# Highly sensitive and selective colorimetric and off-on fluorescent probe for $Cu^{2+}$ based on rhodamine derivative†

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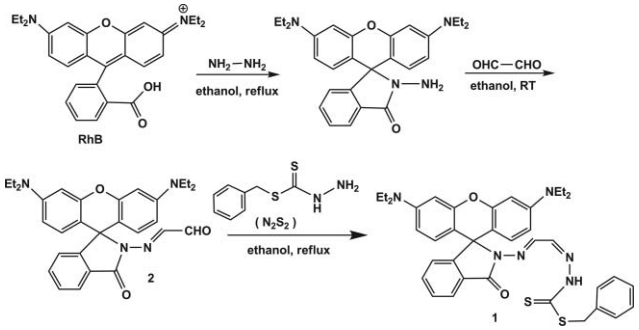
**A new probe for $Cu^{2+}$ based on the $Cu^{2+}$- induced reversible ring-opening mechanism of the rhodamine spirolactam was described. It displayed a highly selective and sensitive "turn-on" fluorescent and colorimetric response toward $Cu^{2+}$.**

Copper plays a critical positive role in several biological processes.[1] However, at certain high concentrations, copper is one of the most toxic and dangerous heavy metal elements to some organisms such as bacteria and viruses,[2] which is also confirmed to be harmful to humans, in that it can cause neurodegenerative diseases (*e.g.*, Alzheimer's and Wilson's diseases) probably by its involvement in the production of reactive oxygen species.[3] Consequently, a great effort has been devoted to the development of efficient and selective methods to assess copper ions in cells and organisms.

Fluorescent probes for $Cu^{2+}$ have been extensively explored since they allow nondestructive and prompt detection by a simple fluorescence enhancement (turn-on) or quenching (turn-off) response. Because of paramagnetic nature of $Cu^{2+}$, most of the reported $Cu^{2+}$ probes exhibit "on-off" signals.[4] In contrast, only few examples of "off-on" type probes are reported.[5] However, in terms of sensitivity and selectivity concerns, probes exhibiting fluorescence enhancement upon $Cu^{2+}$ complexation are favored over those showing fluorescence quenching under $Cu^{2+}$ binding. Among the reported 'turn-on'-type $Cu^{2+}$ probes, few have nanomolar sensitivity.[5a,5b,5c] Thus, it is of great interest to design and synthesis of fluorescent probes with $Cu^{2+}$ induced highly sensitive and "turn-on" fluorescence signals.

Rhodamine dyes have been employed extensively in the conjugation with biomolecules owing to their excellent fluorescence properties such as long absorption and emission wavelength, large absorption coefficient and high fluorescence quantum yield. Recently, various rhodamine-based turn-on fluorescent probes for metal ions have been reported.[6] The sensing mechanism of these probes is based on the change in structure between spirocyclic and open-cycle forms. Inspired by this platform, we envisioned a new rhodamine-based probe **1** (Scheme 1). It showed a reversible "turn-on" fluorescent response for $Cu^{2+}$ in aqueous solution with remarkably high sensitivity and selectivity.

Synthesis of probe **1** is shown in Scheme 1. Reaction of rhodamine B with $NH_2NH_2$ and then glyoxal afforded **2**, which further reacted with $N_2S_2$ to give probe **1** in 80% yield.




**Scheme 1** Synthesis of probe **1**.

A pH titration experiment was first evaluated as shown in Fig. S4 (ESI†), the absorbance of the probe-copper complex displayed a plateau in the pH range from 4.0 to 8.0, and the maximum absorbance toward the $Cu^{2+}$ was obtained under pH 6.0. In the view of sensitivity and the speed time, in our experiment, pH 6.0 was chosen as optimum experimental condition for environmental examples. Therefore, further UV/vis and fluorescent studies were carried out in methanol/HEPES mixed buffer solution (methanol–water = 8/2, pH 6.0, 0.02 M HEPES).

The fluorescence spectra of **1** in the presence of different concentrations of $Cu^{2+}$ in 20% (v/v) water–methanol solution (0.02 M HEPES, pH 6.0) were recorded (Fig. 1). Like most of the spirocycle rhodamine derivatives,[6] the free **1** displayed a very weak fluorescence, which indicated that the spirolactam form (Fig. 2 (bottom)) was the predominant species. When $Cu^{2+}$ was added to the buffer solution of **1**, a significant fluorescence intensity with an emission maximum at 580 nm increased in a $Cu^{2+}$ concentration-dependent way, which indicated the opened-ring

