Supporting Information

Cu$^{2+}$-catalyzed and H$_2$O$_2$-facilitated oxidation strategy for sensing copper (II) based on cysteine-mediated aggregation of gold nanoparticles

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Fig. S1  UV-vis spectra of (a) 2400 μL PEG-AuNPs + 600 μL H$_2$O and (b) 2400 μL PEG-AuNPs with the addition of 600 μL mixtures of buffer (50 mM; pH 5.0) and H$_2$O$_2$ (1 mM). The inset shows the corresponding images of color.
Fig. S2 (a) Absorbance ratio of 2400 μL AuNPs after adding different concentrations of 600 μL NaCl (red). (b) Absorbance ratio of PEG-AuNPs with various amounts of NaCl (blue). Conditions: 2400 μL PEG-AuNPs + 600 μL NaCl. The concentration of PEG in the AuNPs nanodispersion is 0.125 g/L.

Fig. S3 Effect of PEG concentration on the absorbance ratio of AuNPs (2400 μL) added with 600 mL of buffer (50 mM) and cysteine (25 μM). The concentrations of PEG in the AuNPs were 0, 0.05%, 0.125%, 0.15%, 0.2%, 0.3% and 0.5%, respectively.
**Fig. S4** Absorbance ratios of 2400 μL PEG-AuNPs after the addition of 600 μL mixture solutions containing cysteine (25 μM), H₂O₂ (1 mM) and Cu²⁺ (0 μM; 0.4 μM; 1.0 μM; 2.0 μM) in buffer at different reaction time.

**Fig. S5** Effect of pH on the value of A₆₅₀/A₅₂₀ of the PEG-AuNPs-based detection system in the absence (red) and presence (blue) of 0.4 μM Cu²⁺.
Fig. S6 $A_{650}/A_{520}$ values of the PEG-AuNPs solutions as a function of concentration of buffer solution.

Fig. S7 Absorption ratios corresponding to the 2400 μL PEG-AuNPs added with 600 μL of buffer (50 mM) and cysteine (25 μM), treated with different concentrations of $\text{H}_2\text{O}_2$ in the presence of 1 μM and 2 μM Cu$^{2+}$ for a fixed time interval of 10 min, respectively.
Cuprous salt is normally insoluble in water. Even if there is a trace amount of Cu$^+$ dissolved in water, Cu$^+$ would decompose in the sense of the equation: $Cu^+ \rightarrow Cu^{2+} + Cu$. Also, Cu$^+$ might be oxidized readily by oxygen dissolved in water to Cu$^{2+}$. To obtain a high concentration of soluble Cu$^+$, 0.1 M cupric sulfate (CuSO$_4$) aqueous solution was firstly mixed with 0.1 M ascorbic acid (AA) to produce Cu$^+$ in this work. Different concentrations of Cu$^+$ working solutions were freshly prepared by dilution from this stock solution. Fresh solution of AA was prepared daily. For detecting Cu$^+$, the experiment procedure is the same as that for detecting Cu$^{2+}$ described in the manuscript. As we know, AA is a powerful reducing agent. In the presence of AA, Cu$^{2+}$ would be reduced to Cu$^+$. One molecule of AA can react with two molecules of Cu$^{2+}$. In our study, a 1:1 (AA/Cu$^{2+}$) ratio is used. So AA is excessive in amount. Higher levels of AA in the solution could reduce the amount of
Cu$^{2+}$ formed and prevent oxidation of Cu$^+$ by O$_2$ which would reacts with AA. As shown in Fig. S9a, no precipitate produced after Cu$^{2+}$ added with AA. Fig. S9b shows the effect of the reaction between cysteine/H$_2$O$_2$ and Cu$^+$ in buffer solution on the aggregation of the PEG-AuNPs. In the experiment the cysteine/H$_2$O$_2$ system was allowed to react with different concentrations of Cu$^+$ for 10 min in buffer, and subsequently the PEG-AuNPs were added to the reaction mixture and the extension of stimulated aggregation of the PEG-AuNPs was followed after a 5 min interval. Evidently, as the concentration of Cu$^+$ increases, the degree of aggregation was inhibited, as reflected in the UV-vis spectra, with an increased absorption at 520 nm ($A_{520}$) and a decreased absorption at 650 nm ($A_{650}$). The ratio of $A_{650}$/A$_{520}$ decreased as the concentration of Cu$^+$ increased in the cysteine/H$_2$O$_2$ reaction mixture. Therefore, the concentration of Cu$^+$ can also be detected with the naked eye or with UV-vis spectroscopy, and the detection limits of Cu$^+$ were 20 μM and 10 μM, respectively. The detection limits of Cu$^+$ are not the same as that of Cu$^{2+}$. Also, the color change or UV-vis spectroscopy of PEG-AuNPs is different when the concentration of Cu$^{2+}$ is equal to Cu$^+$. So, if Cu$^{2+}$ and Cu$^+$ coexist in water, we would not distinguish the signal of Cu$^{2+}$ from that of Cu$^+$. In other words, we could not detect two kinds of copper ions (Cu$^{2+}$ and Cu$^+$) at the same time in aqueous solution. Fortunately, Cu$^+$ is not stable in aqueous solution, as mentioned above.
Fig. S9  (a) Solutions of 0.1 M CuSO$_4$ and 1 mL of 0.1 M CuSO$_4$ added with 1 mL of 0.1 M AA. (b) Photograph of the sensor solutions for various Cu$^+$ concentrations, which are, in μM, listed next to the respective solutions. (c) Absorbance spectra corresponding to the cysteine-stimulated aggregation of the PEG-AuNPs that probe the Cu$^+$-catalyzed oxidation of cysteine to cystine in buffer by H$_2$O$_2$ in the presence of different concentrations of Cu$^+$. (d) Absorbance ratios between blue (650 nm) and red (520 nm) derived from the colorimetric responses of PEG-AuNPs as a function of concentration of Cu$^+$.

