

# Simple, Rapid and Label-free Colorimetric Assay for Zn<sup>2+</sup> Based on Unmodified Gold Nanoparticles and Specific Zn<sup>2+</sup> binding Peptide

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## Experimental section

### Materials and Measurements

Gold nanoparticles (AuNPs) (13 nm) were prepared by citrate reduction of HAuCl<sub>4</sub><sup>1</sup>. The final concentration of AuNPs was estimated to be about 10.4 nM using UV-Vis spectrometric measurements based on an extinction coefficient of  $\sim 2.7 \times 10^8 \text{ M}^{-1} \text{ cm}^{-1}$  at  $\lambda = 520 \text{ nm}$  for 13 nm particles.<sup>1</sup> All peptides (CCPGCAR, CCPGCA) were synthesized and purified by GL Biochem. Ltd. (Shanghai, China). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and 2,6-pyridinedicarboxylic acid (PDCA) were purchased from Sigma (St. Louis, MO). All chemical reagents were of analytical grade and used without further purification. All solutions were prepared with ultra-pure water (18.25 MΩ cm) from a Millipore system. UV-Vis absorption spectra were obtained on a Beckman DU-800 spectrophotometer.  $\Delta Abs_{600}$  ( $\Delta Abs_{600} = |Abs_x - Abs_0| = Abs_0 - Abs_x$ ,  $Abs_x$  is the absorbance at 600 nm when the sample is treated with  $x \mu\text{M}$  of Zn<sup>2+</sup> and  $Abs_0$  is that treated without Zn<sup>2+</sup>) was used here to monitor the performances of the sensor.

### Typical experimental process for Zn<sup>2+</sup> detection

6 μL solution of peptide (20 μM, prepared in 80 μM TCEP solution) was added to 50 μL 2× buffer (1× buffer: 50 mM borate buffer, 25 mM NaClO<sub>4</sub>, 100 μM TCEP, pH 7.4), the sample was replenished to 90 μL by H<sub>2</sub>O. Then, 10 μL solution of Zn<sup>2+</sup> with different concentration was added. At last, 100 μL AuNPs was introduced to the sensing system accompanying the shake.

- (1) R. Jin, G. Wu, Z. Li, C. A. Mirkin, G. C. Schatz, *J. Am. Chem. Soc.*, 2003, **125**, 1643-1654.

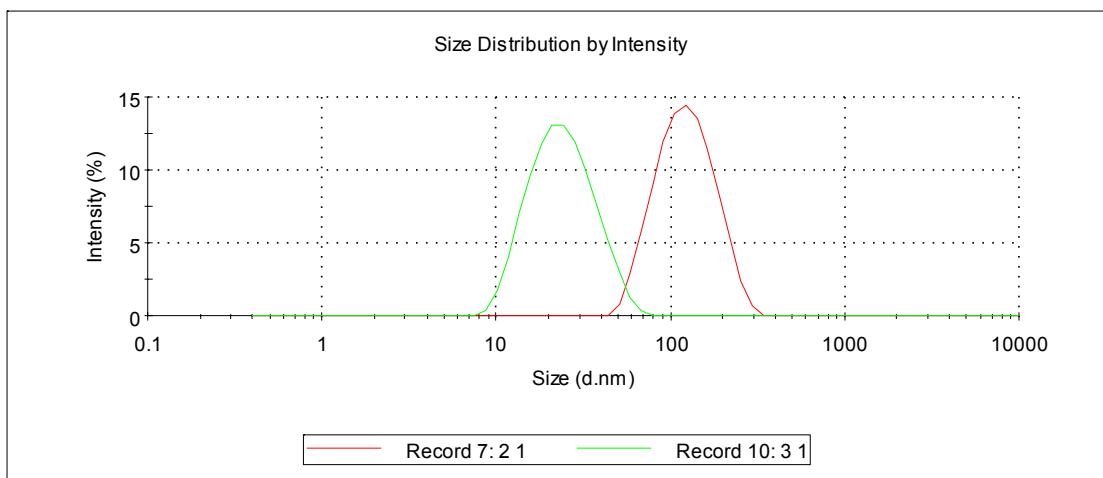


Fig. S1 Characterization of the peptide and AuNPs assembly by dynamic light scattering (DLS). Red: the AuNPs sample treated with peptide; green: the AuNPs sample treated with peptide and  $Zn^{2+}$ .

