Electronic Supplementary Information (ESI)

Simulated enzyme inhibition-based strategy for ultrasensitive colorimetric biothiol detection based on nanoperoxidase

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Experimental Section

Chemicals

$HAuCl_4 \cdot 3H_2O$, thrombin and glutathione were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cysteine, glycine, glucose, cholesterol, tryptophane, Adenosine -5'-triphosphate disodium, Adenosine-5'-diphosphate disodium, Adenosine-5'-monophosphate disodium and HRP buffer (citric acid-Na_2HPO_4, pH 5.0) were purchased from Sangon Biotech. (Shanghai, China). Tyrosine was provided by Huixing Chemical Reagent Co. (Shanghai, China). H_2O_2, sodium citrate and ethanol were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 3, 3, 5, 5-tetramethylbenzidine (TMB) and 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Aladdin Reagent Co. (Shanghai, China). Standard Hg^{2+} solution and glutamic acid were provided by Macklin Reagent Co. (Shanghai, China). Lambda DNA

was obtained from Promega Co. (Wisconsin, USA). All reagents used in this work were of analytical grade or higher and all solutions were prepared or diluted with ultrapure water produced from a Milli-Q system (Millipore Corporation, USA). Pretreated human blood samples were provided by Nanjing Chest Hospital and further treated with Millipore Amicon Ultra centrifugal filter (3 kDa, Millipore Corporation, USA).

Apparatus

Transmission electron microscopy (TEM) images were taken using HRTEM microscope (JEOL-2100F, JEOL, Japan). The UV-vis absorption data were measured by an Agilent Cary 60 spectrophotometer (Agilent Co., USA) and a Thermo Fisher Multiskan GO microplate reader (Thermo Fisher Scientific, USA).

The synthesis of AuNPs

The AuNPs were synthesized according to a general citrate reduction method.[1] The synthesized AuNP colloids with diameters of approximately 13 nm were diluted with ultrapure water, and the concentration of the mixture was estimated based on the UV-vis absorption spectra measured with an Agilent Cary 60 spectrophotometer with a standard quartz cuvette.[2] After that, the AuNP colloid was further diluted with ultrapure water to obtain a 0.6 nM solution.

Procedures for GSH Detection

In a typical procedure, 0.5 μL of 80 μM Hg^{2+} and 99.5 μL of various concentrations of GSH solution were freshly mixed in a 96-well microplate. Then, 5 μL of 0.6 nM AuNP colloids were mixed with the above solution and incubated for 15 minutes. Finally, 80 μL of HRP buffer (pH 5, citric acid-Na_2HPO_4), 5 μL of 20 mM TMB (ethanol solution)

and 10 μL of 10 M H_2O_2 were successively added followed by thorough mixing. After incubation at room temperature (25 ^oC) for 10 minutes, the absorption data were measured with a microplate reader. For the recovery test, the pretreated human blood samples were diluted with ultrapure water (1500 times) and further filtered with a centrifugal filter to remove possible residual protein components. After that, the diluted samples were mixed 1:1 with ultrapure water or GSH solution at the appropriate concentration (200 or 400 nM) for the recovery determination (diluted 3000 times finally and spiked with 0, 100 and 200 nM GSH). For the test based on Ellman's method, the blood samples were diluted with ultrapure water (75 times) and further filtered with the centrifugal filter. After that, the samples were mixed 1:1 with 20 mM pH 7.6 tris buffer or GSH solution (4 μM or 8 μM) prepared by 20 mM pH 7.6 tris buffer and applied for determination.

Figures

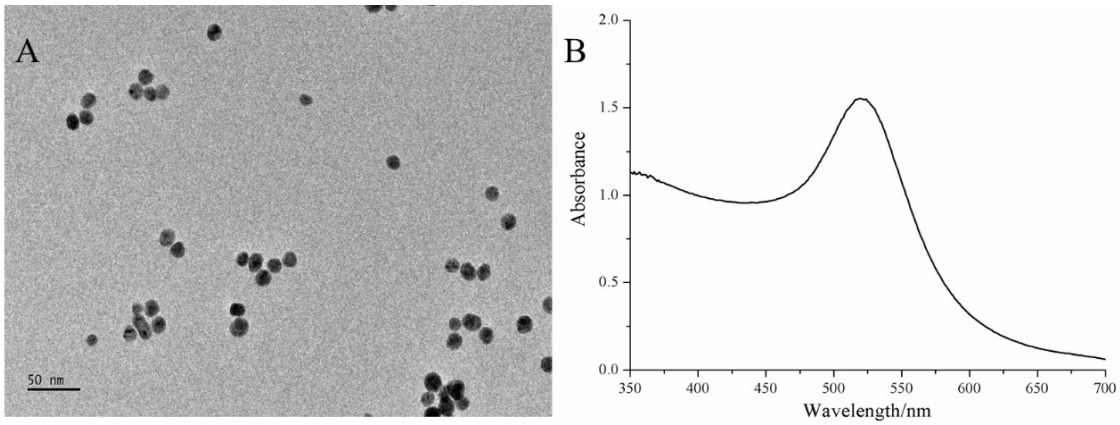

Fig. S1 TEM image (A) and UV-vis absorption spectra (B) of the synthesized AuNPs colloid.

