Multipath colourimetric assay for copper(II) ions utilizing MarR functionalized gold nanoparticles

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We use the multiple antibiotic resistance regulator (MarR), as a highly selective biorecognition elements in a multipath colourimetric sensing strategy for the fast detection of Cu²⁺ in water samples. The colourimetric assay is based on the aggregation of MarR-coated gold nanoparticles in the presence of Cu²⁺ ions, which induces a red-to-purple colour change of the solution. The colour variation in the gold nanoparticle aggregation process can be used for qualitative and quantitative detection of Cu²⁺ by the naked eye, and with UV–vis and smartphone-based approaches. The three analysis techniques used in the multipath colourimetric assay complement each other and provide greater flexibility for differing requirements and conditions, making the assay highly applicable for Cu²⁺ detection. Under optimal conditions, the Cu²⁺ concentration was quantified in less than 5 min with limits of detection for the naked eye, UV–vis and smartphone-based approaches of 1 μM, 405 nM and 61 nM, respectively. Moreover, the sensing system exhibited excellent selectivity and practical application for Cu²⁺ detection in real water samples. Thus, our strategy has great potential for application in on-site monitoring of Cu²⁺, and the unique response of MarR towards copper ions may provide a new approach to Cu²⁺ sensing.

To prevent contaminants from causing damage to human health and environmental catastrophes, it would be ideal to detect such contamination events as quickly as possible to be able to rapidly initiate remedial strategies. Toxic metals such as Cu²⁺, a major environmental pollutant, have adverse effects on the self-purification ability of water systems in nature. In addition, elevated concentrations of Cu²⁺ cause serious gastrointestinal disturbance, liver and kidney damage, and various neurological diseases. The most commonly used detection methods for Cu²⁺ include inductively coupled plasma mass spectrometry, atomic absorption spectroscopy and inductively coupled plasma optical emission spectroscopy (ICP-OES). Although these are very sensitive and quantitative techniques, they require either laborious sample preparation procedures or inconvenient analytical methods and are thus very time-consuming. Therefore, there is an ever-increasing demand for the development of quantitative techniques for the detection of Cu²⁺ that do not require advanced instruments.

In this context, colourimetric assays are very promising on account of their simplicity and cost effectiveness. In particular, functionalised gold nanoparticles (AuNPs) have received considerable attention for use in colourimetric sensing because of their ease of biofunctionalisation, high extinction coefficients and characteristically strong surface plasmon resonance (SPR) absorption. In most cases, AuNPs are used as chemical sensors by monitoring their colour changes on aggregation/dissociation because the colourimetric response can be easily observed with the naked eye. Recently, several AuNP-based colourimetric assays have been developed for Cu²⁺ sensing by modifying the AuNP surfaces with aptamers, polyamines, proteins and other specific reagents. Zhou et al. reported the visual detection of Cu²⁺ using azide- and alkyne-functionalised AuNPs on the basis of a Cu²⁺-catalysed alkyne–azide cycloaddition reaction. This method showed high specificity, but the minimum concentration of Cu²⁺ detectable by eye was approximately 50 μM. Accordingly, based on a similar Cu²⁺-catalysed alkyne–azide cycloaddition reaction, Shen et al. further developed a simple “clickable” biosensor for colourimetric detection of Cu²⁺. This method had a low detection limit of 250 nM and a linear range of 0.5–10 μM. However,
Cells were grown in LB medium containing 30 ml/L E. coli KH2PO4, 8.09 mM Na2HPO4·12H2O, 136.9 mM NaCl, 2.68 mM KCl, pH 7.4) for further experimental use. Greater than 18.2 MΩ was used for all the experiments. All glassware was thoroughly cleaned with freshly pre-equilibrated with 10 mL binding buffer (50 mM Na3PO4, 30 mM NaCl and 10 mM imidazole). The MarR protein was purified by Ni-NTA chromatography and desalted on a PD-10 column (GE Healthcare) by using 50 mM Tris–HCl, pH 7.5, 100 mM NaCl and 1 mM ethylenediaminetetraacetic acid (EDTA) containing 1 mM phenylmethylsulfonyl fluoride. After sonication on ice for 20 min, the cell lysate was centrifuged at 12,000 rpm for 30 min. The cleared lysate was filtered and loaded onto a 1-mL Histag column (GE Healthcare), which was pre-equilibrated with 10 mM binding buffer (50 mM NaPO4, 30 mM NaCl and 10 mM imidazole). The MarR protein was then eluted with a linear imidazole gradient followed by a further purification step using Amicon Ultra (Millipore). The protein was collected and concentrated in phosphate-buffered saline (PBS, 0.15 M, 1.47 mM KH2PO4, 8.09 mM NaHPO4·12H2O, 136.9 mM NaCl, 2.68 mM KCl, pH 7.4) for further experimental use.

