Supplementary Material

Reversion of gold nanoparticle aggregates for the detection of Cu²⁺ and its application in enzyme immunoassays

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Fig. S1. (A) UV-Vis spectra of AuNP solution in the absence and presence of 0.5 mM CA. The inset image corresponds to the colorimetric response. (B) Time course of AuNP aggregation in the absence and presence of 0.5 mM CA.



Fig. S2. Zeta potential of citrate-capped AuNPs. The as-synthesized citrate-capped AuNPs were centrifuged at 12500 rpm for 25 min and the supernatant was discarded. The AuNPs were then resuspended in aqueous solution at different pH values and the zeta potentials of these AuNPs were measured. The results indicated that an increasing pH value could lead to the dissociation of citrate ions on the surface of AuNPs¹, thereby resulting in reduction of the negative zeta potential.



Fig. S3. Detection of Cu^{2+} in ultrapure water. (A) Time-course measurement of A660/A520 for CA-AuNPs upon the addition of different concentrations of Cu^{2+} . To decrease the analysis time, the reaction time was chosen to be 20 min. (B) Absorption spectra of CA-AuNP after the addition of different concentrations of Cu^{2+} . Inset: Photograph of the corresponding solutions. (C) Absorption ratio of CA-AuNP to various concentrations of Cu^{2+} . The detected linear range was observed from 0.4 to 1 μ M. (D) Absorption responses of CA-AuNP after the addition of 1 μ M metal ions. Mix stands for the mixture of other interfering metal ions.



Fig. S4. Absorption spectra of CA-AuNP in different conditions. When CA-AuNP was added to drinking water, a red shift in the absorption maximum from 520 nm to 550 nm was noted in condition (b). Because the original drinking water was free of Cu²⁺, large amounts of Mg²⁺ and Ca²⁺ occurring in water² may have interfered with the response of CA-AuNPs. As a result, we found that final concentrations of 100 μ M Mg²⁺ and 300 μ M Ca²⁺ spiked in the ultrapure water resulted in the redshift of the absorption peak (condition c). This response was similar to condition (b), suggesting that the interference of CA-AuNP in drinking water may arise from Mg²⁺ and Ca²⁺. As shown in conditions (d) and (e), the addition of EDTA effectively masked the interference ions from Mg²⁺ and Ca²⁺.



Fig. S5. Time-course measurement of A660/A520 for CA-AuNPs upon the addition of different concentrations of Cu^{2+} in (A) drinking water and (B) undiluted urine. The results showed that the maximum ratio of A660/A520 was obtained in 6 min (drinking water) and 1 min (undiluted urine) after the addition of Cu^{2+} and exhibited a very slow change with a further increase in reaction time. (C) Effect of dilution on A660/A520 for CA-AuNPs in urine samples.



Fig. S6. (A) Color change with the increase of Cu^{2+} concentration in the urine samples. (B) Response curves for Cu^{2+} detection in the urine samples with a linear range from 33 to 1000 μ M.

	(1)	(2)	(3)	(4)	(5)
		0	0		
H-hCG (50 µL, 16 mIU/mL)	-	+	-	-	+
Cu ²⁺ (50 μL, 3.5 μM)	+	+	+	-	-
HRP-Ab (50 µL)	-	-	+	+	-
Ultrapure water (50 µL)	+	-	-	+	+
CA-AuNP (100 μL)	+	+	+	+	+

Fig. S7. Color responses of CA-AuNPs under different conditions. To investigate the interaction between Cu^{2+} and HRP, different conditions were performed in unmodified wells (without the immobilization of the primary antibody) of the ELISA plate for 15 min incubation. CA-AuNPs were then introduced to the mixture for 5 min.



Figure S8. Absorption spectra of CA-AuNPs in the presence of H-hCG with concentrations ranging from 0 to 16 mIU/mL.

References

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