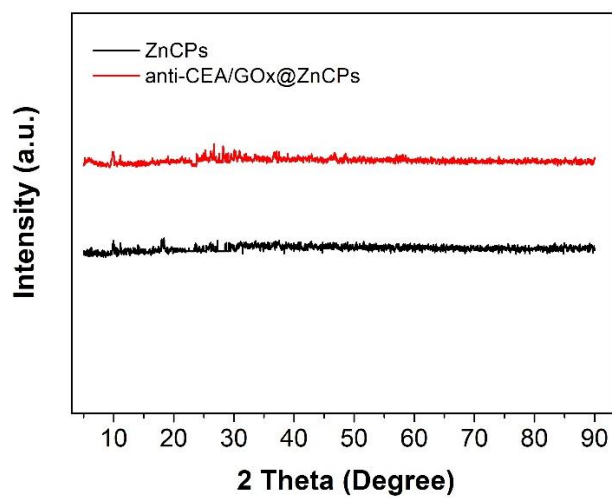


Figure S7. Powder XRD patterns of ZnCPs and anti-CEA/GOx@ZnCPs.

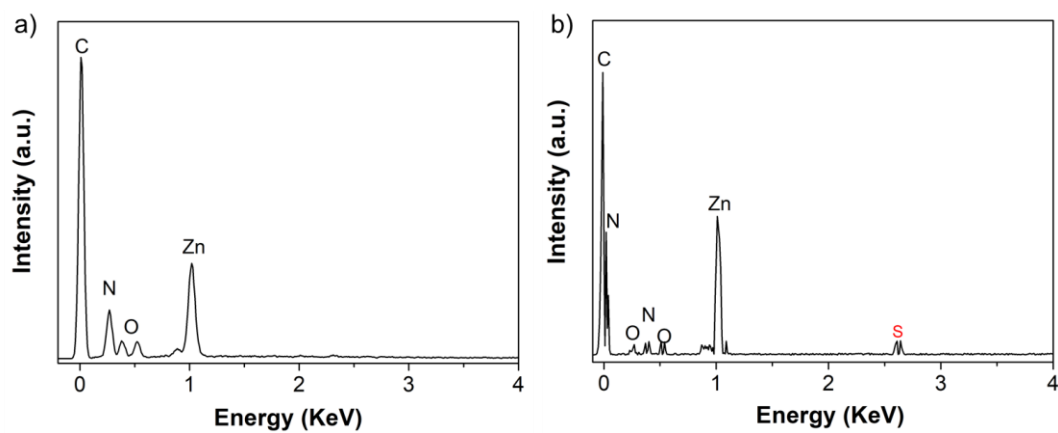


Figure S8. EDS spectra of ZnCPs (a) and anti-CEA/GOx@ZnCPs (b).

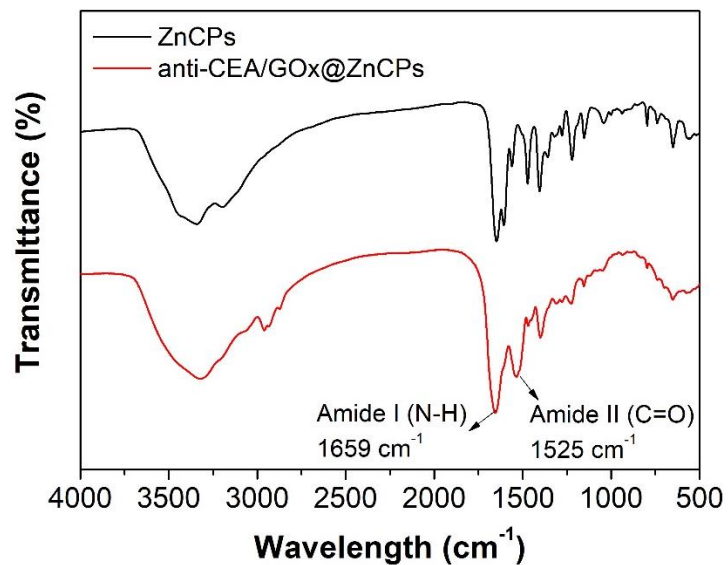


Figure S9. FTIR spectra of ZnCPs and anti-CEA/GOx@ZnCPs.

