Revisit Diaminomaleonitrile Based Fluorophores As Highly Selective Sensing Platform for Cu$^{2+}$

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Abstract

A colorimetric and turn-on fluorescent chemodosimeter 1 based on diaminomaleonitrile was synthesized for Cu$^{2+}$ detection. It showed high selectivity and sensitivity towards Cu$^{2+}$ over the other tested metal ions. Probe 1 in acetonitrile exhibited a strong absorption band at 530 nm and weak fluorescence emission when excited at 480 nm, while Cu$^{2+}$ addition could lead to a 30 nm blue shift of absorption band and a remarkable fluorescence enhancement. Moreover, the detection limit of probe 1 for Cu$^{2+}$ was calculated to be 28 nM. Quite different from the reported mechanism based on a metal-complexation induced fluorescence enhancement, the sensing mechanism was actually proved to be based on the Cu$^{2+}$-promoted hydrolysis reaction, which was confirmed by $^1$H NMR, $^{13}$C NMR and mass spectrum analysis. Studies on probe 2 were carried out to verify the universality of this sensing mechanism.
Introduction

Cu\textsuperscript{2+} ions play very important roles in many different biological processes, such as various enzyme and proteins-catalyzed reactions, while high concentration of Cu\textsuperscript{2+} ions in human body could cause imbalance in cellular processes and lead to increased blood pressure, damage of liver and neurodegenerative diseases\textsuperscript{1–3}. Hence, it is desirable to develop novel approaches for Cu\textsuperscript{2+} detection. Fluorescent probes have been regarded as highly efficient tools for ions detection due to their advantages such as simple synthesis steps, easy manipulating procedures, inexpensive cost, good selectivity and sensitivity, naked eyes detection, and so on\textsuperscript{4–23}. Among them, diaminomaleonitrile derivation was one of the most attractive approaches for its easy modulation, usually turn-on fluorescent response and high selectivity to Cu\textsuperscript{2+}\textsuperscript{24–34}. And so far, most of the reports have attributed their fluorescence enhancement to metal-complexation\textsuperscript{24–25, 33–34}, while the direct evidences still need more investigations.

In present work, we synthesized two prototypical chemodosimeters 1 and 2 (as shown in Scheme 1) to investigate sensing mechanism of diaminomaleonitrile based fluorophores for Cu\textsuperscript{2+}. These two chemodosimeters could be easily synthesized through condensation between diaminomaleonitrile and fluorophores containing aldehyde group by a one-step reaction with excellent yields\textsuperscript{24, 45}. BODIPY and pyrene were chosen as the signaling unit because of their excellent photophysical properties such as high molar absorption coefficient and fluorescent quantum yield\textsuperscript{35–42}.

Fluorophores which contain unbridged C=N bonds usually show nonfluorescent properties due to C=N isomerization, as many studies described\textsuperscript{43–44}. As expected, both chemodosimeters 1 and 2 are nonfluorescent. However, a remarkable fluorescence enhancement was observed in the presence of Cu\textsuperscript{2+} through a Cu\textsuperscript{2+}-promoted hydrolysis reaction. This sensing mechanism was fully confirmed by \textsuperscript{1}H NMR, \textsuperscript{13}C NMR and mass
spectrum analysis. To the best of our knowledge, Cu\(^{2+}\)-promoted hydrolysis sensing mechanism of probes based on diaminomaleonitrile was firstly proposed and would bring new thinking when using diaminomaleonitrile based fluorophores as Cu\(^{2+}\) probes.

