

A visual volumetric hydrogel sensor enables quantitative and sensitive detection of copper ions

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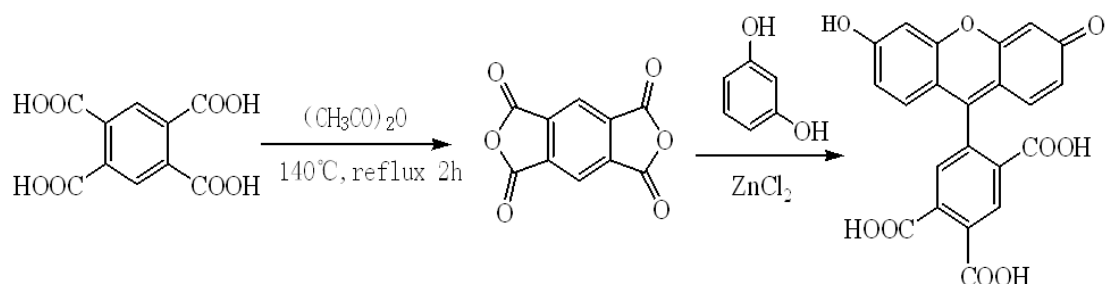
S1. Chemical and general methods

Chemicals Acrylamide and polyethylene glycol-6000 (PEG-6000) from Tianjin Kemiou Chemical Reagent Co., Ltd., potassium persulfate (KPS) and copper sulfate (CuSO_4) from Xi'an Chemical Reagent Factory, sodium hypochlorite (NaClO) from Tianjin Fu-chen Chemical Reagent Science and Technology Co., Ltd., pyromellitic acid ($\text{C}_{10}\text{H}_6\text{O}_8$, 99%) from Quzhou Rui-feng Chemical Co., Ltd, ethanol (EtOH), ethanediolglycol (EG), acetic anhydride (Ac_2O), and m-dihydroxybenzene ($\text{C}_6\text{H}_6\text{O}_2$) from Sinopharm Chemical Reagent Co., Ltd. were used without further purification. All water was distilled deionized water.

General methods Fourier transmission infrared spectra (FTIR) were recorded on a Thermo Nicolet FT-IR330 model spectrophotometer at wave numbers between 400 and 4000 cm^{-1} . The hydrogel structure was characterized by a FEI Quanta 200 model (SEM) and JEM-2100 model (TEM). Photo images were taken using an Olympus SZ-11 model digital camera. The TEM sample was prepared according to the literature method.^[1] Diluted drops of hydrogel suspensions were allowed to dry slowly on carbon coated copper grids. To promote the contrast, the hydrogel was negatively stained with 2% phosphotungstic acid by dropping 10 μl of the phosphotungstic acid on the grid. Then, the grid was air-dried and observed by TEM.

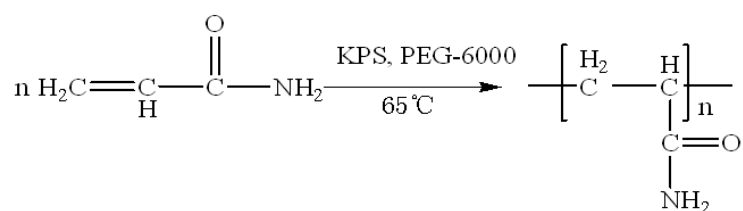
S2. Hydrogel synthesis

Reaction 1



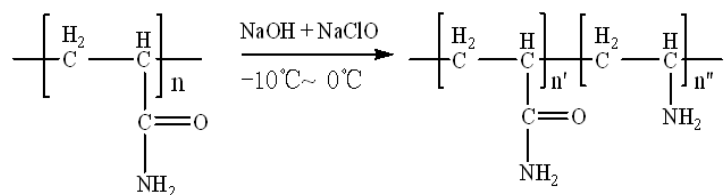
Synthesis of 5,6-dicarboxyfluorescein (5,6-DCF)^[2,3] 25.4 g pyromellitic acid was dissolved in 19 mL acetic oxide, and then underwent reflux for 2 h. The resultant pyromellitic dianhydride was filtered out, washed with diethyl ether, dried in vacuo. A certain amount of pyromellitic dianhydride (5.45 g, 0.025 mol), bright or pale yellow, was added into resorcinol (5.50 g, 0.05 mol) under vigorous agitation, which had been melted at 150°C beforehand (The temperature is reported as bath temperatures). The reaction was then immediately accelerated by the addition of fused ZnCl₂ (6.80 g, 0.05 mol), and the reaction temperature was increased and eventually remained at 180°C for over 20 min until the reactive mixture solidified. The resultant 5,6-DCF was ground and boiled in 100 ml of 0.50 M HCl for 1 h. The red ground powder was collected, washed with water, and dried in vacuo. At last the 5,6-DCF powder was purified on silica chromatographic column with eluent of isopropanol /concentrated ammonia (volume ratio 2:1).