Methods

Chemicals and instrumentation. Chloroauric acid (HAuCl4), trisodium citrate and copper sulfate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Other metal ions salts (Na2SO4, KNO3, Ca(NO3)2, Cd(NO3)2, Mg(NO3)2, Pb(NO3)2, Zn(NO3)2, HgSO4, Mn(NO3)2, and Fe(NO3)3) and environmental anion salts (KCl, KNO3 and K2SO4) were obtained from Aladdin Reagent Co. Ltd. (Shanghai, China). All chemical reagents were of analytical grade and used without further purification. Ultrapure water with an electrical resistance greater than 18.2 MΩ was used for all the experiments. All glassware was thoroughly cleaned with freshly prepared 3:1 HCl/HNO3 and rinsed with ultrapure water before use.

UV–vis spectra were measured on a multifunctional microplate reader SpectraMax M5 (Molecular Devices, USA). Transmission electron microscope (TEM) images were obtained with a JEM-1200EX microscope (JEOL, Japan). The captured images were recorded with a smartphone (Huawei, Ascend P7, China) with back camera (13 Megapixel: 4160 × 3120 pixels) and the RGB values of the colourimetric detection results were acquired using Adobe Photoshop CS6 software.

Preparation of MarR. Wild-type MarR and its mutant (MarRmut) with C-terminal His-tags were expressed in E. coli BL21(DE3), which bears a pET30a vector containing the marR gene from Lysobacter enzymogenes OH11. Cells were grown in LB medium containing 30 μg mL−1 kanamycin at 37 °C to an OD600 of 0.6, and then isopropyl β-D-1-thiogalactopyranoside was added to a final concentration of 1 mM. Protein expression was induced at 18 °C for 12 h before harvesting. The cell pellets were collected and resuspended in protein extract buffer (50 mM Tris–HCl, pH 7.5, 100 mM NaCl and 1 mM ethylenediaminetetraacetic acid (EDTA)) containing 1 mM phenylmethylsulfonyl fluoride. After sonication on ice for 20 min, the cell lysate was centrifuged at 12,000 rpm for 10 min. The cleared lysate was filtered and loaded onto a 1-mL Histag column (GE Healthcare), which was pre-equilibrated with 10 mM binding buffer (50 mM NaPO4, 30 mM NaCl and 10 mM imidazole). The MarR protein was then eluted with a linear imidazole gradient followed by a further purification step using Amicon Ultra (Millipore). The protein was collected and concentrated in phosphate-buffered saline (PBS, 0.15 M, 1.47 mM KH2PO4, 8.09 mM NaHPO4·12H2O, 136.9 mM NaCl, 2.68 mM KCl, pH 7.4) for further experimental use.
Synthesis of AuNPs. AuNPs with an average diameter of 20 nm were prepared by a previously reported citrate-mediated reduction of HAuCl₄ with slight modifications. Briefly, a HAuCl₄ solution (0.01%, 100 mL) was brought to boil in a 250 mL conical beaker with vigorous stirring, and then trisodium citrate solution (1%, 1.8 mL) was added under constant stirring. The colour of the solution changed to wine-red after about 45 s, the solution was boiled for a further 5 min, then the heating source was removed and the colloidal gold solution was stirred for another 10 min at room temperature. The solution was stored in a dark bottle at 4 °C. The concentration of the AuNP colloid was estimated to be about 9.7 nM from UV–vis spectroscopic measurements according to the Beer–Lambert law and based on an extinction coefficient of 8.78 × 10⁸ M⁻¹ cm⁻¹ at λ = 520 nm for 20 nm particles.

