

## Supporting Information

### Visual Determination of Cu<sup>2+</sup> through Copper-Catalysed in-situ Formation of Ag Nanoparticles

Xun Yuan<sup>a</sup> and Yi Chen<sup>\*a,b</sup>

<sup>a</sup> Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P.R. China. Fax: 86-10-6255 9373; Tel: 86-10-6261 8240; E-mail: chenyi@iccas.ac.cn

<sup>b</sup> Beijing National Laboratory for Molecular Science, Beijing 100190, P.R. China

#### S-1 Treatment of serum samples:

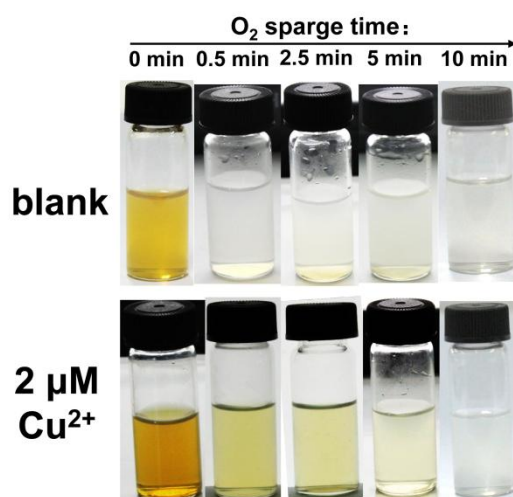
**Pretreatment of serum samples.** Human serum samples (Beijing BioDee biotechnology corporation Ltd., Beijing, China) were first deproteinized by a reported method.<sup>1</sup> Briefly, an aliquot of 0.5 mL serum sample was mixed with 0.35 mL of 2 M HCl by vortex. After stood at room temperature for 10 min it was added and mixed with 0.5 mL of 20 % trichloroacetic acid. After further reaction at 4°C for 20 min, the solution was centrifuged at 8000 rpm for 10 min. The supernatant (ca. 1mL) was then mixed with 0.2 mL H<sub>2</sub>O<sub>2</sub> (30 %) in a 10-mL beaker, and heated to nearly dryness at 150°C. The residual was redissolved in 1 mL water and heated to dryness again. It was finally dissolved in 1 mL water and neutralized with 5 M NaOH (ca. 60 μL).

**Treated by chelating resin.** D751 chelating resin (Aladdin Reagent Company, Shanghai, China) used in this experiment is an iminodiacetic acid (IDA) chelating cation exchange resin on a macro porous styrene divinylbenzene matrix and has a particularly high selectivity for transition metals. Exchange Capacity of sodium-formed resin for Cu<sup>2+</sup> is not less than 0.65 mol/L. Before use, 1 g of chelating resin was successively conditioned in 5 mL Millipore water, 5 mL HNO<sub>3</sub> (2 M) and 5 mL NaOH (1 M) for 3 h each. After rinsed with water till neutral, it was dried at 50°C for ca. 2 h.<sup>2</sup>

In care required, the pretreated human sera, as well as tap and river water samples (for