Reaction 2



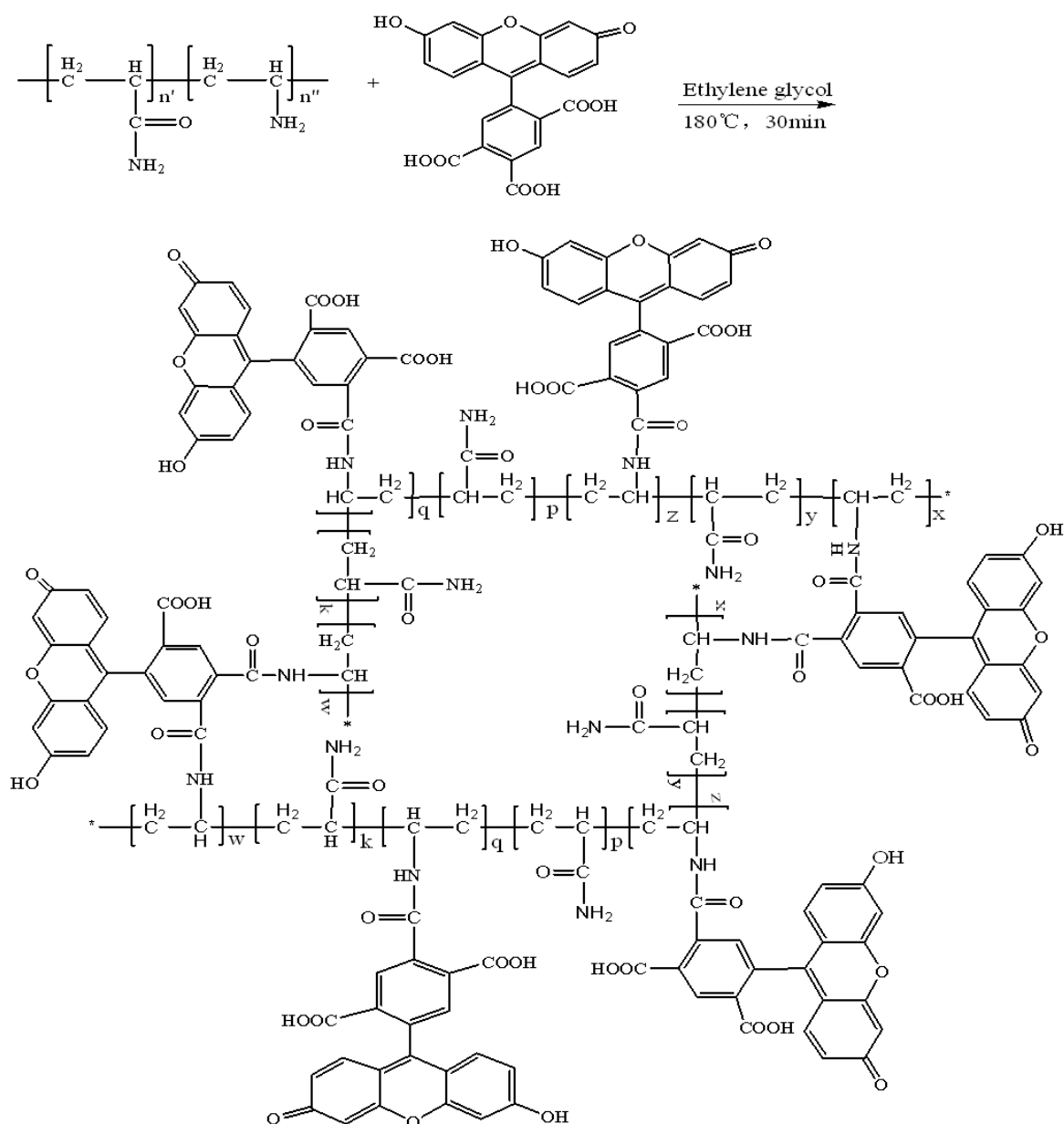
Synthesis of polyacrylamide (PAM) PAM was synthesized according to the conventional method^[4]. Typically, 5.67 g acrylamide was blended with 0.109 g KPS and 0.56 g PEG-6000, the mixture was then dissolved in 120 mL distilled water, and the polymerizations was conducted at 65°C for 2 h. Thereafter, the resultant white solid polyacrylamide was isolated, rinsed by ethanol, collected by filtration and dried in vacuo at 50°C to constant weight.