**Experimental**

*Reagents and chemicals*

Chemical reagents and solvents such as 2,4-Dimethylpyrrole, benzaldehyde, trifluoroacetic acid (TFA), 2,3-dicyano-5,6-dichlorobenzoquinone (DDQ), trimethylamine (TEA), boron trifluoride ether complex (BF\(_3\)·OEt\(_2\)), phosphorus oxychloride (POCl\(_3\)), N,N-Dimethylformamide (DMF), ethylene dichloride, sodium acetate trihydrate (CH\(_3\)COONa), magnesium sulfate (MgSO\(_4\)), sodium carbonate (Na\(_2\)CO\(_3\)), 2,3-diaminomaleonitrile, ethanol (EtOH), dichloromethane (CH\(_2\)Cl\(_2\)), 1-pyrenecarboxaldehyde were offered by Sigma-Aldrich. Silica gels (200-300 mesh) were used for the column chromatography and silica gels coated on aluminum plates were used for the thin layer chromatography (TLC). The tested metal salts were Cu(ClO\(_4\))\(_2\), AgNO\(_3\), Mn(ClO\(_4\))\(_2\), Co(ClO\(_4\))\(_2\), Cd(ClO\(_4\))\(_2\), Zn(ClO\(_4\))\(_2\), Ni(ClO\(_4\))\(_2\), Pb(ClO\(_4\))\(_2\), Mg(ClO\(_4\))\(_2\), Ca(ClO\(_4\))\(_2\), KClO\(_4\), NaClO\(_4\), Fe(ClO\(_4\))\(_3\), Ba(ClO\(_4\))\(_2\).

*Apparatus*

All absorption and fluorescence spectra were recorded on a PerkinElmer Lambda 25 spectrometer and a Perkin Elmer LS 55 spectrometer with Perkin Elmer quartz cells (1 cm), respectively. \(^1\)H NMR and \(^{13}\)C NMR spectra of probe 1 and 2 were measured on a Bruker Advance 400 MHz spectrometer in (CD\(_3\))\(_2\)SO. HRMS spectra of probe 1 and probe 2 were gained on a Bruker amaZon SL instrument using standard conditions (ESI).
Procedure

Synthesis of 1,3,5,7-tetramethyl-6-aminomaleonitrile-8-phenyl-BODIPY 1 (probe 1)

Probe 1 was synthesized according to the reported reference\textsuperscript{45}. In a bottle with a stirrer was added a solution of β-formyl-BODIPY (0.3 g, 0.85 mmol) in the mixture of EtOH (40 mL) and water (9 mL) and acetic acid (0.5 mL). Then the solution of 2,3-diaminomaleonitrile (0.1 g, 0.93 mmol) in EtOH (30 mL) was added to the mixture slowly. The reaction was refluxed for 2 h and then cooled to room temperature. After the completion of reaction, the mixture was concentrated, purified by silica gel column chromatography (CH\textsubscript{2}Cl\textsubscript{2}) to give probe 1 as a red solid (0.26 mg, 0.595 mmol) in 70% yield. \textsuperscript{1}H NMR (400 MHz, (CD\textsubscript{3})\textsubscript{2}SO) δ 8.21 (s, 1H), 7.58 (m, 3H), 7.51-7.26 (m, 4H), 6.35 (s, 1H), 2.73 (s, 3H), 2.51 (s, 3H), 1.56 (s, 3H), 1.36 (s, 3H); \textsuperscript{13}C NMR (101 MHz, (CD\textsubscript{3})\textsubscript{2}SO) δ 159.65, 155.74, 150.25, 146.07, 143.21, 141.17, 134.14, 132.92, 130.19, 130.02, 129.89, 128.28, 125.45, 125.00, 123.91, 115.10, 114.11, 105.27, 55.38, 15.04, 14.76, 14.41, 12.67. HRMS: calculated for [M-H]: 441.1811, measured: 441.1815.