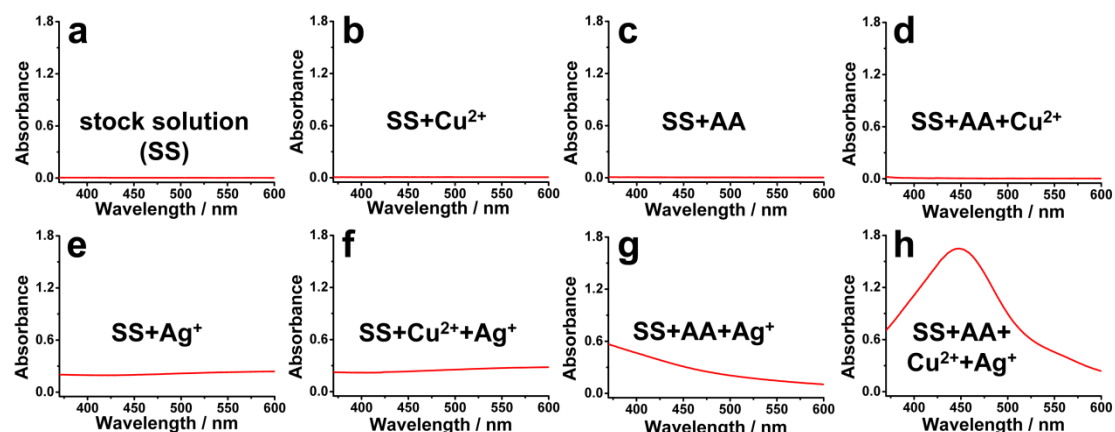
comparison with direct determination methods), are suggested to be treated with the preconditioned chelating resin through following procedure: an aqueous sample was mixed with 0.2 M acetic acid (pH 4.5) at 1 mL: 1 mL. After 20 mg resin was added, it was stirred at 500 rpm at room temperature for 2 h. The resin was collected and dunked in 1 mL water for 3 x 10 min, and then extracted in 0.1 mL HNO<sub>3</sub> (2 M) at room temperature by stirring at 500 rpm for 0.5 h to release the metal ions. The supernatant was collected and neutralized by 5 M NaOH (ca. 30 μL) and made up to 1 mL with water. An aliquot of 0.1 mL of the solution was then subjected to visual or colorimetric determination.

### S-2 Influence of O<sub>2</sub>



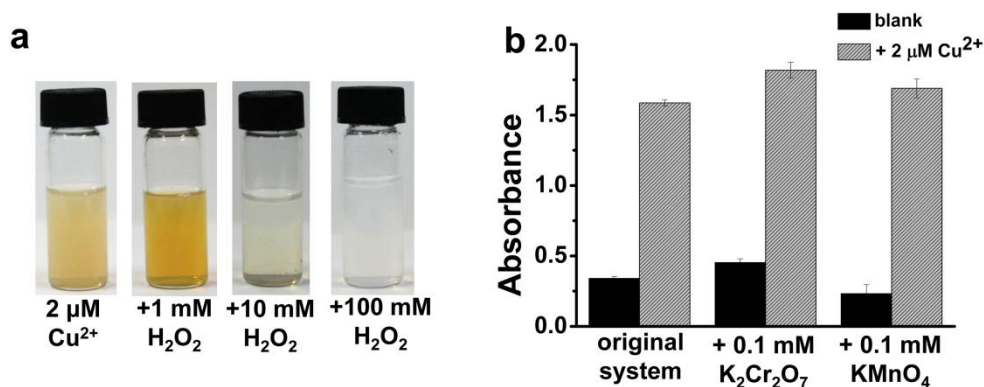
**Fig. S1** Photographs taken after 15-min reaction at 95°C with solutions sparged by O<sub>2</sub> for different time (as indicated in figure) after deaerated by N<sub>2</sub> for 30 min. The flow rates of O<sub>2</sub> and N<sub>2</sub> are both 0.8 L/min. The blank solution is composed of CTAB (0.48 mM), AA (2 mM), and AgNO<sub>3</sub> (0.4 mM). The working solution is composed of CTAB (0.48 mM), AA (2 mM), AgNO<sub>3</sub> (0.4 mM), and Cu<sup>2+</sup> (2 μM).