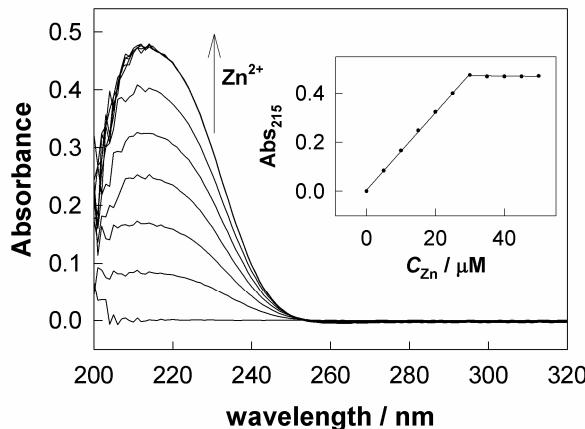


Fig. S2 UV-Vis spectrum during titration of peptide with  $Zn^{2+}$ . 30  $\mu M$  of peptide was titrated with 1 mM stock solution of  $Zn^{2+}$  to final concentration of 0 – 50  $\mu M$   $Zn^{2+}$  in 50 mM borate buffer, pH 7.4 ( $I = 25$  mM from NaClO<sub>4</sub>), 100  $\mu M$  TCEP. The arrow indicates increasing tendency of absorbance after  $Zn^{2+}$  addition. The titration was followed by a 5 min equilibration period after each addition. The inset shows the absorbance changes at 215 nm as a function of the concentrations of the  $Zn^{2+}$ .

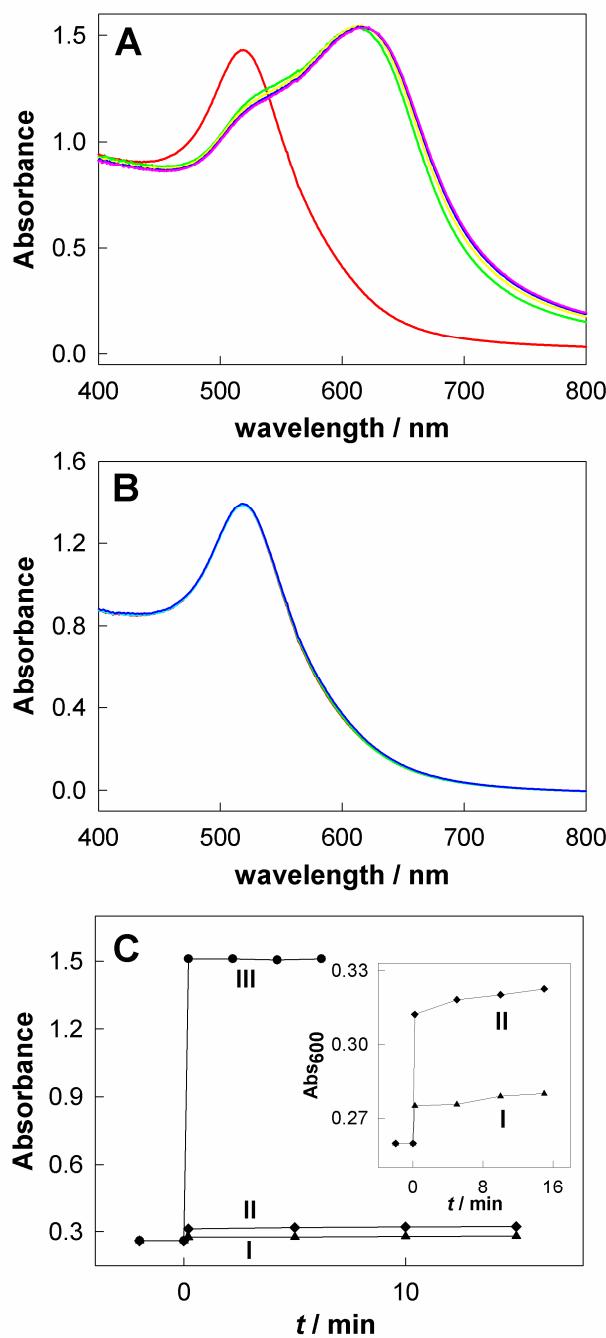


Fig. S3 (A) The absorption spectra of AuNPs at different times after the addition of 2 μM CCPGCAR peptide (green to pink: 0.2, 2, 4, and 6 min; red: AuNPs solution without peptide). (B) The absorption spectra of AuNPs at different times after the addition of 2 μM CCPGCA peptide (green to blue: 0.2, 5, 10, and 15 min; red: AuNPs solution without peptide). (C) The absorbance of 600 nm at different times after the addition of 2 μM CCPGCA peptide (I), 5 μM CCPGCA peptide (II), and 2 μM CCPGCAR peptide (III). Inset of (C): the enlarged figure of (I) and (II).

