Supplementary Information

Ascorbic acid-loaded apoferritin assisted carbon dots-MnO_2 nanocomposites for selective and sensitive detection of trypsin

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Experimental Procedure

Preparation of APOAA

In particular, APO was dispersed in 2 mL of 10 mM PBS (pH 7.4) and the solution was adjusted to pH 2.0 with 0.1 M HCl solution under continuous stirring. The solution was further stirred for 30 min to make the APO completely disassociated. Then 100 μL of 1.5 M AA solution was added into APO solution dropwise with stirring to allow AA to diffuse around the subunits of the APO. After that, the pH of solution was adjusted to 8.5 with 0.1 M NaOH. The obtained mixture was stirred for another 3 h at room temperature to allow AA enters the APO core. Subsequently, to remove free AA out of APO, the mixture solution was dialyzed against 10 mM PBS (pH 7.8) for 48 h using MD10 dialysis bag with a molecular weight cutoff (MWCO) of 8000-14000. The obtained APOAA solution was stored in amber glass bottles at 4 °C for further use.

Preparation of MnO_2 nanosheets

$MnCl_2$ aqueous solution and tetramethylammonium hydroxide (TMAOH) containing 3 wt% of H_2O_2 were mixed quickly and then stirred for 12 h at room temperature. The dried aggregates were washed with methanol and water several times, and following dried at 45 °C. Then the product dispersing in water and following sonicated for 12 h to get the resulting suspension (brown colloid).

Figures and Tables

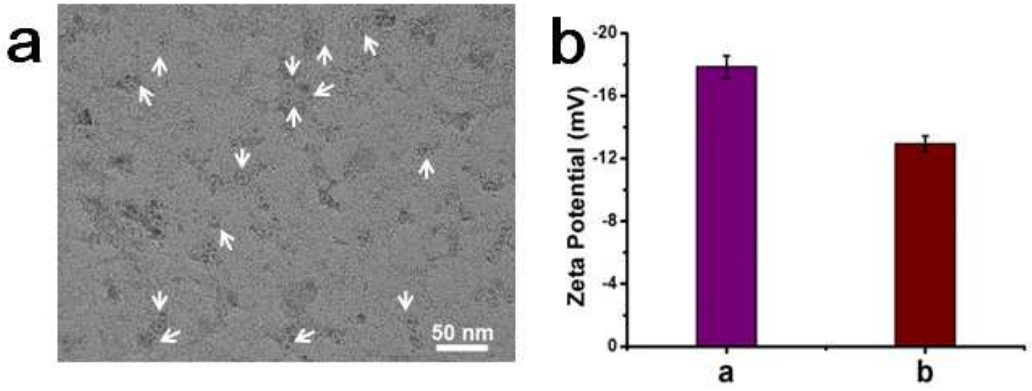

Figure S1. (a) TEM images of APOAA and (b) Zeta Potential of APO (column a) and APOAA (column b)

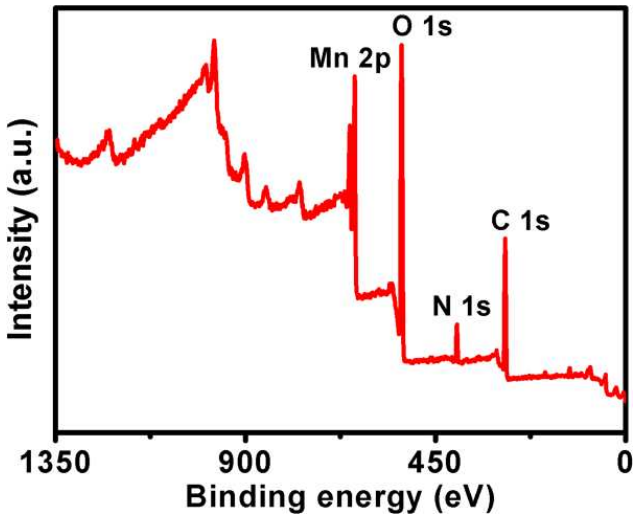

Figure S2. XPS survey spectrum of the CDs-MnO_2

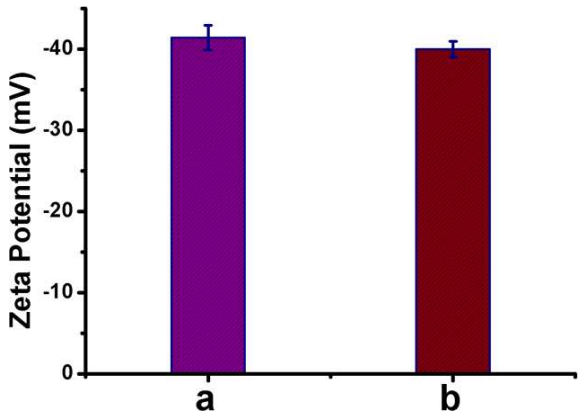

Figure S3. Zeta Potential of MnO_2 synthesized newly (column a) and stored for 6 months (column b)