The presence of trace contaminant iron in buffers and reagents could lead to an inhibitory in rate in studies of Cu$^{2+}$-catalyzed oxidation reactions, as reported in reference 31. To increase the rate of Cu$^{2+}$-catalyzed oxidation of cysteine, buffers should be purified, and all contact with glass should be avoided, furthermore, buffers and reagents were required to be made up in new plastic containers. However, it is difficult, tedious and expensive to use purification procedures. Moreover, as shown in Fig. 3 in the reference above, inhibition of Cu$^{2+}$-catalyzed oxidation of cysteine by
iron is almost saturated when the concentration of iron salts is more than 15 nM. However, in our experiment, all the reagents used were not further purified. We found that iron did not interfere with the detection of Cu$^{2+}$ according to our interference experiment. It is supposed that the iron concentration have exceeded the critical value that could affect the detection of Cu$^{2+}$ in our study. To further investigate the influence of Fe$^{3+}$, different concentrations of Fe$^{3+}$ on the cysteine-stimulated aggregation of the PEG-AuNPs have been examined. The reaction conditions were the same as those of the typical assay described in the experiment section of the manuscript. Different concentrations of Fe$^{3+}$ were added alone or together with Cu$^{2+}$ (10 µM) to the reaction mixtures including cysteine/H$_2$O$_2$ and buffer for a 10 min reaction time, and then incubated with PEG-AuNPs for a further 5 min. As can be seen from Fig. S10, the sole addition of Fe$^{3+}$ to the reaction mixtures could not lead to any change of color and the UV−vis spectra of the PEG-AuNPs dispersion. Contrarily, the addition of 10 µM Cu$^{2+}$ together with Fe$^{3+}$ clearly resulted in a decrease in $A_{650}$ and an increase in $A_{520}$ at the same time. Accordingly, the color of the dispersion also changed from blue to the initial wine red. It can also be seen from Fig. S10 that the spectra almost remained the same when the concentration of Fe$^{3+}$ was varied from 0.01 µM to 1000 µM. These results probably suggest that Fe$^{3+}$ could not affect Cu$^{2+}$-catalyzed oxidation of cysteine by H$_2$O$_2$ in our assay.
Fig. S10 (a) Photographs of PEG-AuNPs prepared by adding Fe$^{3+}$ alone or along with Cu$^{2+}$ to the mixtures containing cysteine, buffer and H$_2$O$_2$. The concentrations of Fe$^{3+}$ are, in μM, listed above the respective solutions. And the concentration of Cu$^{2+}$ which is added together with Fe$^{3+}$ is 10 μM. (b) UV−vis spectra of PEG-AuNPs prepared by adding different concentrations of Fe$^{3+}$ to the reaction mixtures. (c) UV−vis spectra of PEG-AuNPs prepared by adding different concentrations of Fe$^{3+}$ along with Cu$^{2+}$ to the reaction mixtures.

The pH of real water samples was measured with a PHS-3C digital pH meter manufactured by INESA Scientific Instrument Co., Ltd. (Shanghai, China). The pH of ground water and tap water were 6.71 and 7.43, respectively. The analysis of the real samples was performed according to the typical conditions optimized in the manuscript. So buffer should be added into the real samples. When buffer was added, the pH value of the mixture solution could be maintained stable. Also the appropriate ionic strength is essential for the detection of Cu$^{2+}$. If buffer was not added, the PEG-AuNPs would not aggregate in our study. This has been discussed in the manuscript. The spectra of the real samples before and after addition of Cu$^{2+}$ are shown in Fig. S11.
Fig. S11 Absorbance spectra of the PEG-AuNPs for probing Cu$^{2+}$ in real samples using our sensor. The reaction conditions were the same as that of the typical assay described in the manuscript. The detection of Cu$^{2+}$ in ground water (a, c, e, g) and tap water (b, d, f, h) has been performed, respectively. The concentrations of Cu$^{2+}$ were (a, b) 0, (c, d) 0.2 $\mu$M, (e, f) 0.5 $\mu$M and (g, h) 1.0 $\mu$M. Absorbance spectra of three replicated experiments have been done for every sample.