Reaction 3



PAM amination Via Hoffman degradation,^[4] amide groups in PAM were partially converted into amine groups. 22.5 mL of 2.5 M sodium hypochlorite solution (Titrated through iodometry prior to use) was added into 150 mL of 0.37 M sodium hydroxide solution. The mixture was stirred and cooled to -10°C- 0°C. A solution of PAM aqueous solution (0.00523 g/mL) was added to the mixture. One hour later, 41.8 g of sodium hydroxide was gradually added into the reaction system, and the reaction was kept at -10°C-0°C for 6 hours. After completion of Hoffman degradation, the resultant product was isolated by precipitation from ethanol, dissolved under stirring in a 6 M hydrochloric solution while keeping the pH of the solution lower than 2, precipitated with ethanol again. In the finish, the product was rinsed with ethanol and dried in vacuo at 50°C to a constant weight.

Reaction 4



Where, k , n' , n'' , p , q , w , x , y and z represent the number of chain links.

Synthesis of hydrogel^[5] The hydrogel was synthesized through the dehydration between the amine group of aminated PAM and carboxylic group of 5,6-DCF. 1.2 g aminated PAM was dissolved in 30 mL ethylene glycol and heated to 110°C , and then 60 mg 5,6-DCF was added and dissolved. After it, the solution was gradually heated to 180°C and kept for 30 min with in vacuo. The obtained yellow product was kept in 1 M sodium hydroxide for 2 h at 100°C . The obtained hydrogel was alternatively cleaned with ethanol and water until the water eluate become colorless. Hydrogel obtained was dried in vacuo at 50°C to a constant weight, then ground to 80-120 mesh particles.

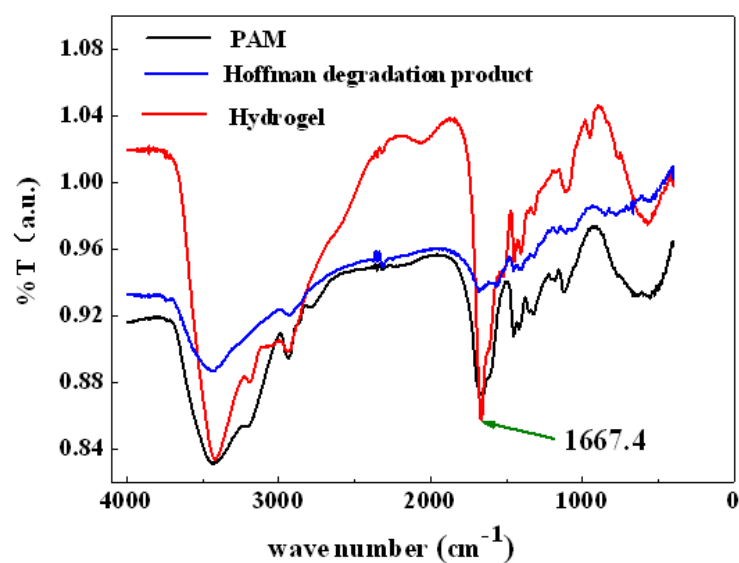


Fig. S1 FTIR spectra of PAM, Hoffman degradation product, and hydrogel

FTIR Characterization The successes of Reaction 3 and 4 were verified via FTIR characterization of PAM, Hoffman degradation product, and hydrogel as showed above. Peaks at 3199.4 cm⁻¹ and 3416.6 cm⁻¹ represent the symmetric and asymmetric stretching vibration of amide N-H; peak at 1667.4 cm⁻¹ represents the symmetric stretching vibration of C=O. In comparison with PAM and its Hoffmann degradation product, the obvious peak strength change at 1667.4 cm⁻¹ supports the disappearance of amide groups and the formation of amine groups. Similarly, the strengthen absorption at 1667.4 cm⁻¹ points to the formation of amide groups in Reaction 4.

Hydrogel appearance The appearance of hydrogel was showed in the following Fig. S2.