### S-3 Validation of proposed mechanism:



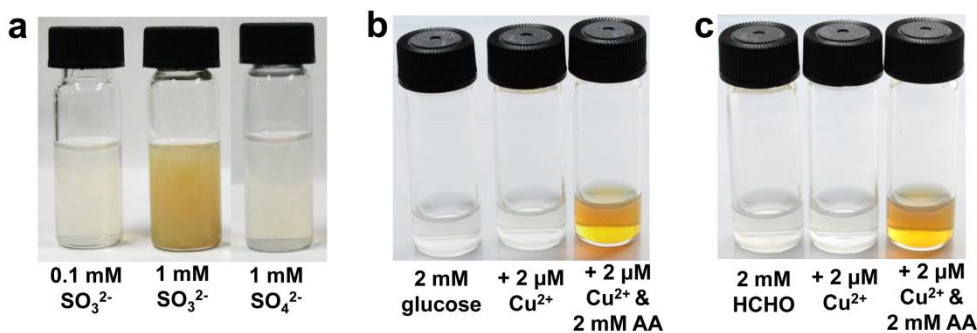
**Fig. S2** Absorption spectra of following solution after heating at 95°C for 15 min: stock solution (SS, a), SS plus Cu<sup>2+</sup> (b), SS plus AA (c), SS plus AA and Cu<sup>2+</sup> (d), SS plus Ag<sup>+</sup> (e), SS plus Cu<sup>2+</sup> and Ag<sup>+</sup> (f), SS plus AA and Ag<sup>+</sup> (g), and SS plus AA, Cu<sup>2+</sup> and Ag<sup>+</sup> (h). The concentrations of Cu<sup>2+</sup>, AA and Ag<sup>+</sup> are 2 μM, 2 mM and 0.5 mM, respectively.

### S-4 Influence of oxidizers



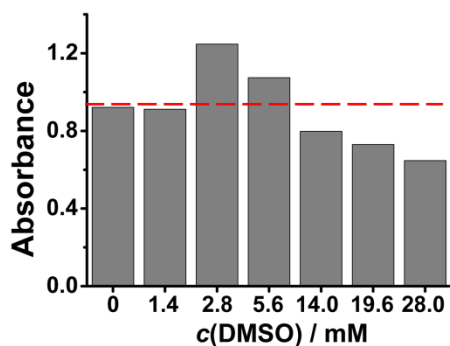
**Fig. S3** (a) Photographs taken after 15-min reaction at 95°C with solutions composed of CTAB (0.48 mM), AA (2 mM), AgNO<sub>3</sub> (0.4 mM), Cu<sup>2+</sup> (2 μM) and H<sub>2</sub>O<sub>2</sub> (as indicated in the figure). (b) Plots of absorbance at 440 nm vs. different oxidizers. The reaction conditions are similar with the typical experiment described in Experimental Section with 2 μM of Cu<sup>2+</sup> (or water for blank), except the oxidizer added.

### S-5 Influence of reductants



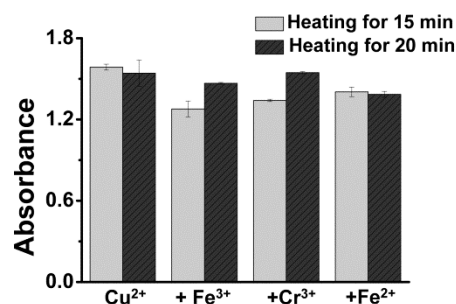
**Fig. S4** Photographs taken after 15-min reaction at 95°C with solutions composed of (a) CTAB (0.48 mM), AA (2 mM),  $\text{AgNO}_3$  (0.4 mM) and chemicals (as indicated in the figure), (b, c) CTAB (0.48 mM),  $\text{NaNO}_3$  (96 mM), BSA (2.4  $\mu\text{g}/\text{mL}$ ),  $\text{AgNO}_3$  (0.5 mM) and chemicals (as indicated in the figure).

### S-6 Influence of radical scavengers



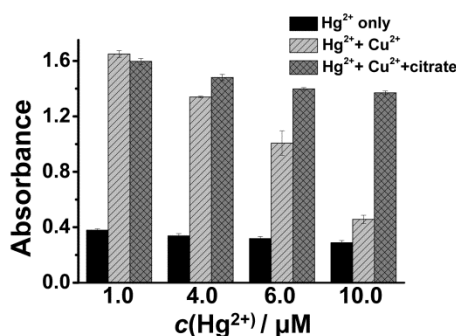
**Fig. S5** Plot of absorbance at 435 nm vs. concentration of DMSO. The reaction conditions are similar with the typical experiment described in Experimental Section with 1  $\mu\text{M}$  of  $\text{Cu}^{2+}$ , except the different amount of DMSO. The dash line indicates the absorbance of solution without DMSO.