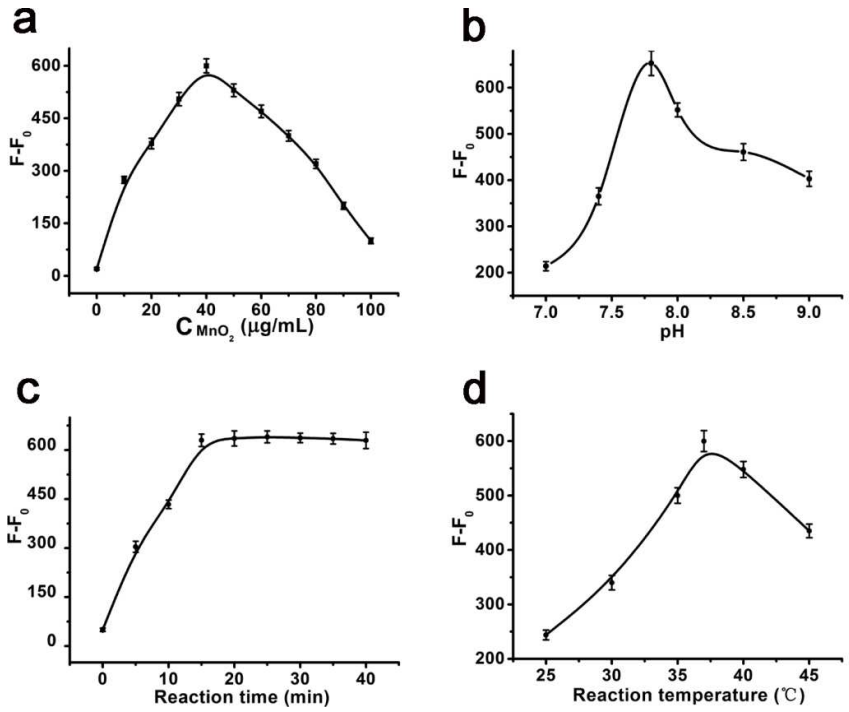

Figure S4. Dependence of the fluorescence recovery value of CMA on (a) MnO_2 nanosheets concentration (b) reaction pH (c) reaction time (d) reaction temperature (the concentration of trypsin is 300 ng/mL). The error bars were derived from the standard deviation of three measurements. Error bar = SD (n = 3). F_0 and F are the fluorescence intensities of CMA in the absence and presence of trypsin, respectively.

Table S1 Comparison of this method with the newly reported approaches for trypsin determination.

Materials	Methods	Linear range (ng/mL)	LOD (ng/mL)	Ref.
Cytochrome c	Chemiluminescence	0-100	0.7	[1]
CdTe QDs	Photoelectrochemial	30-450	2.7	[2]
Cytochrome c-Heme-TMB	Colorimetric	5-2000	4.5	[3]
Au nanoclusters	Colorimetric	9×10^2-1×10^6	600	[4]
Au nanoclusters	Fluorescence	1×10^4-1×10^8	2	[5]
GFP	Fluorescence	0-400	0.0282	[6]
CDs-MnO_2	Fluorescence	1-500	0.3411	This work

GFP: green fluorescent protein

Table S2 Recoveries results of the determination of trypsin in serum samples.

Sample	Added (ng/mL)	Found (ng/mL)	Recovery[a] (%)
Sample 1	0	0	-
Sample 2	50	48 ± 0.5	96
Sample 3	100	103±0.4	103
Sample 4	200	196±0.2	98
Sample 5	350	355±0.3	101.4
Sample 6	450	446±0.4	99.1

[a] Mean value of three independent measurements.

References

[1] Zhang, Q.; Li, W.; Chen, J.; Wang, F.; Wang, Y.; Chen, Y.; Yu, C. An ultrasensitive chemiluminescence turn-on assay for protease and inhibitor screening with a natural substrate. *Chem. Commun.* **2013**, *49*, 3137-3139.

[2] Chen, J.; Zhao, G. Nano-encapsulant of ascorbic acid-loaded apoferritin-assisted photoelectrochemical sensor for protease detection. *Talanta* **2017**, *168*, 62–66.

[3] Zhang, L.; Du, J. A sensitive and label-free trypsin colorimetric sensor with cytochrome c as a substrate. *Biosens. Bioelectron.* **2016***, 79*, 347–352.

[4] Wang, G.; Jin, L.; Dong, Y.; Wu, X.; Li, Z. Intrinsic enzyme mimicking activity of gold nanoclusters upon visible light triggering and its application for colorimetric trypsin detection. *Biosens. Bioelectron.* **2015**, *64*, 523–529.

[5] Hu, L.; Han, S.; Parveen, S.; Yuan, Y.; Zhang, L.; Xu, G. Highly sensitive fluorescent detection of trypsin based on BSA-stabilized gold nanoclusters. *Biosens. Bioelectron.* **2012**, *32*, 297–299.

[6] Huang, S.; Lia, F.; Liao, C.; Zheng, B.; Dua, J.; Xiao D. A selective and sensitive fluorescent probe for the determination of HAS and trypsin. *Talanta*, **2017**, *170*, 562–568.