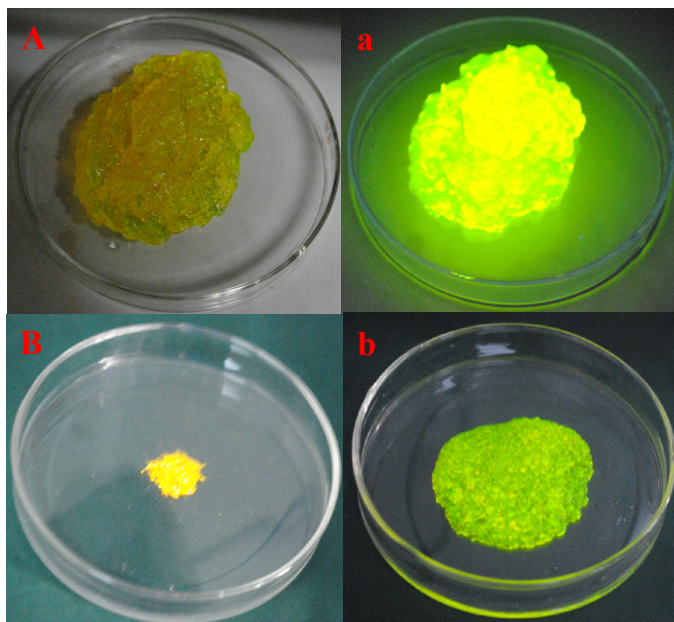


Fig. S2 (A) hydrogel under natural and (a) ultraviolet light; (B) dried and (b) water swelled hydrogel particles under ultraviolet light.

S3. Amine and carboxylic groups in hydrogel

According to literature method^[6], the contents of amine group in Hoffmann degradation product were measured. It was found that about 19.4% of amide groups in PAM were converted into amine groups. By measuring the contents of 5,6-dicarboxyl fluorescein in water eluent mentioned in “Synthesis of hydrogel” section, and considering each 5,6-dicarboxyl fluorescein reacted with two amine groups, we calculated that about 16.3% of amine groups reacted with the carboxylic groups. As such, the shares or ratios of amide, amine and carboxylic groups in hydrogel are 80.6 : 13 : 3.2, or 25 : 4 : 1.

For each sensor, about 0.06 g dry hydrogel particle is needed. The contents of amide, amine and carboxylic groups in molar concentration were also estimated as follows.

The molar mass of the acrylamide, vinylamine and 5,6-dicarboxyl fluorescein were 71, 43, and 489, respectively.

The average molar mass of the single chain link in hydrogel can be estimated as,

$$M_r = 71 \times (1 - 19.4\%) + 43 \times (19.4\% - 3.2\% \times 2) + 489 \times 3.2\% = 78.46 \text{ g/mol}$$

Then,

$$n(-\text{CONH}_2) = 0.06 / 78.46 \times 80.6\% = 6.16 \times 10^{-4} \text{ mol}$$

$$n(-\text{NH}_2) = 0.06 / 78.46 \times 13\% = 9.94 \times 10^{-5} \text{ mol}$$

$$n(-\text{COO}) = 0.06 / 78.46 \times 3.2\% = 2.45 \times 10^{-5} \text{ mol}$$

In a sensor, the actual volume occupied by water swelled hydrogel is 4.5 mL. So, the concentration of amine and carboxylic groups can be calculated.

$$C_{\text{amine}} = 9.94 \times 10^{-5} \div 0.0045 = 2.21 \times 10^{-2} \text{ M}$$

$$C_{\text{carboxyl}} = 2.45 \times 10^{-5} \div 0.0045 = 5.44 \times 10^{-3} \text{ M}$$

Above results indicate that the hydrogel is abundant in amine and carboxylic groups. For Cu^{2+} detection, the sensor is immersed in 100 mL sample solution. The assumed concentrations of hydrogel amine and carboxylic groups in 100 mL sample solution are $9.94 \times 10^{-4} \text{ M}$ and $2.45 \times 10^{-4} \text{ M}$, respectively.

S4. Apparent hydrogel swelling rate

Since the difficulty of measuring the volumes of a 3D irregular dried or a fully swelled particle. Here, the apparent swelling rate was measured in consistency with the followed sensor establishment. A certain amount of dry hydrogel particles was weighed and their volume was measured by using a 1.0 mL calibrated graduated pipette whose out mouth was sealed with paraffin. After immersing the pipette in water for 30 minutes, the volume of fully swelled hydrogel was measured by using a 5 mL calibrated graduated pipette. The hydrogel swelling rate in volume was calculated to be 70 times in water.

Following the same procedure, the hydrogel apparent swelling rate in 2 mM borate buffer was estimated to be 67 times.

S5. Sensor preparation and detection procedures

Dry hydrogel particles underwent thorough swelling in 2 mM borate buffer. With the aid of a drip pipe, a proper amount of hydrogel particles was filled into a 5 mL calibrated graduated pipette, of which one end had been stopped beforehand with cotton. Programmed vibration of the pipette involving up and down direction was then conducted to make sure the sensor was close-packed with its initial volume readout at 0.0 mL. The success of a sensor preparation was checked as follows: after being immersed into 100 mL borate buffer for 10 min, the same vibration program was done to make sure the sensor' volume readout is at its original 0.0 mL.