### S-7 Influence of metal ions



**Fig. S6** Impact of 100 μM Cr<sup>3+</sup>, Fe<sup>3+</sup> or Fe<sup>2+</sup> on the absorbance of Ag NPs formed by Cu<sup>2+</sup> (2 μM, the first pillar) catalysis, measured at 440 nm and different heating time as indicated. The data were averaged over 3 replicates with error bar to show the standard deviation.

Some ions such as Fe<sup>3+</sup> and Cr<sup>3+</sup> can lower the reaction speed but can be recovered by ca. 5 min prolonging the heated reaction time as shown in **Fig. S8**. However, this is not applicable to Fe<sup>2+</sup> which can fortunately be oxidized back to Fe<sup>3+</sup>.



**Fig. S7** Plots of absorbance at 440 nm vs. the content of Hg<sup>2+</sup>. The reaction solution was composed of CTAB (0.48 mM), NaNO<sub>3</sub> (96 mM), BSA (2.4 μg/mL), AA (2 mM), AgNO<sub>3</sub> (0.5 mM), and Hg<sup>2+</sup> (as indicated in figure, labeled as Hg<sup>2+</sup> only), or Hg<sup>2+</sup> plus Cu<sup>2+</sup> (2 μM, labeled as Hg<sup>2+</sup>+Cu<sup>2+</sup>), or Hg<sup>2+</sup> plus Cu<sup>2+</sup> (2 μM) and citrate (50 μM, labeled as Hg<sup>2+</sup>+Cu<sup>2+</sup>+citrate), then mixed and heated at 95°C for 15 min. The data were averaged over 3 replicates with error bar to show the standard deviation.

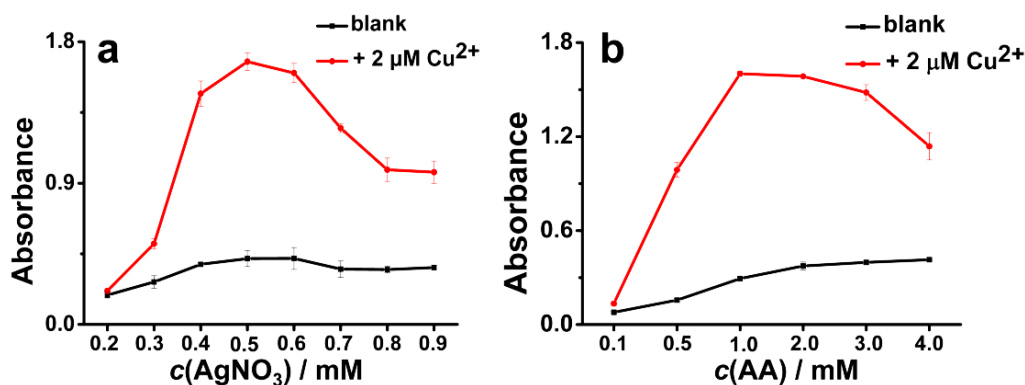
### S-8 influence of anions

**Table S1** Tolerance of coexisting anions measured at 2  $\mu\text{M}$   $\text{Cu}^{2+}$ <sup>a</sup>.

Anions	Tolerable concentrations / $\mu\text{M}$	Anions	Tolerable concentrations / $\mu\text{M}$
$\text{SO}_4^{2-}$	1000	$\text{H}_2\text{PO}_4^-$	100
$\text{Cl}^-$	500	$\text{Br}^-$	50
$\text{Ac}^-$	100	citrate	50
$\text{CO}_3^{2-}$	100	$\text{ClO}_3^-$	50
$\text{HPO}_4^{2-}$	100	$\text{NO}_2^-$	25

<sup>a</sup> By tolerance was meant the maximum concentration of coexisting substances causing a 5% absorbance decrease at 440 nm.

