## Supporting Information

# Gold nanoparticles-based nanoconjugates for enhanced enzyme cascade and glucose sensing

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#### **Experimental details**

Horseradish peroxidase, glucose oxidase, and 2, 2'-azinobis (3-ethylbenzthiazoline-6-sulfonate acid) (ABTS) were purchased from Sigma-Aldrich. Other reagents were attained from Sinopharm Group Co. Ltd.

#### Preparation of AuNPs and HRP-AuNPs.

AuNPs solutions were prepared with the typical citrate reduction method as follows: 5 mL of 11.6 mg/mL trisodium citrate solution was added to a boiling, rapidly stirring solution of 50 mL, 0.826 mM HAuCl<sub>4</sub>. The solution was kept boiling and stirring for another 30 min. After being cooled to room temperature overnight, the prepared AuNPs (concentration: ~11 nM) were stored at 4 °C. For preparation of HRP-AuNPs, 5  $\mu$ L of HRP (Sigma-Aldrich) solution of certain concentration (1~10  $\mu$ g/mL) was directly mixed with 45  $\mu$ L as-prepared AuNPs. The mixture (containing 0.1~1  $\mu$ g/mL HRP and 10 nM AuNPs) was incubated at room temperature for 1 hour if not mentioned otherwise, and was then stored at 4 °C for further use.

#### Characterization of HRP-AuNPs.

The morphology of AuNPs was characterized with a Philips CM300 FEG transmission electron microscopy. The absorption spectra of AuNPs and HRP-AuNPs were determined with a HITACHI U-3010 UV-Vis spectrophotometer. Their sizes were measured using a dynamic light scattering (DLS) particle sizer (Beckman Coulter Delsa Nano C A53878) at room temperature. The Fourier transform infrared spectroscopy (FTIR) measurements were carried out with a Thermo Nicolet Avatar 370 FTIR spectrophotometer.

#### Enzyme kinetic analysis.

For the enzyme assay,  $50\mu$ L of HRP-AuNPs was mixed with  $50\mu$ L 2, 2'-azinobis (3-ethylbenzthiazoline-6-sulfonate acid) (ABTS, Sigma-Aldrich) (1 mM in 10 mM sodium citrate buffer, pH 5.0) and 15  $\mu$ L H<sub>2</sub>O<sub>2</sub> of certain concentration. This mixture was incubated at 37°C for 10 min, whose absorbance (at 410 nm) was then read with a Tecan microplate reader.

#### Determination of gluconic acid.

AuNPs or HRP-AuNPs (0.1  $\mu$ g/mL HRP vs. 10 nM AuNPs) were firstly let reacted with 20  $\mu$ L glucose (0.5 M) for 30 min at 37 °C. Next, 250  $\mu$ L of solution 1 (5 mM EDTA and 0.15 M Et<sub>3</sub>N in water) and 25  $\mu$ L of solution 2 (3 M NH<sub>2</sub>OH in water) were added to the product solution, then the mixture was allowed to react for 15min. After that, 125  $\mu$ L of solution 3 (1 M HCl, 0.1 M FeCl<sub>3</sub>, and 0.25 M CCl<sub>3</sub>COOH in water) was added in to react for another 5min. Finally the absorbance of the resulting mixture at 505 nm was recorded.

#### **Detection of glucose.**

The glucose detection was proceeded as follows: (1) 50  $\mu$ L of HRP-AuNPs (0.1  $\mu$ g/mL HRP vs. 10 nM AuNPs) and 20  $\mu$ L glucose of certain concentration (0, 0.4, 0.8, 2, 4, 8, 20, 40, 80 mM) were mixed and incubated at 37 °C for 30 min; (2) 50  $\mu$ L of 1 mM ABTS solution (pH 5.0) was then added to the solution, the mixture was allowed to react for another 10 min at 37 °C; (3) the absorbance (at 410 nm) of the resulting solution was measured with the microplate reader.

### **Additional Figures**



Figure S1. TEM image of naked gold nanoparticles.



Figure S2. Visual spectroscopic analysis of naked AuNPs (black) and HRP-AuNPs (red).



Figure S3. Size distribution histograms of naked gold nanoparticles (a) and HRP-AuNPs (b).



Figure S4. FT-IR spectra of (a) HRP and (b) HRP-gold nanoparticles.



**Figure S5.** The detecting results of control (water) and 0.4 mM of glucose. Error bars represent the standard error derived from at least three independent measurements. The dash line indicates the threshold of the detection system (control mean  $+ 3 \times$  standard deviation).