When the sensor was applied to sample, likewise, the sensor was immersed in 100 mL sample solution (in 2 mM borate buffer) in a 100 mL graduated cylinder. 10 min later, the sensor was taken out and underwent the established operation. Then we can readily readout the shrinkage volume, even the Cu^{2+} concentration if the premade auxiliary concentration scale bar was used (as in Fig. S8). All the procedures for sensor preparation and detection are summarized in Fig. S3.

Figure S3

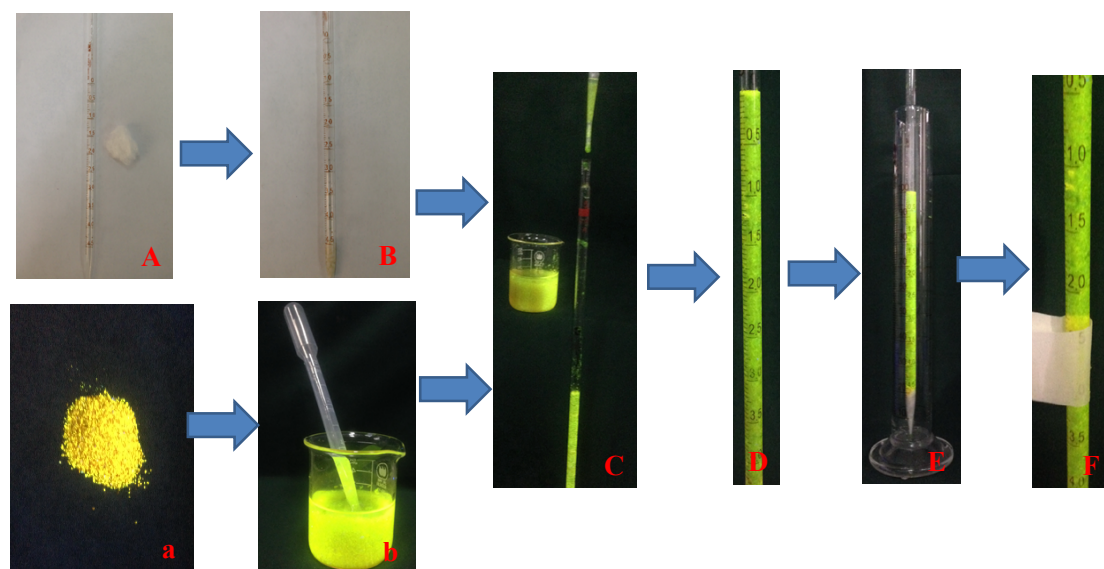


Fig. S3 Scheme of the sensor preparation and detection operation. A, a 5.0 mL graduated pipette and cotton; B, the end mouth sealed pipette; a, dried hydrogel particles; b, fully water swelled hydrogel; C, filling swelled particles into pipette with a drip pipe; D, the constructed sensor; E, sensor immersed in 100 mL sample solution in a 100 mL graduated cylinder; F, removing drainage water with filter paper before volume readout.

Figure S4

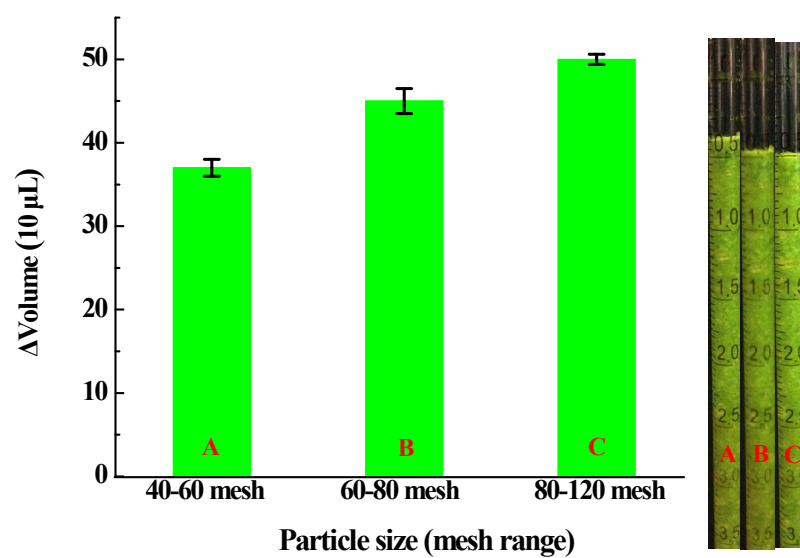


Fig. S4 Effect of hydrogel particle size on sensitivity. Cu^{2+} concentration, 1.0 μM ; red letters indicating the correspondence to experiment records.