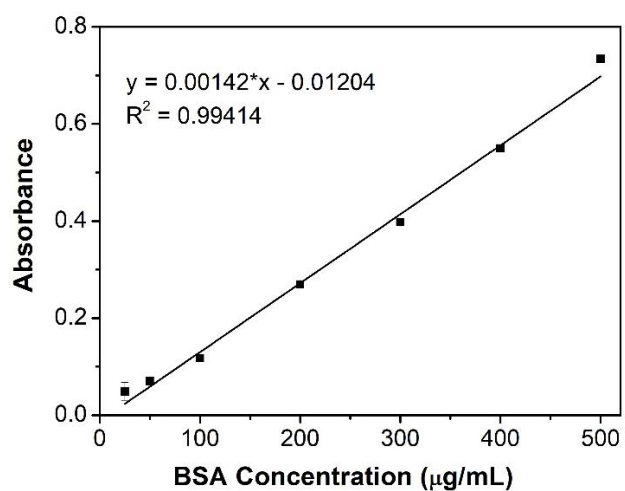


Figure S10. Calibration curve of the absorbance of BCA reagent at 560 nm as a function of BSA concentrations.

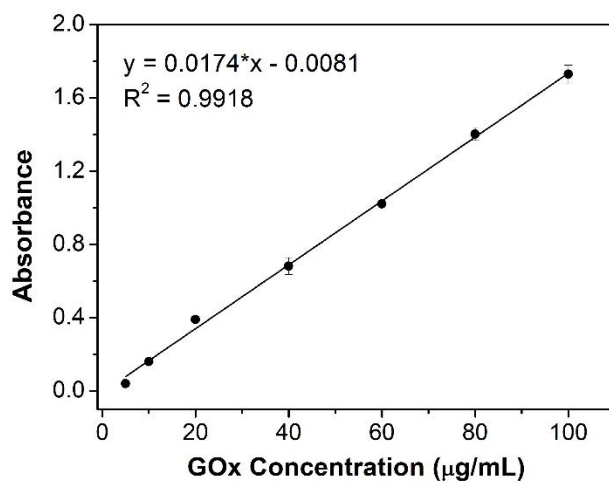


Figure S11. Calibration curve of the absorbance of oxTMB at 615 nm as a function of GOx concentrations.

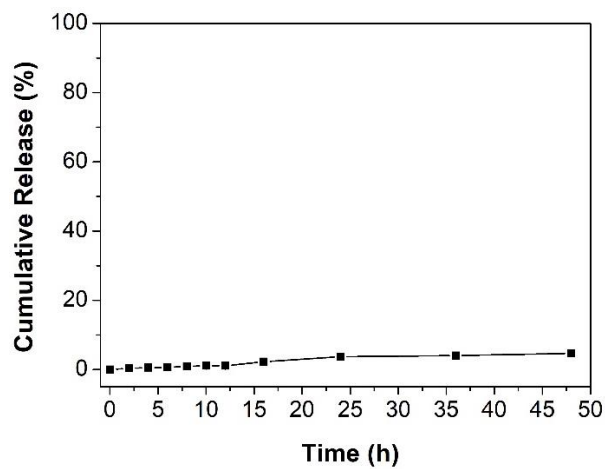


Figure S12. Cumulative release rate of proteins from anti-CEA/GOx@ZnCPs in HEPES buffer (10 mM, pH 7.4).

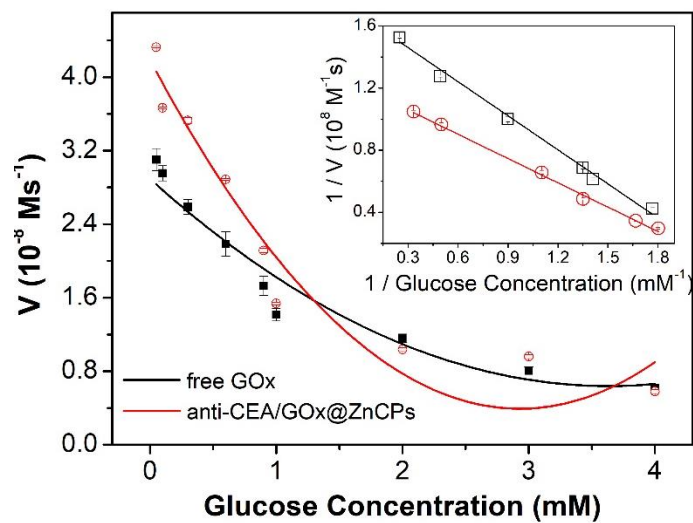


Figure S13. Steady-state kinetic assay of free GOx and anti-CEA/GOx@ZnCPs and their corresponding double-reciprocal plots (inset).