Synthesis of 2-((pyren-1-yl)methyleneamino)-3-amino-maleonitrile 2 (probe 2)

Probe 2 was synthesized according to the reported reference\textsuperscript{46}. In a bottle with a stirrer was added a solution of 1-pyrenecarboxaldehyde (1.0 g, 4.3 mmol) in the mixture of EtOH (120 mL) and water (27 mL) and acetic acid (0.5 mL). Then the solution of 2,3-diaminomaleonitrile (0.57 g, 5.2 mmol) in EtOH (90 mL) was added to the mixture slowly. The reaction was refluxed for 2 h and then cooled to room temperature. A yellow precipitate was formed and filtered, then washed with EtOH four times. The product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 1 : 1, v/v) to give probe 2 as a yellow solid (1.1 g, 3.4 mmol) in 80% yield. \textsuperscript{1}H NMR (400 MHz, (CD\textsubscript{3})\textsubscript{2}SO) δ 9.23 (s, 1H), 8.98 (d, J = 8.2 Hz, 1H), 8.75 (d, J = 9.4 Hz,
1H), 8.37-8.27 (m, 5H), 8.22-8.19 (m, 1H), 8.13-8.09 (m, 3H). $^{13}$C NMR (101 MHz, (CD$_3$)$_2$SO) δ 152.43, 133.47, 131.22, 130.50, 130.28, 129.73, 129.62, 127.97, 127.86, 127.28, 127.11, 126.93, 126.72, 126.65, 125.52, 124.42, 124.13, 122.16, 115.08, 114.42, 104.26. HRMS: calculated for [M-H]$^-$: 319.0984, measured: 319.0988.

**General UV–vis absorption and fluorescence spectra measurement**

Acetonitrile was used for preparing the stock solution of probe 1 (1 mM). The tested ions such as Cu$^{2+}$, Mn$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Ba$^{2+}$, Ca$^{2+}$, Ag$^+$, Fe$^{3+}$, K$^+$, Na$^+$, Mg$^{2+}$ (10 mM) were prepared in Milli-Q water. To study the selectivity of probe 1 for Cu$^{2+}$ detection, the mixture solution of probe 1 and metal ions were prepared by adding the stock solution of probe 1 (20 μL) and the stock solution of each metal ion (10 μL) to the cell (1 cm) and then diluted by addition of 2.5 mL acetonitrile. Absorption and fluorescence spectra were recorded after shaking the mixture for 5 min. For Cu$^{2+}$ titration experiment, a solution of probe 1 (8 μM) in acetonitrile was prepared in a Perkin Elmer quartz cells (1 cm) and then was added the stock solution of Cu$^{2+}$ (0.1 equiv.), the mixture was shaken for 5 min before measuring UV–vis absorption and fluorescence spectra, this protocol was repeated until addition of 8 equiv. totally$^{47}$. The fluorescence quantum yields ($\Phi_F$) of probe 1 and probe 1 with Cu$^{2+}$ were measured by the standard method using Rhodamine 6G ($\Phi_{std} = 0.95$) in ethanol as a reference$^{33}$.

**Results and Discussion**

**Synthesis of probe 1**

Synthetic procedure of probe 1 was depicted in Scheme 1. Probe 1 was easily
prepared in 70% yield by the nucleophilic addition reaction of BODIPY-aldehyde with 2,3-diaminomaleonitrile in the presence of acetic acid as catalyst in EtOH/H₂O mixed solution. It was fully characterized by ¹H NMR, ¹³C NMR and HRMS, as shown in SI.