Figure S5

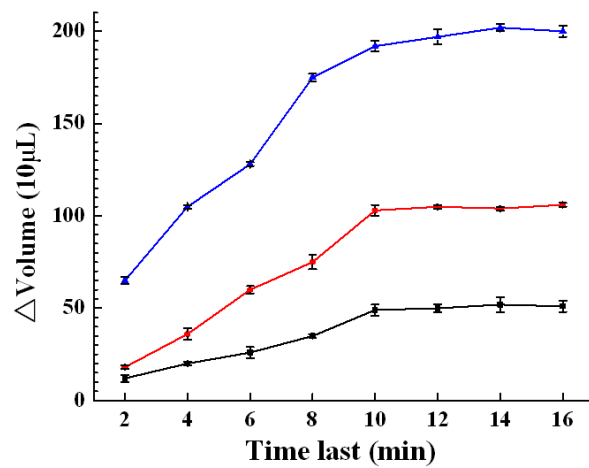


Fig. S5 Responsive kinetics of hydrogel volumetric sensor with respect to Cu^{2+} concentrations of 1.0 μ M (black), 5.0 μ M (red) and 12.0 μ M (blue) in 2 mM pH=7.4 borate saline buffer.

Figure S6

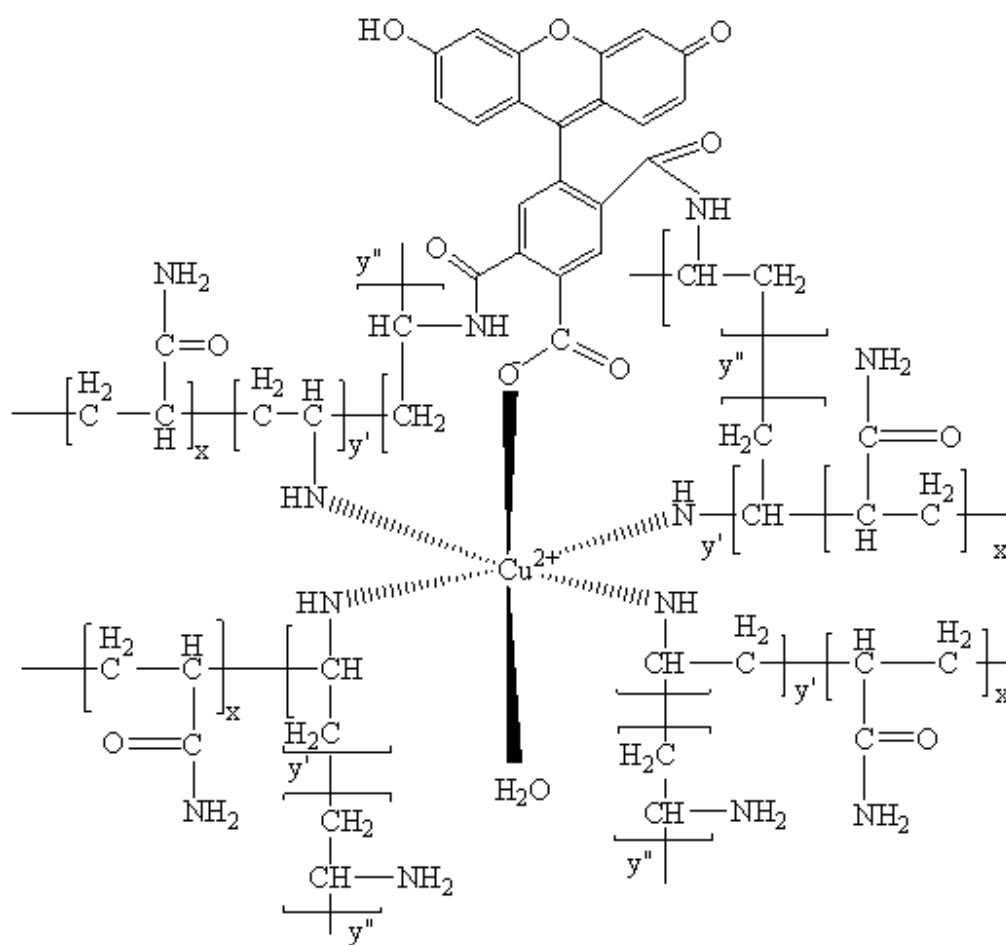


Fig. S6 Assumed schematic structure of Cu^{2+} chelate with amine and carboxylic groups in hydrogel.

