

## Supporting Information

### **A Fluorescence “off-on-off” sensing platform based on bimetallic gold/silver nanoclusters for ascorbate oxidase activity monitoring**

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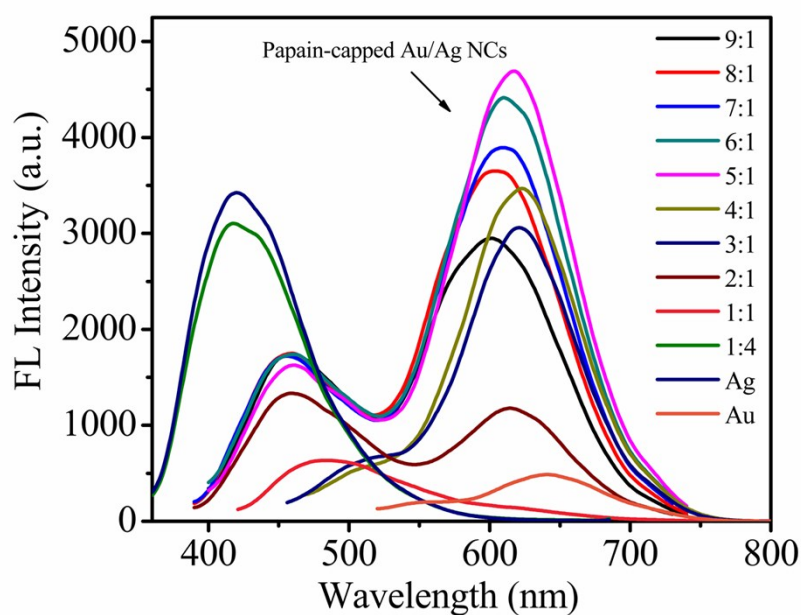
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## Reagents

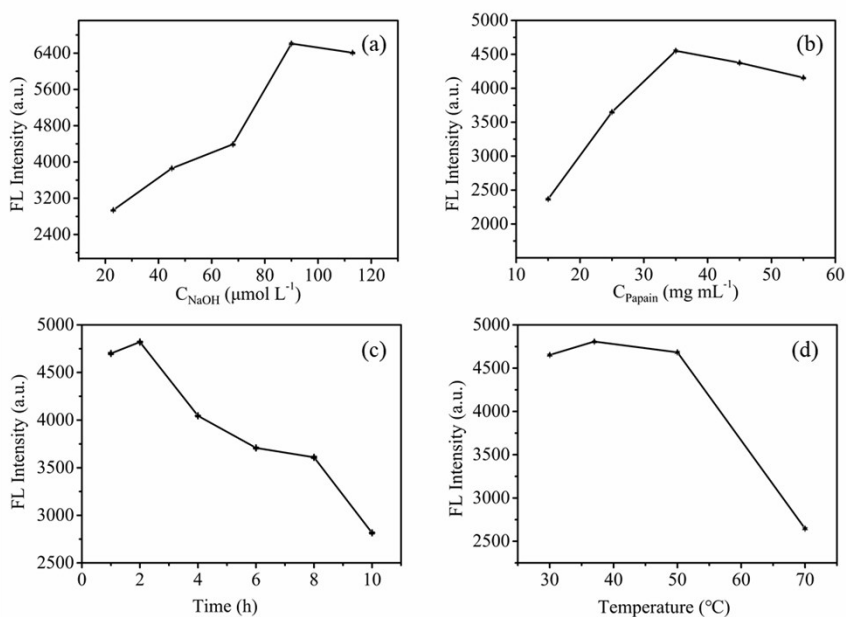
Reduced L-glutathione (GSH), trypsin (Try), hyaluronidase (HAase), bovine serum albumin (BSA), hemoglobin (Hb), urease (Urea), lysozyme (Lys) and Human serum albumin (HSA) AgNO<sub>3</sub> were ordered from Sangon Biotech (Shanghai) Co. Ltd. NaH<sub>2</sub>PO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub> and pepsin (pep) were obtained from Sino-pharm Co. (Shanghai, China). Phosphate buffer saline (PBS, 100 mmol L<sup>-1</sup>) were prepared with different volume ratio of 100 mmol L<sup>-1</sup> NaH<sub>2</sub>PO<sub>4</sub> and 100 mmol L<sup>-1</sup> Na<sub>2</sub>HPO<sub>4</sub>. Protamine (pro) was purchased from Shanghai Aladdin biochemical Co. Ltd. HAuCl<sub>4</sub> was ordered from Acros Organics. Ascorbic acid and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were obtained from Beijing Dingguo Biotechnology Co. Ltd. Ultrapure water with good resistivity ( $\rho \geq 18 \text{ M}\Omega \text{ cm}^{-1}$ ) throughout this experiment was used. The pH values were recorded by PHS-3C (Hangzhou, China). All chemicals are obtained from formal chemical suppliers and can be used directly without any further purification.