Absorption and fluorescence titrations of probe 1 for Cu²⁺

According to the literatures 24-34, 46, fluorophores with diaminomaleonitrile group could be used as probes for Cu²⁺ detection. Therefore, we firstly used absorption and fluorescence spectroscopy to study its sensing ability towards Cu²⁺ in acetonitrile. As depicted in Fig. 1, probe 1 shows four absorption bands, which peaked at 340, 374, 421 and 530 nm, respectively. With addition of Cu²⁺ continually, absorption bands of probe 1 at 340, 374, 421 nm decreased progressively, while the absorption band at 530 nm decreased gradually, meanwhile new band at 508 nm appeared firstly, then shifted to 498 nm finally. Two isosbestic points at 511 and 435 nm were clearly observed, which indicated the formation of complex between probe 1 and Cu²⁺. Fluorescence spectral response of probe 1 towards Cu²⁺ was also examined in acetonitrile. As depicted in Fig.2, probe 1 shows no emission upon excitation at 480 nm. With incremental addition of Cu²⁺, a new emission band peaked at 522 nm appeared and increased gradually, which achieved a balance when the concentration of Cu²⁺ reached 22 µM. Moreover, the fluorescence quantum yield (Φ_F) was increased from 0.0003 to 0.54, over 1000-fold enhancement (SI). As shown in Fig.3, the variation of fluorescent intensity ratio (I/I₀) with the concentrations of Cu²⁺ ranging from 0 to 8.0 µM exhibits good linear relationship (R² = 0.9913). According to the equation of 3σ/k, the detection limit of probe 1 towards Cu²⁺ was obtained to be 28 nM, where σ is the standard deviation of 13 times fluorescence intensity ratio (I/I₀) of probe 1 and k is the slope of the fluorescence intensity ratio (I/I₀) versus Cu²⁺ concentrations plot. This limit of detection of probe 1 is
much lower than many reported probes for Cu$^{2+}$ detection (Table 1), which indicates high sensitivity of probe 1.

**Selectivity**

Selectivity of probe 1 to various metal ions was investigated by absorption and fluorescence spectroscopy. As shown in Fig. 4, among the tested metal ions such as Cu$^{2+}$, Mn$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Ba$^{2+}$, Ca$^{2+}$, Ag$^{+}$, Fe$^{3+}$, K$^+$, Na$^+$ and Mg$^{2+}$, only addition of Cu$^{2+}$ to the solution of probe 1 leads to the disappearance of absorption band at short wavelength range (275-430 nm) and 32 nm blue shift from 530 nm to 498 nm. While the absorption spectrum of probe 1 shows no changes when added other metal ions. Moreover, as shown in Fig. 5, probe 1 exhibits pink color, while after addition of the same amount of different metal ions, only Cu$^{2+}$ could induce an obvious color change from pink to light yellow, which indicated that probe 1 could be used for the “naked eye” detection of Cu$^{2+}$. Furthermore, only Cu$^{2+}$ results in a remarkable fluorescence enhancement among the tested metal ions (Fig. 6). Meanwhile, addition of Fe$^{3+}$ ions into the solution of probe 1 could also lead to a relatively weak fluorescence enhancement. In addition, probe 1 with Cu$^{2+}$ exhibits bright green fluorescence color under illumination using a 365 nm UV-lamp, while probe 1 with other metal ions did not show any fluorescence color changes (Fig. 7). The absorption and fluorescence response towards the tested metal ions indicated that probe 1 had good selectivity for the detection of Cu$^{2+}$.

**Competition study**

To investigate whether other metal ions have interference on Cu$^{2+}$ detection, fluorescence response of probe 1 with other metal ions towards Cu$^{2+}$ was measured. As
depicted in Fig. 8, addition of various metal ions, such as Mn$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, Ba$^{2+}$, Ca$^{2+}$, Ag$^{+}$, K$^{+}$, Na$^{+}$ and Mg$^{2+}$, into probe 1 did not induce any fluorescence emission intensity changes except Fe$^{3+}$. Further addition of Cu$^{2+}$ into the mixed solution of 1 and other metal ions could lead to a remarkable fluorescence enhancement. The slight interference of Fe$^{3+}$ ions can be avoided in its lower concentration range. As shown in Fig.S1, Cu$^{2+}$ (10 μM) could induce a remarkable fluorescence enhancement of probe 1, while addition of Fe$^{3+}$ (10 μM) still shows almost no fluorescence emission. This phenomenon further confirmed that probe 1 had ability of detecting Cu$^{2+}$ over the other metal ions in the complicated environment.

**Kinetic study**

The reaction kinetics between probe 1 and Cu$^{2+}$ was monitored by the fluorescence spectroscopy. As depicted in Fig.9, after addition of Cu$^{2+}$ (16 μM) into probe 1 (8 μM), the fluorescence intensity at 522 nm increased quickly and reached a plateau within 5 min, which exhibited promising applications in real-time detection for Cu$^{2+}$.

