Supporting Information

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

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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$_3$ were ordered from Sangon Biotech (Shanghai) Co. Ltd. NaH$_2$PO$_4$, Na$_2$HPO$_4$ and pepsin (pep) were obtained from Sino-pharm Co. (Shanghai, China). Phosphate buffer saline (PBS, 100 mmol L$^{-1}$) were prepared with different volume ratio of 100 mmol L$^{-1}$ NaH$_2$PO$_4$ and 100 mmol L$^{-1}$ Na$_2$HPO$_4$. Protamine (pro) was purchased from Shanghai Aladdin biochemical Co. Ltd. HAuCl$_4$ was ordered from Acros Organics. Ascorbic acid and hydrogen peroxide (H$_2$O$_2$) were obtained from Beijing Dingguo Biotechnology Co. Ltd. Ultrapure water with good resistivity ($\rho \geq 18$ M$\Omega$ 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$_2$O$_2$ system in the presence and absence of AA.
<table>
<thead>
<tr>
<th>Detection mode</th>
<th>Material</th>
<th>Linear range (mU mL⁻¹)</th>
<th>LOD (mU mL⁻¹)</th>
<th>References</th>
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<td>Ratiometric fluorescent</td>
<td>C-dots/ oxOPD</td>
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<td>and colorimetric Fluorometric and colorimetric</td>
<td>DNA-Au/Ag NCs</td>
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<td>728</td>
<td>[3]</td>
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<td>Fluorescence</td>
<td>Papain-capped Au/Ag NCs</td>
<td>5–80</td>
<td>1.72</td>
<td>This work</td>
</tr>
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Reference