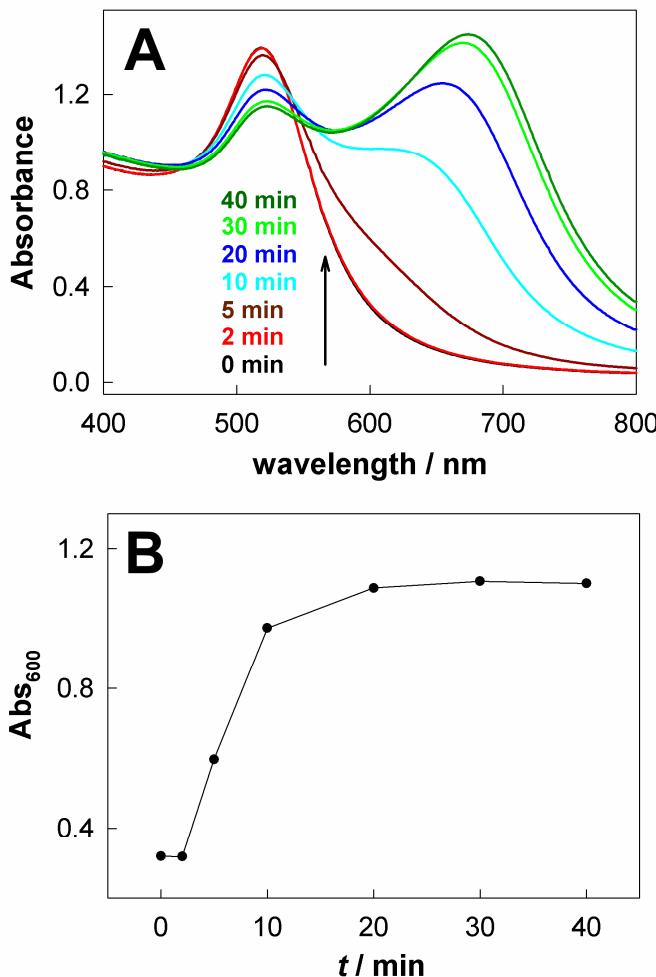


Fig. S4 (A) The absorption spectra of AuNPs at different times after the addition of 20  $\mu\text{M}$  DTT. (B) The absorbance of 600 nm at different times after the addition of 20  $\mu\text{M}$  DTT.

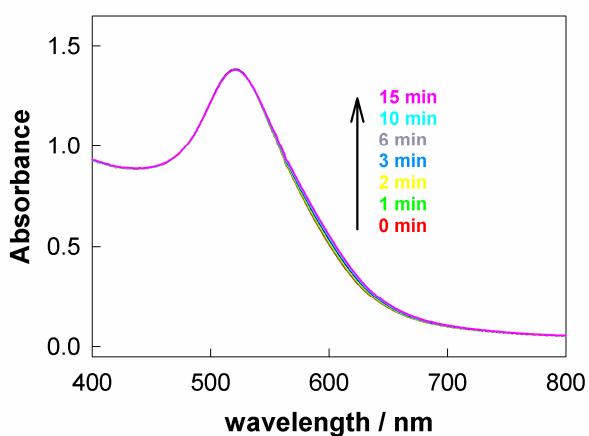


Fig. S5 The stability of AuNPs in the presence of peptide-Zn<sup>2+</sup> complex.

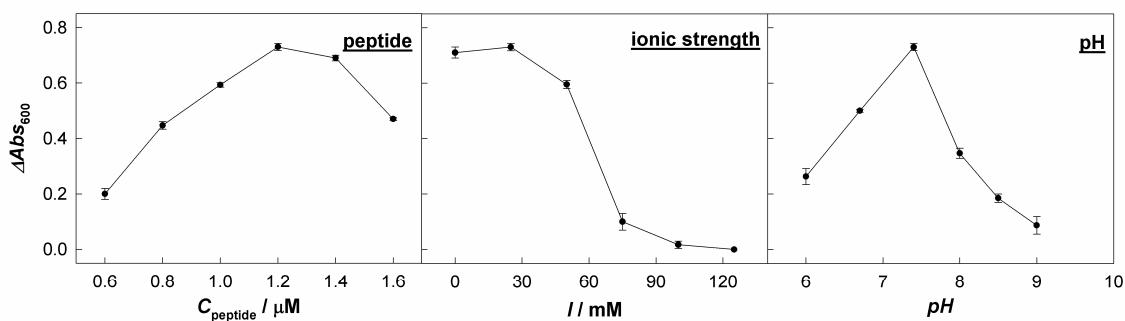


Fig. S6 The effect of some factors of the sensing system on the absorbance change at 600 nm (from left to right): the concentration of the peptide probe (0.6, 0.8, 1.0, 1.2, 1.4, and 1.6  $\mu\text{M}$ , respectively), ionic strength from NaClO<sub>4</sub> (0, 25, 50, 75, 100, and 125 mM, respectively), and pH value of borate buffer (6.0, 6.7, 7.4, 8.0, 8.5, and 9, respectively).