**Mechanism study**

Diaminomaleonitrile based Cu$^{2+}$ fluorescent sensors were generally constructed through a metal-complexation induced fluorescence enhancement mechanism, with C=N group as the binding site. However, if this happened, it would enhance the intramolecular charge transfer and usually lead to a red shift of the absorption spectra, which was contrast to many reported results. In our present work, we observed a blue shift of the absorption band and a remarkable fluorescence enhancement, which was quite similar to that in the presence of ClO$^{-}$. Combining the blue shift of the absorption band and fluorescence enhancement, the Cu$^{2+}$-promoted hydrolysis of...
probe 1 to produce aldehyde product was proposed to explain these spectral responses. This was also consistent with the mechanism reported by Feng’s group. To verify our hypothesis of the mechanism, the fluorescent product of probe 1 in the presence of Cu\(^{2+}\) was isolated and analyzed. As shown in Fig. S2, the \(^1\)H NMR spectrum of the isolated product 1-Cu\(^{2+}\) was analyzed and matched well with that of carboxyl-BODIPY 4 (Scheme 2). Peaks at 7.81-7.80 (m, 2H) and 7.44-7.42 (m, 2H) are the protons of phenyl group at 8-position; peak at 6.37 (s, 1H) is the proton of BODIPY at 2-position; peaks at 2.55 (s, 3H), 2.53 (s, 3H), 1.40 (s, 3H), 1.39 (s, 3H) are protons of methyl and singlet peak at 14.31 is probably the proton of carboxyl group. From the \(^{13}\)C NMR spectrum (Fig. S3), all the carbon peaks of BODIPY 4 can be found and peak at 159.92 is attributed to carboxyl group. In addition, the isolated product 1-Cu\(^{2+}\) was further confirmed to be BODIPY-acid by the mass spectrometry. The peak at m/z 369.1521, which corresponded to calculated m/z 369.1586 of BODIPY 4, was detected by mass spectrometry. (Fig. S4). The isolated product 1-Cu\(^{2+}\) (8 \(\mu\)M) and probe 1 (8 \(\mu\)M) with Cu\(^{2+}\) (80 \(\mu\)M) not only have the same absorption band and similar absorption intensity (Fig. S5), but also have the same emission band and intensity (Fig. S6), which demonstrated spectral response of probe 1 to Cu\(^{2+}\) was due to the formation of oxidation product 1-Cu\(^{2+}\). Consequently, the proposed mechanism is depicted as follows (Scheme 2): Cu\(^{2+}\) firstly coordinates with two adjacent nitrogen of diaminomaleonitrile moiety followed by the cleavage of C=N bond to yield the aldehyde-BODIPY 3, which was further oxidized into 4 by Cu\(^{2+}\) in acetonitrile.

Job’s plot experiment was carried out to determine the binding stoichiometry of the reaction. As shown in Fig. S7, absorption variation at 530 nm reached a maximum when the molar fraction was 0.6, which indicated a 1:2 ratio for 1-Cu\(^{2+}\) complex. This is consistent with our proposed sensing mechanism. For the first step, Cu\(^{2+}\) ion
coordinated with probe 1 with C=N group and led to the hydrolysis of probe 1 to give aldehyde-BODIPY 3, which was oxidized by the other Cu$^{2+}$ ion into final product 4. Therefore in the first step the apparent binding constant between probe 1 and Cu$^{2+}$ could be calculated based on 1:1 binding stoichiometry, which was then calculated to be \(6.3 \times 10^5\) M\(^{-1}\).