### S-9 Optimization of the prime system.



**Fig. S8** Plots of absorbance at 440 nm vs. concentrations of  $\text{AgNO}_3$  (a) and AA (b) averaged from 3 replicates with error bars to show the standard deviation. Detailed conditions are as follows: (a) The solution was composed of CTAB (0.48 mM),  $\text{NaNO}_3$  (96 mM), BSA (2.4  $\mu\text{g}/\text{mL}$ ), AA (2 mM),  $\text{AgNO}_3$  (as indicated in the figure), and  $\text{Cu}^{2+}$  (2  $\mu\text{M}$ ) or water (as blank), then mixed and heated at 95°C for 15 min; (b) The solution was composed of CTAB (0.48 mM),  $\text{NaNO}_3$  (96 mM), BSA (2.4  $\mu\text{g}/\text{mL}$ ), AA (as indicated in the figure),  $\text{AgNO}_3$  (0.5 mM), and  $\text{Cu}^{2+}$  (2  $\mu\text{M}$ ) or water (as blank), then mixed and heated at 95°C for 15 min.

### S-10 Calculation of dissolved $\text{O}_2$ concentration:

Dissolved  $\text{O}_2$  concentration was calculated by following formulas:<sup>3</sup>

$$\begin{cases} C_p = C^* \times P \times K \\ K = \left[ \frac{(1 - P_{wv} / P)(1 - \theta P)}{(1 - P_{wv})(1 - \theta)} \right] \\ \theta = 9.75 \times 10^{-4} - 1.426 \times 10^{-5} t + 6.436 \times 10^{-8} t \end{cases}$$

where  $C_p$  is the equilibrium oxygen concentration (mg/L) at a nonstandard pressure,  $P$  (atm),  $C^*$  the equilibrium oxygen concentration (mg/L) at the standard pressure (1 atm),  $t$  temperature ( $^{\circ}\text{C}$ ) and  $P_{wv}$  the partial pressure (atm) of water vapor, computed by:

$$\ln P_{wv} = 11.8571 - [3840.70 / (273.15 + t)] - [216,961 / (273.15 + t)^2]$$

According to China Meteorological Data Sharing Service System (<http://cdc.cma.gov.cn/index.jsp>), the atmospheric pressure of each month in Beijing was kept nearly constant but varied slightly from month to month as shown in **Table S2**. By these data, the related oxygen concentration and its dependence on temperature can be estimated in any a month as shown in **Table S3**.

**Table S2** Atmospheric pressure each month in Beijing averaged from 1971 to 2000.

Month	average pressure / kpa	Month	average pressure / kpa
1	102.42	7	99.97
2	102.20	8	100.37
3	101.74	9	101.05
4	101.00	10	101.67
5	100.57	11	102.13
6	100.12	12	102.38

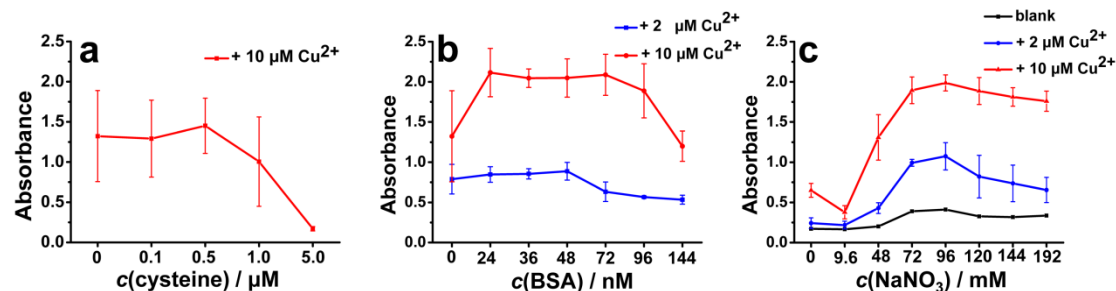
**Table S3** Dependence of dissolved  $\text{O}_2$  concentration on temperature for experiments performed in January.