Preparation of MarR-coated gold nanoparticles (M–AuNPs). To estimate the minimum quantity of MarR required to prevent the aggregation of colloidal gold, a salt-induced AuNP aggregation test was performed. In brief, the pH of the AuNP solution (20 nm mean diameter) was adjusted to pH 8.2 with K₂CO₃ (0.2 mol L⁻¹), and serially diluted MarR solutions (30 μL, 62.5–1000 mg L⁻¹) were added to portions of the AuNP solution (125 μL). After 5 minutes, NaCl solution (10%, 125 μL) was added to the mixture. The absorbance at 520 nm was measured before and after NaCl addition. More than 10% of minimum amount of MarR (0.22 mg) was added to the colloidal gold solutions (6 mL, pH 8.2) with rapid stirring, and this was followed by incubation for 4 h. The M–AuNPs were centrifuged three times (10,000 × g) at 4 °C for 25 min. After removal of the supernatant, the sediment was resuspended in ultrapure water at a concentration of 3.2 nM.

Assay procedure for the colourimetric determination of Cu²⁺. In a typical experiment, a Cu²⁺ sample solution was added to a solution of M–AuNPs (120 μL), to give final Cu²⁺ concentrations in the range of 100 nM to 20 μM. After vibration for 2 min at room temperature, the colour changes were detected by the naked eye, UV–vis absorption spectroscopy and the smartphone-based approach. For detection with the naked eye, photographs were taken with a digital camera. The UV–vis analysis used absorption measurements obtained on a UV–vis spectrophotometer between 450 and 800 nm. For the smartphone-based approach, photographs were taken under artificial white light without flash using a smartphone equipped with a high-resolution camera.

Figure 1. Schematic representation of the proposed Cu²⁺ sensing mechanism of the multipath colourimetric assay.

Figure 2. Colourimetric response of wild-type MarR (MarR⁰⁻) and mutant (MarR⁰⁻) functionalized gold nanoparticles versus the Cu²⁺ concentration.
Image processing was performed by calculation of the RGB values by placing a 100 × 100 pixel box on each well using Adobe Photoshop CS6 software. The linear relationship was calculated from the A600/A520 absorbance ratio and the blue/red ratio versus the Cu²⁺ concentration, respectively.

Analysis of real water samples. Tap water and commercial bottled water samples were analysed without filtration or any other pre-treatment. River water samples (Xuanwu Lake, Nanjing, China) were filtered through 0.45 µm cellulose acetate filters and the pH was adjusted to pH 7.0. Because the concentrations of Cu²⁺ in the water samples were lower than the detection limit of the proposed method, various amounts of Cu²⁺ standard
solution were spiked into the water samples. These sample solutions were mixed with the pre-concentrated M–AuNPs to give a final concentration of 3.2 nM, then analysed with the multipath colourimetric assay.

Results and Discussion

Principle of the colourimetric assay. As shown in Fig. 1, MarR is a protein that is easily attached to the surface of AuNPs, and the M–AuNPs show good dispersion with a visible red colour. The single surface plasmon absorption peak is centred at 520 nm. In the presence of Cu^{2+}, the M–AuNPs are aggregated and the colour changes from red to purple as the Cu^{2+} concentration is increased. As a result of the aggregation, the intensity of surface plasmon resonance band at around 520 nm decreases and a new peak appears at 600 nm. The colourimetric assay was also recorded using a smartphone under artificial white light without flash. The smartphone images and the absorbance ratios of the M–AuNP solutions versus the concentrations of Cu^{2+} were recorded using a smartphone and processed with Adobe Photoshop CS6 by calculation of the RGB values. Thus, the colour variation of the M–AuNP solution can be used for qualitative and quantitative detection of Cu^{2+}. Error bars at n = 3.

Figure 5. Colourimetric sensing of Cu^{2+}. (A) Photographs of multiple antibiotic resistance regulator (MarR)-coated AuNP (M–AuNP) solutions with different concentrations of Cu^{2+} in the range from 100 nM to 20 μM. (B) UV–vis spectra of the M–AuNP solutions. (C) Plot of the A_{600}/A_{520} absorbance ratios of M–AuNP solutions versus the concentrations of Cu^{2+}. (D) Photographs of M–AuNP solutions with different concentrations of Cu^{2+} using a smartphone under artificial white light without flash. (E) and (F) Plot of blue/red colour values of the smartphone photographs versus the concentrations of Cu^{2+}. Error bars at n = 3.
were used as the detection probe, and we postulated that the aggregation of M–AuNPs was induced by bridge formation between two M–AuNPs through catalytic oxidation of the cysteine residues promoted by Cu$^{2+}$. To validate this assumption, we mutated the only cysteine residue of MarR to serine and used this mutant MarR in the Cu$^{2+}$-induced colourimetric assay. As shown in Fig. 2, the wild-type MarR (MarRcys) probe showed a marked red-to-purple colour change with increasing of Cu$^{2+}$ concentration, whereas the mutant (MarRser) probe showed a colour change only when the Cu$^{2+}$ concentration exceeded 20 μM. This indicates that the MarRcys probe exhibits a more sensitive response to Cu$^{2+}$ than does the MarRser probe. Therefore, we deduced that the aggregation mechanism in the colourimetric assay is based on the catalytic ability of Cu$^{2+}$ to oxidise the cysteine residue of MarR.