To further confirm the reaction mechanism between diaminomaleonitrile based probe 1 for Cu$^{2+}$ detection, probe 2 was synthesized in 80% yield through condensation between 1-pyrenecarboxaldehyde and 2,3-diaminomaleonitrile. After obtaining the probe 2, we firstly investigated its spectral response to Cu$^{2+}$. In the presence of Cu$^{2+}$ in acetonitrile, blue shift of the absorption band and fluorescence enhancement were both observed, which were consistent with the reported results by Wu’s group, who clarified that the sensing mechanism of Cu$^{2+}$ by probe 2 was based on metal-complexation\(^2\). Here we then further investigated the sensing mechanism of probe 2 in the presence of Cu$^{2+}$. If the sensing mechanism is metal-complexation, the fluorescence spectra would be easily restored by addition of EDTA or CN$^-$ solution. As shown in Fig. S8, probe 2 exhibits no fluorescence upon excitation at 350 nm; further addition of Cu$^{2+}$ resulted in a remarkable fluorescence enhancement centered at 417 nm, while no spectral change was observed followed by addition of EDTA or CN$^-$ solutions. Moreover, after reaction with Cu$^{2+}$ in acetonitrile, the TLC was carried out firstly. As shown in Fig. S9, the position of the main product of the reaction corresponded to 1-pyrenecarboxaldehyde. Furthermore, the isolated product 2-Cu$^{2+}$ was analyzed by $^1$H NMR, $^{13}$C MNR and HRMS and compared with 1-pyrenecarboxaldehyde. As shown in Fig. S10 and Fig. S11, the proton peaked at 10.80 ppm and the carbon peaked at 192.93 ppm corresponded to the proton and carbon of aldehyde group, respectively. Other $^1$H and $^{13}$C NMR peaks of the isolated product 2-Cu$^{2+}$ could match well with those of 1-pyrenecarboxaldehyde.
The product 2-Cu$^{2+}$ was confirmed by the mass spectrometry, where a peak at m/z 230.0728 was observed, which was reasonably attributed to the signal of 1-pyrenecarboxaldehyde (Fig. S12). All these results fully supported the sensing mechanism between probe 2 and Cu$^{2+}$ was also based on a Cu$^{2+}$-promoted hydrolysis reaction as depicted in Scheme 3. From the results of these two prototypical Cu$^{2+}$ chemodosimeters, we demonstrated that Cu$^{2+}$-promoted diaminomaleonitrile based fluorophores hydrolysis in acetonitrile was widely applicable in these systems.

**Water samples test**

As mentioned above, probe 1 showed high selectivity and sensitivity towards Cu$^{2+}$ in acetonitrile. To evaluate its sensing ability in real water samples, lake water, tap water and mineral water was examined. A spike and recovery experiment was studied by the fluorescence spectroscopy. As listed in Table 2, the tested concentrations of Cu$^{2+}$ were 4 and 8 µM, respectively. The recovery in all sample was found to be in the range of 97—100.12, and the RSD% was less than 3.6, indicating that probe 1 could be used for Cu$^{2+}$ detection in real water samples.

**Conclusions**

In conclusion, a colorimetric and fluorescence turn-on chemodosimeter 1 was synthesized for Cu$^{2+}$ detection. Among the tested metal ions, probe 1 showed high selectivity towards Cu$^{2+}$. Only addition of Cu$^{2+}$ to probe 1 in acetonitrile resulted in a remarkable fluorescence enhancement and a blue shift of the absorption band from 530 nm to 500 nm, concomitant with a color change from pink to light yellow. The sensing mechanism was based on a Cu$^{2+}$-promoted hydrolysis, which was fully investigated by $^1$H NMR, $^{13}$C NMR and mass spectrum analysis. Studies on probe 2
were carried out to verify the universality of the proposed sensing mechanism in diaminomaleonitrile based Cu$^{2+}$ sensing systems.