$t/^{\circ}\text{C}$	$P_{wv}$	$\theta/10^{-4}$	K	$C^*/\text{mg} \cdot \text{L}^{-1}$ <sup>a</sup>	$C_p/\text{mg} \cdot \text{L}^{-1}$	$\text{O}_2$ concentration / mM
15	0.01683	7.756	1.00017	10.05	10.16	0.318
20	0.02307	7.155	1.00024	9.08	9.18	0.287
25	0.03126	6.587	1.00034	8.23	8.32	0.260
30	0.04188	6.051	1.00046	7.51	7.592	0.237
35	0.05549	5.547	1.00062	6.94	7.02	0.219
40	0.07280	5.076	1.00083	6.45	6.53	0.204
45	0.09458	4.636	1.00111	5.98	6.05	0.189

<sup>a</sup> Data from the Table 5.1, Section 5 in “Lange's Handbook of Chemistry (15th Edition)”, Dean, J. A.(Eds.), McGraw-Hill, Inc.,1999.

By **Table S2** and **Table S3**, it is clear that the dissolved O<sub>2</sub> concentration is controlled mainly by C\* due to its large dependence on temperature. The other factors have only a slight influence and are negligible. Accordingly, the final Ag NP concentration or color varied with the temperature as shown in **Fig. 6** in the main text.