Characterization of M-AuNPs. To investigate the viability of our strategy, the M–AuNPs were characterised in the presence and absence of Cu$^{2+}$ by UV–vis spectroscopy and TEM. As shown in Fig. 3A, the characteristic band of the AuNPs and M–AuNPs was observed at approximately 520 nm (curves a, c), and the colour of the solution appeared red (photographs a, c). After addition of an appropriate amount of Cu$^{2+}$, the colour of the M–AuNP solution changed from red to purple (photograph d), and this colour change was confirmed by the dramatic decrease in the absorbance at 520 nm and the appearance of an intense new peak at about 600 nm (curve d). In contrast, the colour of the AuNP solution remained red after Cu$^{2+}$ treatment (photograph b), indicating that the aggregation of AuNPs was mainly caused by the interaction between MarR and Cu$^{2+}$. TEM studies (Fig. 3B) clearly indicated the Cu$^{2+}$-induced aggregation of M–AuNPs, and thus confirmed the validity of our strategy.

Optimisation of assay conditions. To optimise the performance of the colourimetric assay, it was necessary to consider the concentration of the M–AuNPs, the pH value and the reaction temperature. To investigate the influence of the M–AuNP concentration, the ratio of the absorbances at 600 and 520 nm ($A_{600}/A_{520}$) was used to reflect the signal response of the sensor. As shown in Fig. 4A, the maximum $A_{600}/A_{520}$ absorbance ratio was observed when the concentration of M–AuNPs was 3.2 nM. An M–AuNP concentration of 3.2 nM was thus chosen for further experiments to achieve highly sensitive Cu$^{2+}$ detection.

Table 1. Comparison of different AuNPs-based colourimetric methods for the detection of Cu$^{2+}$. *Not available. $^b$Limit of detection.

| Probe unit              | Linear range | LOD$^b$ | Time (min) | Interference ions | Analytical approach       | Ref. |
|------------------------|--------------|---------|------------|-------------------|---------------------------|------|
| Papain–AuNPs           | N/A$^*$      | 200 nM  | N/A$^*$    | Hg$^{2+}$, Pb$^{2+}$ | naked eye and UV-vis       | 17   |
| DNA–AuNPs              | 20–100 μM    | 20 μM   | 120        | None              | naked eye and UV-vis       | 18   |
| Azide and alkyl–AuNPs  | 0.5–10 μM    | 250 nM  | 20–60      | None              | naked eye and UV-vis       | 20   |
| DNAzyme–AuNPs          | N/A$^*$      | 5 μM    | 90         | Zn$^{2+}$         | naked eye and UV-vis       | 35   |
| Thermally treated AuNPs| 0.2–6.0 μM   | 40 nM   | 5          | Hg$^{2+}$, Pb$^{2+}$ | naked eye and UV-vis       | 36   |
| Azide-tagged AuNPs     | 1.8–200 μM   | 1.8 μM  | 30         | Cr$^{3+}$, Pb$^{2+}$, Cd$^{2+}$ | naked eye and UV-vis       | 37   |
| MarR–AuNPs             | 0.1–10 μM    | 61 nM   | <5         | None              | naked eye, UV-vis and smartphone-based | This work |

Figure 6. $A_{600}/A_{520}$ absorbance ratios of multiple antibiotic resistance regulator (MarR)-coated AuNPs (M–AuNPs) on addition of Cu$^{2+}$ (4 μM), other metallic ions (10 μM; Mg$^{2+}$, Mn$^{2+}$, Hg$^{2+}$, Pb$^{2+}$, Zn$^{2+}$, Fe$^{2+}$, Ca$^{2+}$, Fe$^{3+}$, Al$^{3+}$, Na$^{+}$, K$^{+}$) or environmental anions (1 mM; NO$_3^-$, SO$_4^{2-}$, Cl$^-$). Error bars at n = 3. The inset shows the corresponding photographic images of the M–AuNP solutions.
Next, we investigated the colourimetric response of M–AuNPs with Cu$^{2+}$ at different pH values (pH 3.0–11.0).