**Acknowledgements**

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References


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Table 1 Comparison Cu$^{2+}$ detection limit of 1 with some reported probes

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<th>Sensors</th>
<th>Detection limit</th>
<th>Ref.</th>
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<tr>
<td>Naphthalene diimide-based</td>
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<tr>
<td>Fluorescein-based</td>
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<td>Rhodamine hydrazide-based</td>
<td>0.95 μM</td>
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<td>Azo-phenol based</td>
<td>0.72 μM</td>
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<td>Quinaldine-indole-based</td>
<td>0.63 μM</td>
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<td>Quinolin-based</td>
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<td>Tetrathiafulvalene-based</td>
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<td>Benzildihydrazone-based</td>
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<td><strong>BODIPY-based</strong></td>
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<td>present work</td>
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Table 2 Recovery data for Cu\textsuperscript{2+} detection in spiked water samples

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<tr>
<th>Samples</th>
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<th>Found (µM)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
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<tr>
<td>Lake water</td>
<td>4.0</td>
<td>3.94 ± 0.15</td>
<td>97.5 ± 3.5</td>
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<td></td>
<td>8.0</td>
<td>7.97 ± 0.05</td>
<td>99.25 ± 0.63</td>
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<td>Minerals water</td>
<td>4.0</td>
<td>3.88 ± 0.03</td>
<td>97.08 ± 1.08</td>
<td>0.95</td>
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<tr>
<td></td>
<td>8.0</td>
<td>8.01 ± 0.07</td>
<td>100.12 ± 1.37</td>
<td>1.2</td>
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<tr>
<td>Tap water</td>
<td>4.0</td>
<td>3.88 ± 0.05</td>
<td>97.0 ± 1.25</td>
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<td></td>
<td>8.0</td>
<td>7.88 ± 0.16</td>
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</table>
**Figure Captions**

Fig. 1 Absorption spectra changes of 1 (8 μM) in acetonitrile with addition of Cu$^{2+}$ from 0 to 60 μM.

Fig. 2 Fluorescence changes of 1 (8 μM) in acetonitrile by addition of Cu$^{2+}$ increasingly; Inset: fluorescence intensity ratio (I/I₀) versus concentrations of Cu$^{2+}$ from 0 to 60 μM.

Fig. 3 Linearity between fluorescence intensity ratio (I/I₀) of 1 and Cu$^{2+}$ (0 to 8 μM).

Fig. 4 Absorption changes of probe 1 (8 μM) in acetonitrile with addition of tested metal ions (80 μM).

Fig. 5 Color changes of probe 1 (8 μM) in acetonitrile solution in with addition of tested metal ions (80 μM).

Fig. 6 Fluorescence spectra of probe 1 (8 μM) in acetonitrile with addition of tested metal ions (80 μM).

Fig. 7 Fluorescence color changes of probe 1 (8 μM) in acetonitrile with addition of tested metal ions (80 μM) under illumination by using a 365 nm UV lamp.

Fig. 8 Fluorescence intensity of probe 1 (8 μM) in acetonitrile with addition of metal ions (80 μM), followed by addition of Cu$^{2+}$ (80 μM).
Fig.9 Time-dependent fluorescent intensity at 520 nm of probe 1 (8 μM) in acetonitrile solution in the presence of Cu^{2+} (16 μM).

Scheme 1 Design and synthesis of probe 1 and 2.

Scheme 2 The proposed mechanism of the reaction between probe 1 and Cu^{2+}

Scheme 3 The proposed mechanism of the reaction between probe 2 and Cu^{2+}.
Fig. 1

Fig. 2
Fig. 3

![Graph showing the relationship between copper concentration and absorbance. The equation is $y = 2.4E7x + 3.38$, and the $R^2$ value is 0.9813.]

Fig. 4

![Graph showing absorbance spectra for various ions. The x-axis represents wavelength in nm, and the y-axis represents absorbance. Peaks are shown for different ions, including Cu$^{2+}$, K$^+$, Na$^+$, Ag$^+$, Fe$^{3+}$, Mg$^{2+}$, Ca$^{2+}$, Mn$^{2+}$, Ba$^{2+}$, Cd$^{2+}$, Zn$^{2+}$, and Cd$^{2+}$.]

Fig. 5

Fig. 6

Fig. 7
Fig. 8

Fig. 9
Scheme 1

Scheme 2

Scheme 3
Chemodosimeters 1 and 2 were highly selective and sensitive to Cu$^{2+}$ which based on a Cu$^{2+}$-promoted hydrolysis reaction other than formation of complex.