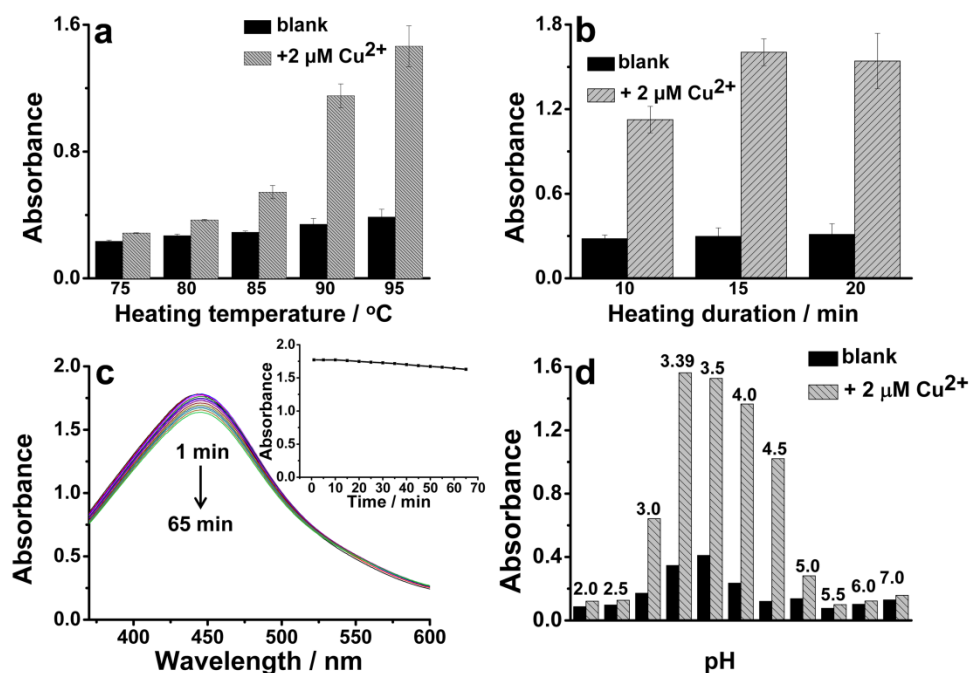
### S-11 Optimization of the additives



**Fig. S9** Plots of absorbance at 430 nm (a, b) and 440 nm (c) vs. the concentration of cysteine (a), BSA (b) and NaNO<sub>3</sub> (c) averaged from 3 replicates with error bars to show the standard deviation. Detailed conditions are as follows: (a) The solution was composed of CTAB (0.48 mM), NaNO<sub>3</sub> (96 mM), AA (2 mM), AgNO<sub>3</sub> (0.4 mM), Cu<sup>2+</sup> (10 μM), and cysteine (as indicated in the figure), then mixed and heated at 90°C for 15 min; (b) The solution was composed of CTAB (0.48 mM), NaNO<sub>3</sub> (96 mM), AA (2 mM), AgNO<sub>3</sub> (0.4 mM), Cu<sup>2+</sup> (2 or 10 μM), and BSA (as indicated in the figure), then mixed and heated at 90°C for 15 min; (c) The solution was composed of CTAB (0.48 mM), NaNO<sub>3</sub> (as indicated in the figure), BSA (2.4 μg/mL), AA (2 mM), AgNO<sub>3</sub> (0.4 mM), and Cu<sup>2+</sup> (2 or 10 μM) or water (as blank), then mixed and heated at 95°C for 15 min.



**S-12 Influence of other parameters (heating temperature and duration, the stability of formed Ag NPs, and pH values)**



**Fig. S10** Plots of absorbance at 440 nm vs. heating temperature (a), heating duration (b), and pH values (d) averaged from 3 replicates with error bars to show the standard deviation. The spectra in (c) were all measured from a 2-μM Cu<sup>2+</sup>-catalyzed reaction solution stored for 1-65 min at an interval of 5 min, where the inset shows the change of the absorbance at 440 nm versus time. Detailed conditions are as follows: (a) The solution was composed of CTAB (0.48 mM), NaNO<sub>3</sub> (96 mM), BSA (2.4 μg/mL), AA (2 mM), AgNO<sub>3</sub> (0.4 mM), and Cu<sup>2+</sup> (2 μM), then mixed and heated at different temperature (as indicated in the figure) for 15 min; (b) The reaction conditions are similar with the typical experiment described in Experimental Section, except the heating duration (as indicated in the figure). (c) The reaction condition is same with the typical experiment described in Experimental Section with Cu<sup>2+</sup> (2 μM). (d) The reaction conditions are similar with the typical experiment described in Experimental Section with Cu<sup>2+</sup> (2 μM) or water (as blank), except the pH (as indicated in the figure and adjusted with NaOH or HNO<sub>3</sub>). The pH value of 3.39 is that of original system.

### S-13 Calculation of recovery in real samples determination

**Preparation of spiked samples.** For spiked water samples, 10, 12.5, 15, 17.5 or 20  $\mu\text{L}$  of  $\text{CuCl}_2$  standards (1 mM) were added into 1 mL samples, respectively, resulting in a spiked concentration of 9.9, 12.3, 14.8, 17.2 or 19.6  $\mu\text{M}$ . However, when samples were treated by chelating resin and be made up to 1 mL, the spiked concentration should be 10.0, 12.5, 15.0, 17.5 or 20.0  $\mu\text{M}$ .

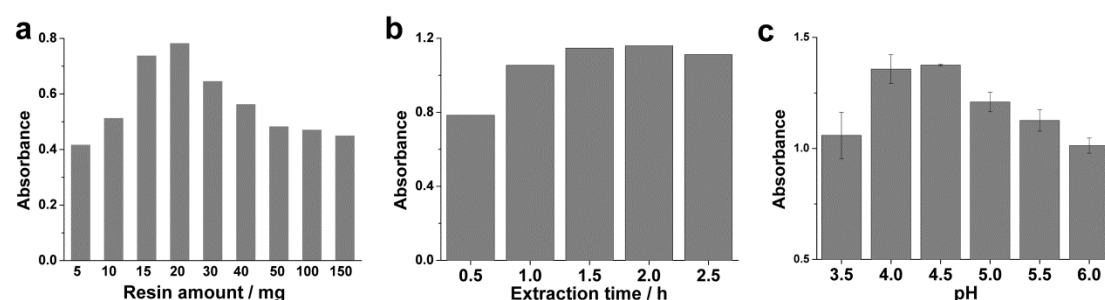
For sera samples, 2.5 or 5.0  $\mu\text{L}$  of  $\text{CuCl}_2$  standards (1 mM) were added into 0.5 mL samples before deproteinization. When samples were treated by chelating resin and be made up to 1 mL, the final spiked concentration should be 2.5 or 5.0  $\mu\text{M}$ . But for the original samples (0.5 mL), it should be 5.0 or 10.0  $\mu\text{M}$ .