## Instruments

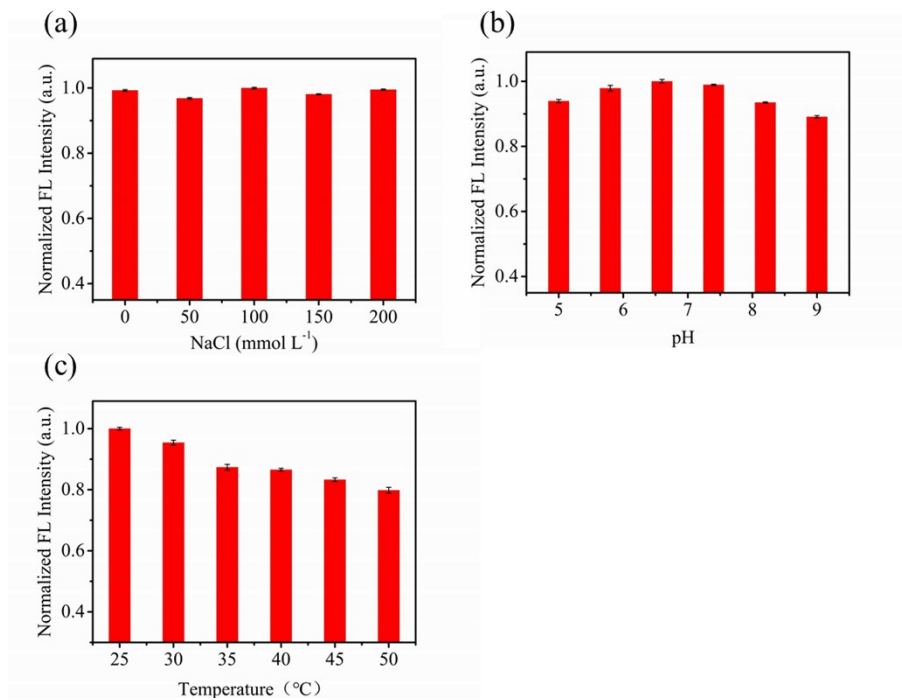
The ultraviolet-visible (UV-vis) absorption spectra, Photoluminescence (PL) spectra and Fourier transform infrared (FT-IR) spectra were obtained by a Varian GBC Cintra 10e UV-vis Spectrophotometer (Shimadzu Co., Ltd. Japan), RF-5301 fluorescence spectrophotometer and Thermo Nicolet 360 FTIR spectrometer, respectively. Transmission electron microscope (TEM) was carried on JEM-2100F. Fluorescence quantum yield and Fluorescence lifetime data were obtained on Edinburgh FLS920.



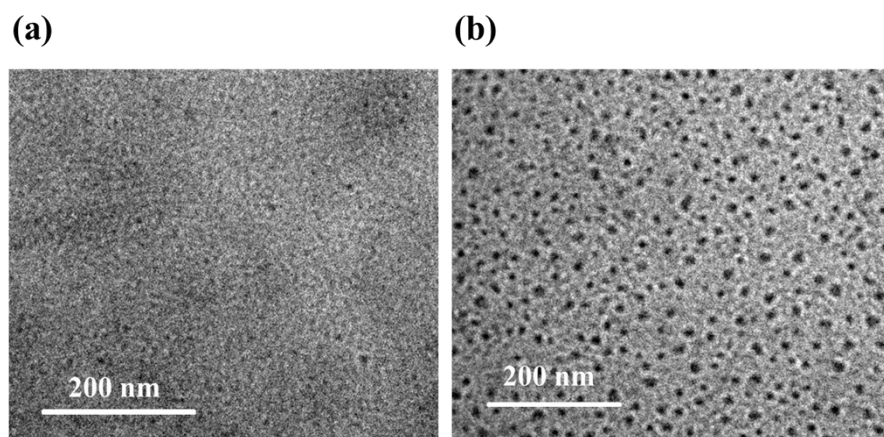
**Fig. S1** The fluorescence spectra of papain-capped Au/Ag NCs with different molar ratio of gold and silver.