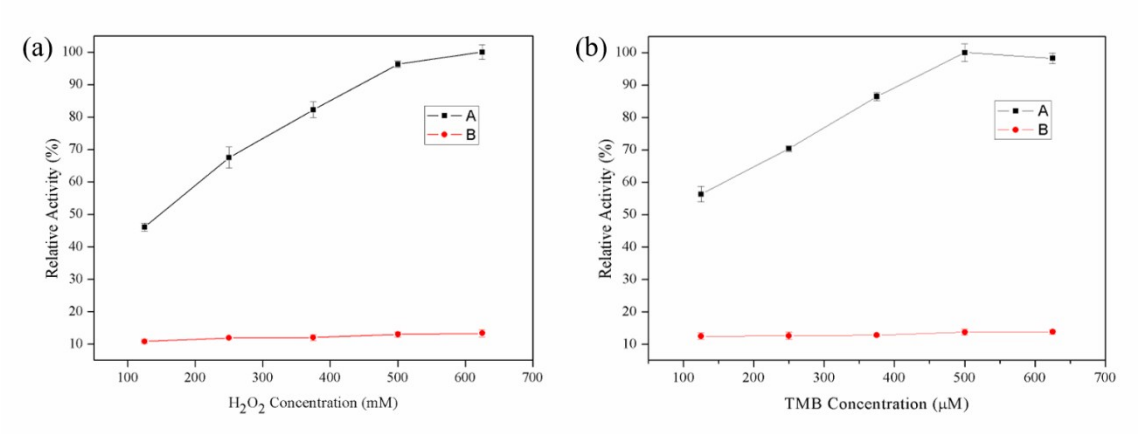

Fig. S2 Optimization for the H_2O_2 (a) and TMB (b) concentration. Curve A: Au-Hg, Curve B: AuNPs. The experimental conditions are the same with that discribed in the **Procedures for GSH Detection** expect that ultrapure water was applied instead of GSH solution.

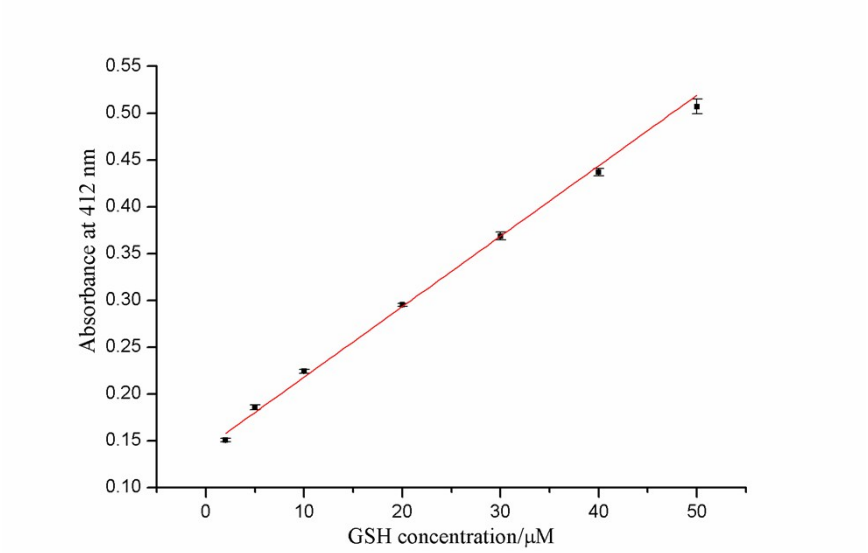

Fig. S3 Standard curve for a series concentrations of GSH obtained by Ellman's method.

Tables

Table 1 Comparision of different works for GSH detection

Methods	Linear range	Detection limit	Reference
colorimetric	0.05 - 15 μM	0.5 μM	3
colorimetric	0.1 - 40 μM	100 nM	4
colorimetric	0 - 20 μM	0.68 μM	5
colorimetric	0 - 40 μM	500 nM	6
colorimetric	0.1 - 20 μM	58 nM	7
SERS	0 - 1.0 μM	0.25 μM	8
fluorescence	20 - 2000 nM	6.7 nM	9
electrochemiluminescence	0.01 - 2.0 μM	3.7 nM	10
electrochemiluminescence	0.1 -1.0 μM	54.3 nM	11
colorimetric	20 - 500 nM	12.5 nM	This work

Table 2 Recovery test for GSH detection in diluted human blood samples.

Sample *No.*	Ellman's method [a] (μM)	This method (nM)	RSD (n=3)	Added GSH (nM)	Found (nM)	RSD (n=3)	Recovery (%)	Ellman's method (μM)
1	2.77	147.0	2.91	100	247.5	4.28	100.5	4.74
				200	353.2	2.93	103.1	6.75
2	2.58	138.6	4.09	100	234.4	2.83	95.8	4.53
				200	345.7	4.12	103.6	6.47

[a] The standard curve for the Ellman's method was obtained as follows: 20 μL 10 mM DTNB was mixed with 180 μL different concentrations of GSH prepared by 10 mM pH 7.6 tris buffer. After incubated for 20 min, the absorbance values at 412 nm were monitored by the microplate reader. The standard curve was obtained as y=0.1428+0.0075x, with R^2 = 0.995. The different samples with 20-fold concentrations were measured by the same procedure as above and the determined concentration is the mean value of 3 parallel experiments.

References

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