Figure S7

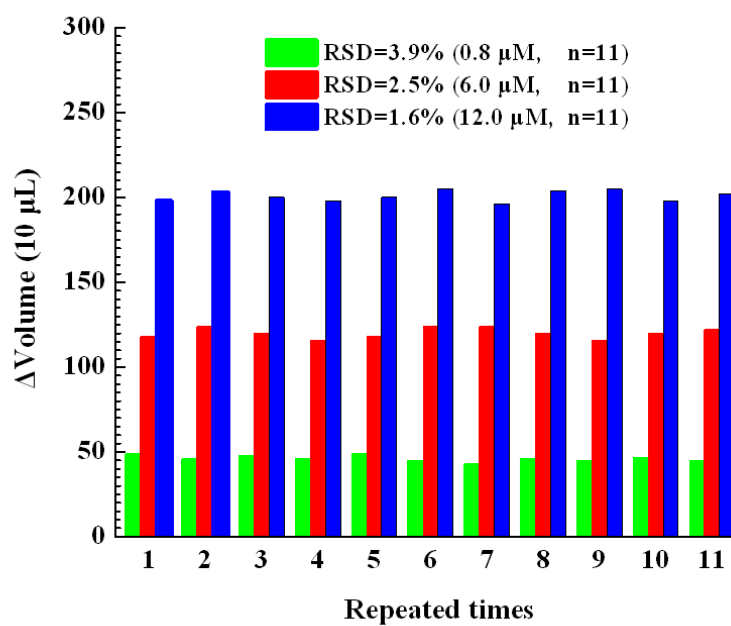


Fig. S7 Investigation of sensor reproducibility with respect to Cu^{2+} concentrations of 0.8 μM (green), 6.0 μM (red) and 12.0 μM (blue).

Figure S8

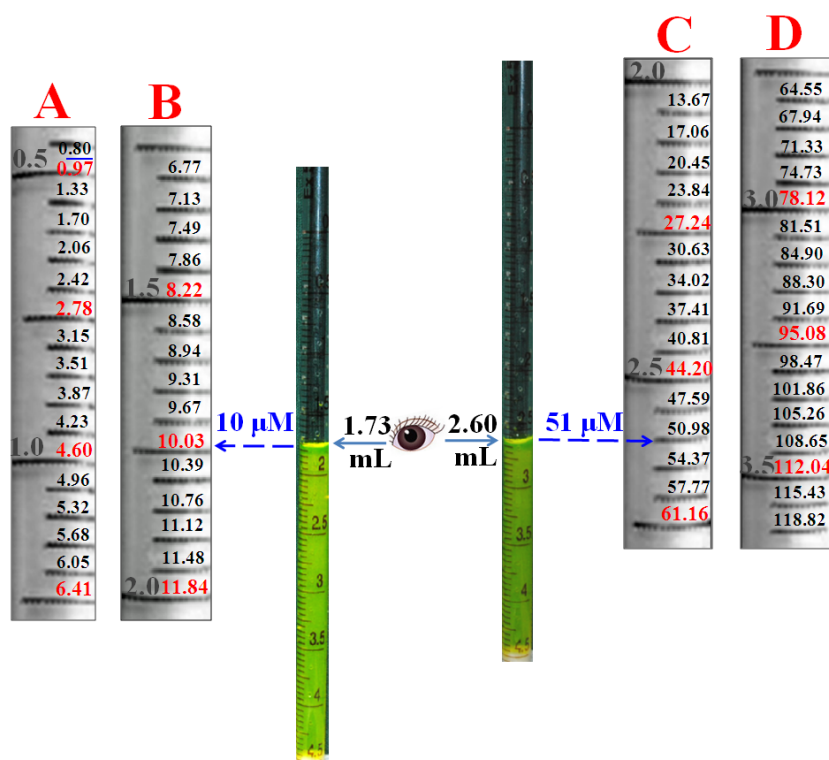


Fig. S8 Scale bar and its use for direct Cu²⁺ concentration readout.

A, B, C, and D are the divided sections of an entire scale bar, they correspond to four concentration ranges of 0.8 to 6.41 μM, 6.41 to 12.00 μM, 12.0 to 61.16 μM, and 61.16 to 120 μM), respectively. The bigger dark figures (0.5, 1.0,...3.0) on the left side of scale bar are the volume graduations of pipette, while those smaller on the right side are the corresponding copper concentrations in μM.