The correlation of the A$_{600}$/A$_{520}$ absorbance ratio with and without Cu$^{2+}$ at a range of pH values is shown in Fig. 4B. The absorbance ratio was low and constant in the absence of Cu$^{2+}$ from pH 3.0 to 11.0, which indicates that the M-AuNPs were stable in this pH range. Addition of Cu$^{2+}$ resulted in a high A$_{600}$/A$_{520}$ absorbance ratio at pH 7 and 8, which indicates that the highest catalytic efficiency of Cu$^{2+}$ towards the cysteine residue of MarR occurs at these pH values. In our experiments, a higher absorbance ratio was observed at pH 7.0 than at pH 8.0, so pH 7.0 was considered to be the optimal pH value.

We also tested the effect of temperature, from 0 to 68 °C, on the sensor performance. As shown in Fig. 4C, the A$_{600}$/A$_{520}$ absorbance ratio changed only very slightly over the studied temperature range, suggesting that the catalytic efficiency of Cu$^{2+}$ towards MarR was retained over a wide temperature range. For easy implementation and extensive application of our assay, we chose room temperature for the following experiments.

**Sensitivity of the Cu$^{2+}$ sensor.** To determine the sensitivity of the multipath colourimetric assay, Cu$^{2+}$ concentrations between 100 nM and 20 μM were tested under the optimised conditions. For naked-eye detection and UV–vis analysis, the A$_{600}$/A$_{520}$ absorbance ratio was recorded for each concentration of Cu$^{2+}$, as well as the corresponding colourimetric response. As shown in Fig. 5A, with increasing concentrations of Cu$^{2+}$, the colour of the M–AuNPs solution gradually changed from red to purple and finally to grey, which suggests an increase

![Figure 7. Sensing results for the naked eye detection of Cu$^{2+}$ in tap water, bottled water and river water samples.](image-url)

| Sample          | Cu$^{2+}$ (μM) | Measured ± SD$^a$ (μM) | Recovery$^b$ (%) | CV$^c$ (%) | Measured ± SD$^a$ (μM) | Recovery$^b$ (%) | CV$^c$ (%) |
|-----------------|---------------|------------------------|------------------|-----------|------------------------|------------------|-----------|
| **Tap water**   | 4             | 3.66 ± 0.19            | 91.50            | 5.19      | 3.81 ± 0.65            | 95.25            | 17.0      |
|                 | 8             | 8.27 ± 0.54            | 103.38           | 6.53      | 8.02 ± 0.49            | 100.25           | 6.11      |
| **Bottled water** | 4             | 4.26 ± 0.24            | 106.50           | 5.63      | 4.42 ± 0.25            | 110.50           | 5.66      |
|                 | 8             | 8.06 ± 0.41            | 100.75           | 5.09      | 8.21 ± 0.28            | 102.63           | 3.41      |
| **River water** | 4             | 3.95 ± 0.15            | 98.75            | 3.80      | 3.61 ± 0.35            | 90.25            | 9.70      |
|                 | 8             | 7.41 ± 0.39            | 92.62            | 5.26      | 7.71 ± 0.50            | 96.38            | 6.50      |