Table S1. Kinetic data of free GOx and anti-CEA/GOx@ZnCPs

Catalyst	$K_m$ (mM)	$V_{max}$ ( $10^{-8} \text{ M s}^{-1}$ )
free GOx	0.44	0.53
anti-CEA/GOx@ZnCPs	0.43	0.82



Table S2. Comparison of various immunoassays for CEA detection.

Antibody labels	Readout signal	Linear range (ng/mL)	LOD (pg/mL)	Refs.
Gold nanoparticle	Colorimetric	0.05 - 50	48	1
HRP labeled AuNP	Colorimetric	6 - 781	12000	2
HRP	Electrochemical	0.5 - 167	100	3
AuNP	Chemiluminescence	5 - 20	100	4
Eu <sup>3+</sup>	Fluorescence	1 - 1000	500	5
anti-CEA/GOx@ZnCPs	Colorimetric	2 - 160	50	This work

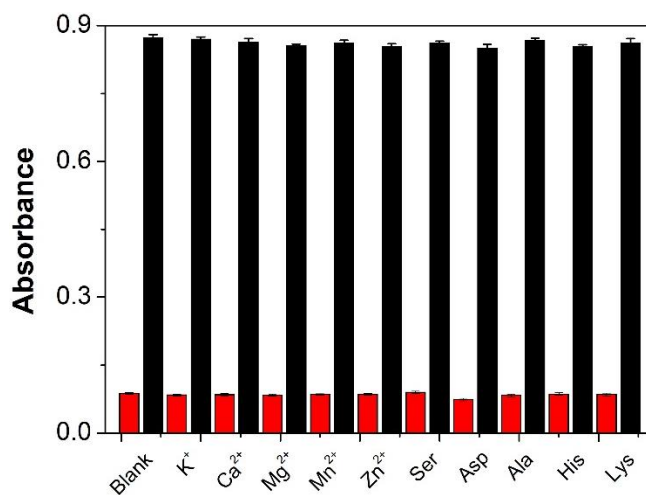


Figure 14. Effects of metal ions and biological species on the colorimetric immunoassay based on anti-CEA/GOx@ZnCPs as a detection antibody. Black bars represent the addition of single interfering material (100 μM). Red bars represent the addition of the mixture of CEA (150 pg/mL) and interfering materials (100 μM).

Table S3. Determination of CEA in serum samples

Added (ng/mL)	Detected (ng/mL)	Recovery (%)	RSD (n=3, %)	Kit assay (ng/mL)
5	5.0	100.0	2.6	4.9
30	30.8	102.7	2.8	30.1
120	120.5	100.4	0.8	121.3

## References

1. Liu, M.; Jia, C.; Jin, Q.; Lou, X.; Yao, S.; Xiang, J.; Zhao, J., Novel colorimetric enzyme immunoassay for the detection of carcinoembryonic antigen. *Talanta* **2010**, *81*, 1625-1629.
2. Liu, M.; Jia, C.; Huang, Y.; Lou, X.; Yao, S.; Jin, Q.; Zhao, J.; Xiang, J., Highly sensitive protein detection using enzyme-labeled gold nanoparticle probes. *Analyst* **2010**, *135*, 327-331.
3. Dai, Z.; Yan, F.; Yu, H.; Hu, X.; Ju, H., Novel amperometric immunosensor for rapid separation-free immunoassay of carcinoembryonic antigen. *J. Immunol. Methods* **2004**, *287*, 13-20.
4. Yang, X.; Guo, Y.; Wang, A., Luminol/antibody labeled gold nanoparticles for chemiluminescence immunoassay of carcinoembryonic antigen. *Anal. Chim. Acta* **2010**, *666*, 91-96.
5. Hou, J.-Y.; Liu, T.-C.; Lin, G.-F.; Li, Z.-X.; Zou, L.-P.; Li, M.; Wu, Y.-S., Development of an immunomagnetic bead-based time-resolved fluorescence immunoassay for rapid determination of levels of carcinoembryonic antigen in human serum. *Anal. Chim. Acta* **2012**, *734*, 93-98.