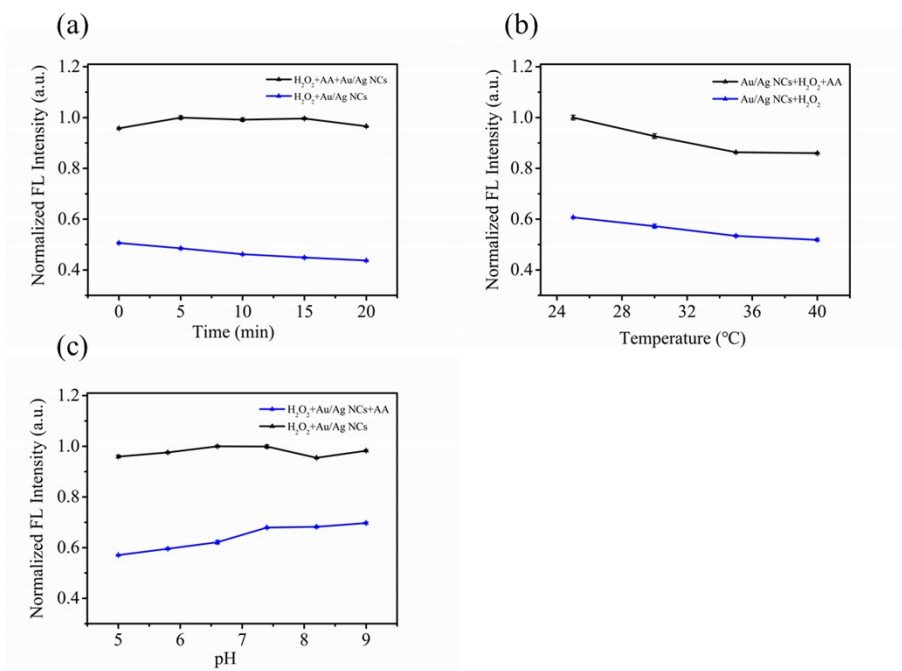
**Fig. S2** Optimal conditions for preparing papain-protected Au/Ag NCs. Effect of NaOH concentration (a), the concentration of papain (b), reaction time (c) and reaction temperature (d) on the FL intensity of papain-protected Au/Ag NCs.



**Fig. S3** The effect of NaCl concentration (a), pH (b), and temperature (c) on the Normalized FL intensity of papain-capped Au/Ag NCs.



**Fig. S4** TEM images of papain-protected Au NCs (a) and papain-protected Au/Ag NCs



**Fig. S5** Effect of reaction time (a), temperature (b) and pH (c) on the fluorescence intensity of papain-capped Au/Ag NCs/H<sub>2</sub>O<sub>2</sub> system in the presence and absence of AA.

**Table S1** Comparison of our method with other methods for the determination of AAO

Detection mode	Material	Linear range (mU mL <sup>-1</sup> )	LOD (mU mL <sup>-1</sup> )	References
Ratiometric fluorescent and colorimetric	C-dots/ oxOPD	0.04–5 0.04–8	0.017 0.012	[1]
Fluorometric and colorimetric	DNA-Au/Ag NCs	10–200	4.8	[2]
Fluorescence	Mn@ZnGe NPs	1250–2500	728	[3]
Fluorescence	Papain-capped Au/Ag NCs	5-80	1.72	This work

## Reference

1. Y. Wang, Y. Yang, W. Liu, F. Ding, P. Zou, X. Wang, Q. Zhao and H. Rao, *Mikrochim Acta*, 2019, **186**, 246.
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3. X. Y. Han, Z. H. Chen, Q. X. Fan, K. N. Li, F. Y. Mu, Q. Luo, Z. Jin, G. Shi and M. Zhang, *Mikrochim Acta*, 2019, **186**, 466.