Table 2. Recovery studies of real water samples spiked with various Cu$^{2+}$ concentrations, comparing the proposed colourimetric method with ICP-OES analyses. $^a$Mean measured concentration of three replicates ± standard deviation. $^b$Mean recovery (%) = 100 × ($c_{\text{mean measured}}/c_{\text{added}}$). $^c$Coefficient of Variation of three determinations.
in the aggregation of the M–AuNPs at higher Cu\(^{2+}\) concentrations. The intensity of the maximum absorption at 520 nm decreased and that of the new peak at 600 nm increased with increasing Cu\(^{2+}\) concentration (Fig. 5B), which also indicates the gradually increasing aggregation of the particles. Fits of the \(A_{520}/A_{600}\) ratio versus the concentration of added Cu\(^{2+}\) exhibit good linear relationships in the range 0.1–8 \(\mu\)M Cu\(^{2+}\) (Fig. 5C), and the limit of detection (LOD) for Cu\(^{2+}\) was calculated to be 405 nM based on the following formula: LOD = \(3 \times \sigma / S\) (average response of the blank + standard deviation of the blank)\(^{11}\). In the smartphone-based approach, the blue/red ratio obtained from the RGB values of each image (Fig. 5D) was calculated based on the colour change of the solution, and correlated with the increase in the concentration of Cu\(^{2+}\). The graphs in Fig. 5E and F show that the blue/red ratio increased as the concentration of Cu\(^{2+}\) increased, and a linear relationship was observed in the range 0.1–10 \(\mu\)M. The LOD was estimated to be 61 nM using the smartphone-based approach. These results compare favourably with other reported AuNP-based colourimetric methods (Table 1), and thus suggest that our MarR-functionalised AuNP-based colourimetric assay is superior to most previously reported methods in terms of speed, selectivity, sensitivity and number of analytical approaches.

**Selectivity of the Cu\(^{2+}\) sensor.** To evaluate the selectivity of the colourimetric assay towards Cu\(^{2+}\), the effects of potential interfering metal ions Mg\(^{2+}\), Mn\(^{2+}\), Hg\(^{2+}\), Pb\(^{2+}\), Zn\(^{2+}\), Fe\(^{3+}\), Ca\(^{2+}\), Fe\(^{2+}\), Al\(^{3+}\), Na\(^{+}\) and K\(^{+}\) (10 \(\mu\)M), and environmental anions, Cl\(^{-}\), NO\(_3\)^{-}, and SO\(_4\)^{2-} (1 mM) on the detection were investigated by UV–vis analysis. The concentration of Cu\(^{2+}\) was 4 \(\mu\)M. As shown in Fig. 6, addition of Cu\(^{2+}\) caused a colour change and the solution became purple, whereas none of the other metal ions markedly changed the colour of the M–AuNP solutions. The corresponding absorbance data also showed that only Cu\(^{2+}\) addition yielded a large increase in the \(A_{520}/A_{600}\) ratio. It is worth noting that Pb\(^{2+}\) and Hg\(^{2+}\) caused a slightly larger colourimetric response than did the other metal ions. This may be attributed to their stronger coordination to cysteine residues and other functional groups (e.g., carboxyl, amino, hydroxyl) of the MarR protein\(^{17,34}\). However, the interaction between these groups and Pb\(^{2+}\) and Hg\(^{2+}\) is expected to be much weaker than the interaction between MarR and Cu\(^{2+}\) under the present conditions. The observation of a high absorbance ratio for Cu\(^{2+}\) demonstrates the excellent selectivity of our proposed method for Cu\(^{2+}\) over other competing metal ions.

**Application to real water samples.** To illustrate the practical application of the multipath colourimetric assay, three water samples (tap water, river water and commercial bottled water) were spiked with different concentrations of Cu\(^{2+}\) and tested using the developed assay. The obtained results for the three analytical approaches are shown in Fig. 7 and Table 2. The results obtained with the present colourimetric method were in excellent agreement with those obtained by ICP-OES, and the spike-recoveries were 90.25–110.50%. These results demonstrate the potential of the developed multipath colourimetric assay for the visual detection of Cu\(^{2+}\) in real water samples.

**Conclusions**

Using MarR as highly selective recognition element for Cu\(^{2+}\) detection based on the Cu\(^{2+}\)-induced tetramer formation of MarR, we have developed a straightforward, rapid, sensitive and highly selective multipath colourimetric assay for Cu\(^{2+}\) in water samples. The Cu\(^{2+}\) assay can be easily implemented using the naked eye, UV–vis spectroscopy and a smartphone-based approach, which endows the colourimetric assay with great flexibility and applicability under an extensive range of conditions. The detection limit of the colourimetric assay is as low as 61 nM, which is far below the maximum recommended limit in drinkable water (20 \(\mu\)M) defined by the U.S. Environmental Protection Agency. Moreover, the whole test can be completed within 5 min. We envisage that this new Cu\(^{2+}\)-regulated specific recognition element (MarR) may provide a simple and general approach to the selective detection of Cu\(^{2+}\), as well as a promising new tool for Cu\(^{2+}\) analysis based on the developed multipath colourimetric assay.

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