Figure S9

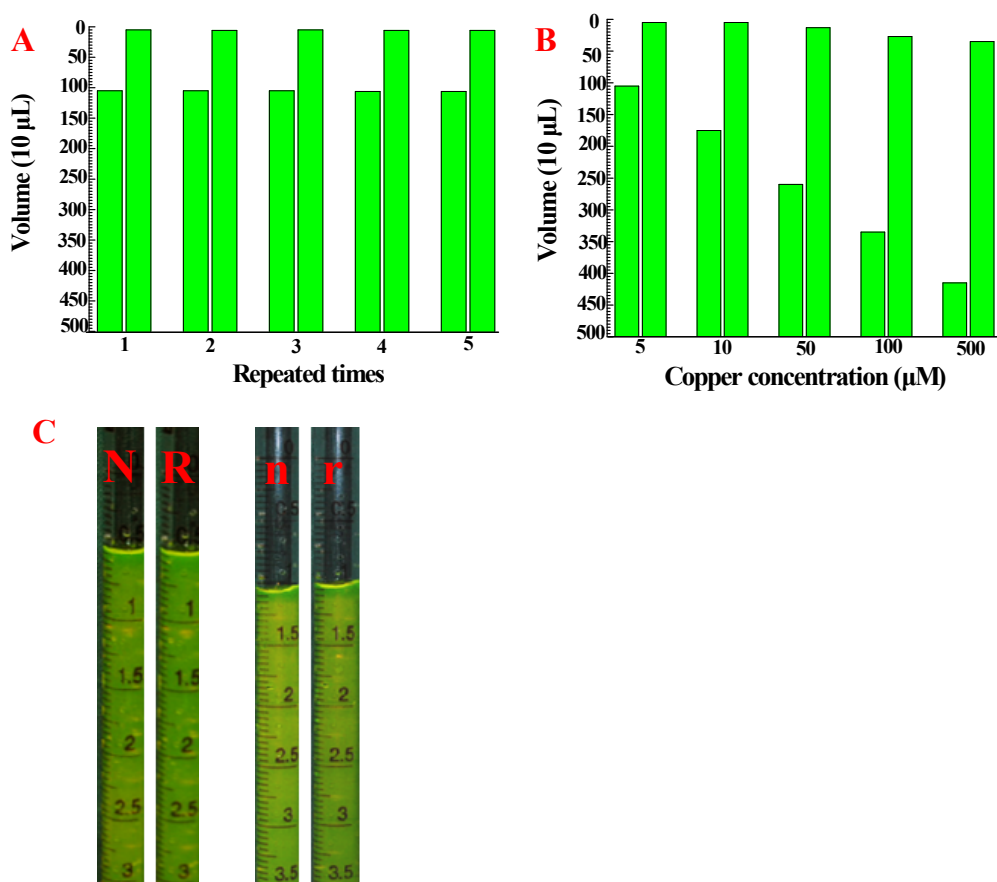


Fig. S9 Recovery of sensor. A, a sensor response to 5.0 μM Cu^{2+} (left column) and that after recovery with 0.1 mM EDTA (right column) for 5 times; B, sensors response to 5.0 μM , 10 μM , 50 μM , 100 μM , and 500 μM Cu^{2+} (left column) and those after recoveries (right column) with 0.1 mM EDTA; C, the fresh hydrogel (N and n) and recovered hydrogel (R and r) response to 1.0 μM (N and R) and 5.0 μM Cu^{2+} (n and r). For A and B, hydrogel was recovered in the pipette. For C, hydrogel particles from many sensors were collected and recovered together.

Table S1

Tab. S1 Applications of present sensor to environmental water samples in contrast to ICP-MS method

Sample	Present work ($\mu\text{M}\pm\text{SD}$) ^b	ICP-MS ($\mu\text{M}\pm\text{SD}$) ^b	Difference (%)
Swimming pool water	1.47 \pm 0.15	1.40 \pm 0.01	+2.4%
Tap water ^a	0.884 \pm 0.041	0.863 \pm 0.019	+5.0%
Tap water	undetected	0.0833 \pm 0.0029	-

a, the initial flow of over night tap water from a brass faucet.

b, triplicate measurements.

Table S2**Tab. S2** Determination of Cu²⁺ in spiked water samples (n=3).

Sample	Cu ²⁺ spiked (μM)	Cu ²⁺ Found (μM) ^a	Recovery (%)
Tap water	1.00	1.09 ±0.09	109
	3.00	3.06±0.11	102
	5.00	5.03±0.15	100.6
Lake water	1.00	0.95±0.08	95
	3.00	2.98±0.10	99.3
	5.00	4.96±0.15	99.2
River water	1.00	1.03±0.06	103
	3.00	3.13±0.12	104.3
	5.00	5.25±0.16	105

a, results from triplicate measurements.

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