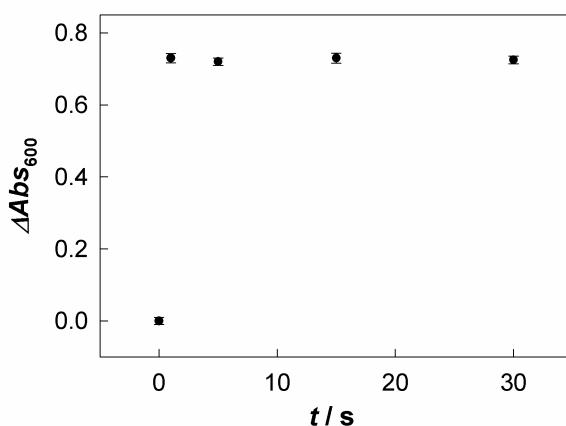


Fig. S7 The effect of the coordination time of  $\text{Zn}^{2+}$  with peptide on the absorbance change at 600 nm.

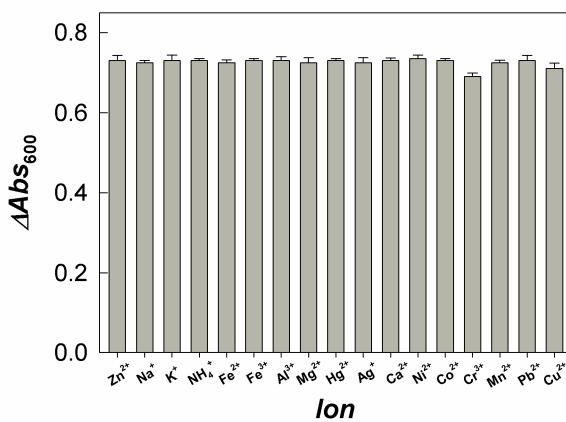


Fig. S8 The absorbance change of the sensing system in the presence of 1.2  $\mu\text{M}$   $\text{Zn}^{2+}$  and 1.2  $\mu\text{M}$  of other metal ions.

Table S1 Determination of Zn<sup>2+</sup> in urine and water samples

	Sample	Added ( $\mu\text{M}$ )	Found ( $\mu\text{M}$ )	Recovery
(A) Urine	1-1	0.3	$0.32 \pm 0.03$	107%
	1-2	0.7	$0.71 \pm 0.03$	102%
	1-3	1.0	$1.04 \pm 0.04$	104%
(B) Water	2-1	0.4	$0.42 \pm 0.02$	103%
	2-2	0.96	$0.93 \pm 0.05$	97%
	3-1	0.4	$0.43 \pm 0.03$	108%
	3-2	0.96	$0.99 \pm 0.05$	103%

(A) Urine samples were first employed as the model complex system (sample 1-1, 1-2, and 1-3). Fresh urine samples were obtained from healthy volunteers. Each sample was filtered through a 0.2- $\mu\text{m}$  membrane to remove particulate matters. The solution of Zn<sup>2+</sup> was prepared in human-urine samples diluted by a factor of 50. Before detection, the sample was equilibrated for 30 min. (B) Recovery experiments in tap waters (sample 2-1 and 2-2) and pond waters (sample 3-1 and 3-2).

Table S2 Assay of Zn<sup>2+</sup> in nutrient sample (1) and drug sample (2).

Sample	Content	Found	Zincon spectrophotometry
1	35.3 mg/100 mL	$35.8 \pm 0.53$ mg/100 mL	$35.7 \pm 0.2$ mg/100 mL
2	10 mg/pcs	$9.7 \pm 0.22$ mg/pcs	$10.5 \pm 0.15$ mg/pcs

Sample 1 and 2 are zinc gluconate oral liquid and zinc gluconate tablets obtained from Harbin Pharmaceutical Co. Ltd (Harbin, China), respectively. The oral liquid was diluted with water and the tablet was directly dissolved in water without further treatment. Before detection, the samples were equilibrated for 30 min.