## Specific pH effect for selective colorimetric sensing assayof glutathione using anti-aggregation of label-free gold nanoparticles

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a 
$$NH_2$$
  $NH_2$   $NH_2$   $NH_2$   $COOH$   $COOH$   $Cye$   $NH_2$   $COOH$   $Cye$   $COOH$   $Cye$   $COOH$ 

Fig.S1 Structures of (a) cysteine (Cys), (b) homocysteine (Hcys), (c) glutathione (GSH) and (d) cystine (Cye)

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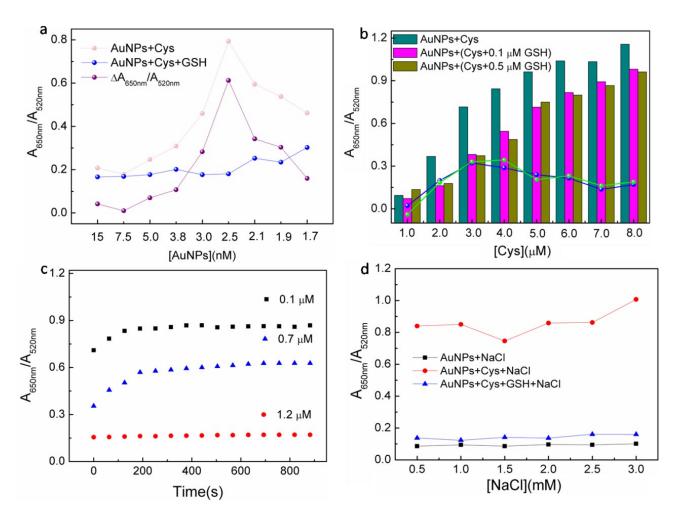


Fig.S2 Optimization of assay conditions: (a) effect of AuNPs concentration in the presence of 0.4 μM Cys and 1.0 μM GSH, (b) effect of Cys in the presence of 0 ( $\blacksquare$ ), 0.1 ( $\blacksquare$ ) and 0.5 μM ( $\blacksquare$ ) GSH, PBS (0.01 M)), (c) effect of time in the presence of 0.1, 0.7 and 1.2 μM GSH, (d) effect of NaCl concentration.

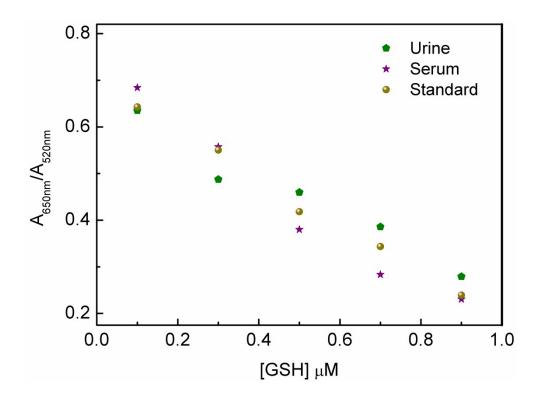


Fig. S3 Calibration curve plotted between intensity ratio ( $A_{650nm}/A_{520nm}$ ) of Cys-AuNPs and concentrations of GSH with human urine, human serum and PBS solution.

## Preparation of real samples

Human urine sample (1.0 mL) was collected from healthy adult female volunteers, and the analysis was conducted immediately after the sample collection. 1.0 mL of urine sample was added into a centrifuge tube, and 1.0 mL of acetonitrile was added to remove proteins in urine. The mixture was centrifuged at 12,000 rpm for 10 min. The supernatant was filtered through a 0.22  $\mu$ m filter, and then dried under vacuum at 50 °C for 10 h, and finally diluted to 5 mLwith deionized water before analysis.

Human serum was obtained from Nanchang University Hospital from healthy donors. Human serum (0.5 mL) was placed in acentrifuge tube and acetonitrile (2.0 mL) was added to precipitate proteins. After vortex-mixing, the sample was centrifuged at 12,000 rpm for 15 min, and the supernatant was transferred into a 25.0 mL volumetric flask and diluted to the mark with deionized water. For the colorimetric detection, different concentration of GSH (10<sup>-4</sup> M) and Cys of 4.0 μM were mixed with 10 μL of the as-pretreated human urine samples or human serum samples, and subsequently added into AuNPs solution and the final detective volume was 2 mL.

Table S1 Analytical results of GSH in real samples

Sample	Added	Measured	Recovery	RSD
	(µM)	$(\mu M)$	(%)	(%, n=3)
Human serum	0.2	0.22	110	3.1
	0.4	0.38	95.0	2.8
	0.6	0.61	102	3.9
Human urine	0.3	0.27	90.0	4.5
	0.5	0.47	94	3.5
	0.7	0.72	103	2.9