**Calculation of recovery.** For the samples was diluted tenfold (0.1 mL to a final volume of 1 mL), the determined concentration ( $c_{\text{determined}}$ ) was one-tenth of the original value. So the recovery can be calculated as follows:

$$\text{recovery} = \frac{10 \times c_{\text{determined}}}{c_{\text{spiked}}} \times 100\%$$

where  $c_{\text{spiked}}$  is the spiked concentration mentioned above.

**S-14 Selection of some critical factors for sample preparation:** In the treatment of a sample with a high content of disturbing anions, some critical conditions should be better optimized including the amount of resin required, pH value and extraction time. **Fig. S11** shows that the resin finds its maximum at 20 mg per 1 mL sample and pH value at pH 4.5. The extraction time can be selected at 1.5-2 h.



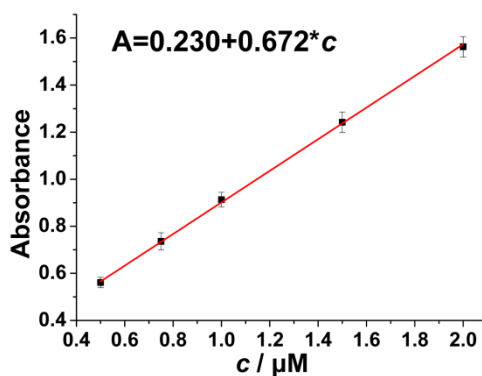
**Fig. S11** Influence of resin amount (a), extraction time (b) and pH value (c) on the back-extraction efficiency measured from 1 mL mimic serum solution (**Table S4**) added with 1 mL acetic buffer at pH 5 (a, b) or variable pH values as indicated in (c). The resin used in b and c was weighed 20 mg, and the extraction durations for a and c were 0.5 and 2 h, respectively.

**Table S4** Content of serum mimic solution used in chelating resin treatment experiments.

Salts	Concentration/mM	Salts	Concentration/mM
NaCl	100	NaH <sub>2</sub> PO <sub>4</sub>	1
NaHCO <sub>3</sub>	27	MgSO <sub>4</sub>	1
NaAc	10	FeCl <sub>3</sub>	0.03
KCl	4	ZnCl <sub>2</sub>	0.02
CaCl <sub>2</sub>	2.5	CuCl <sub>2</sub>	0.02

### S-15 Re-established calibration curve for resin-treated samples

As mentioned in S-1, in resin-treated experiments, 0.1 mL HNO<sub>3</sub> (2 M) was used to elute the metal ions from chelating resin, then neutralized with NaOH, and made up to 1 mL solution with water, the salt (NaNO<sub>3</sub>) concentration in the final solution was 200 mM. The same concentration of NaNO<sub>3</sub> was hence added to the standard Cu<sup>2+</sup> solution to establish a new calibration curve for the quantitation of Cu<sup>2+</sup> in samples treated in this way.



**Fig. S12** The linear relationship ( $R^2 = 0.999$ ) between the absorbance at 435 nm and Cu<sup>2+</sup> concentration. 200 mM of NaNO<sub>3</sub> is added into Cu<sup>2+</sup> standards in accordance with the samples treated by resin.

### References

- 1 Peterson, R. E.; Bollier, M. E. *Anal. Chem.* **1955**, 27, 1195–1197.
- 2 Mumford, K. A.; Northcott, K. A.; Shallcross, D. C.; Snape, I.; Stevens, G. W. *J. Chem. Eng. Data* **2008**, 53, 2012–2017.
- 3 <http://www.waterontheweb.org/under/waterquality/oxygen.html>, and references therein.