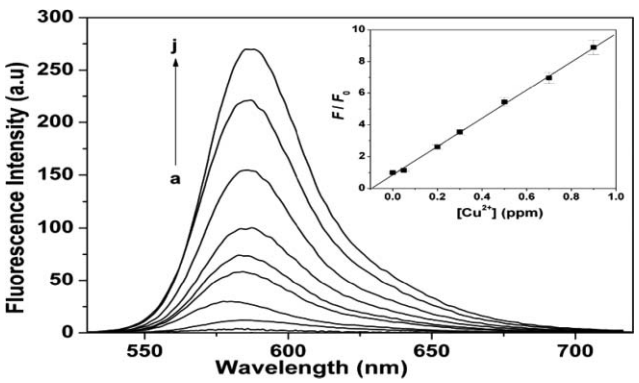



**Fig. 1** Emission spectra of **1** (1.0 μM) in the presence of various concentrations of $Cu^{2+}$ in 20% (v/v) water–methanol solution (0.02 M HEPES, pH 6.0). [$Cu^{2+}$]: (a) 0 to (j) 6.0 μM. Inset: Linear fluorescence intensity ($F/F_0$) of **1** (1.0 μM) upon addition of $Cu^{2+}$ (0–0.9 μM). The response ($F$) is normalized to the emission of the free probe **1** ($F_0$).

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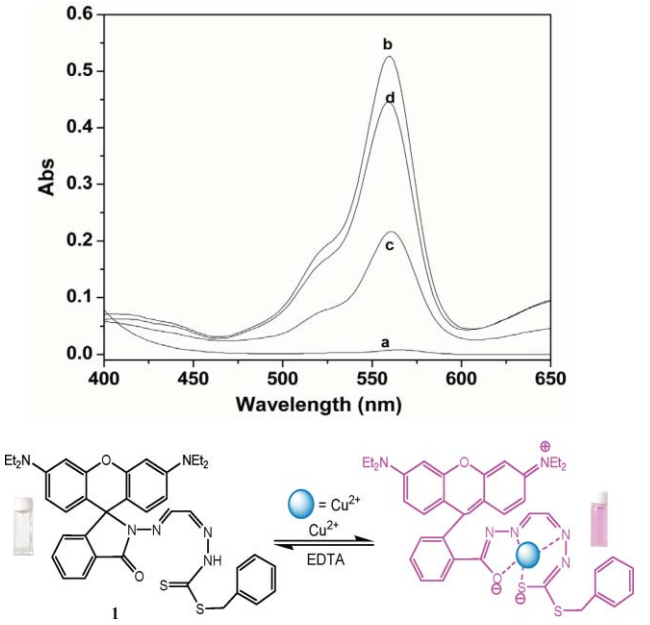


**Fig. 2** (top) Reversible titration response of **1** to $Cu^{2+}$ in methanol-HEPES buffer (0.02 M, pH 6.0) (8 : 2, v/v): (a) **1** (10 μM); (b) **1** (10 μM) with $Cu^{2+}$ (50 μM); (c) **1** (10 μM) with $Cu^{2+}$ (50 μM) and then addition of EDTA (0.1 mM); (d) **1** (10 μM) with $Cu^{2+}$ (50 μM) and EDTA (0.1 mM) and then addition of 0.2 mM $Cu^{2+}$. (bottom) Proposed binding mechanism of $Cu^{2+}$ with **1**.

form of **1** became the main species in the examined solution, and also a highly delocalized π-conjugated structure of **1** was formed (Fig. 2 (bottom)). Furthermore, the $F/F_0$ was well proportional to the amount of $Cu^{2+}$ ($5.0 \times 10^{-8}$–$9.0 \times 10^{-7}$ M) with a good linear correlation ($R = 0.9993$). The detection limit was 3 nM (based on S/N = 3, inset of Fig. 1). The result showed that the probe **1** was capable of detecting both qualitatively and quantitatively of $Cu^{2+}$.

The method of continuous variation (Job's method) was used (Fig. S1, ESI†) to determine the stoichiometry of **1**–$Cu^{2+}$ complex. As expected, the result indicated that a 1 : 1 stoichiometry of $Cu^{2+}$ to **1** in the complex, which was also supported by the Benesi–Hildebrand method (Fig. S2, ESI†).[7] The formation of a 1 : 1 complex was further confirmed by ESI(+)-MS analysis: an ethanol solution containing **1** and **1** equiv. of $Cu^{2+}$ (Fig. S3, ESI†) showed a strong peak at $m/z$ 738.06, assigned to [$Cu^{2+}$ + 1 − $H^+$]. The association constant $K$ was determined from the slope to be $1.7 \times 10^5$ $M^{-1}$, corresponding to a stronger binding capability toward $Cu^{2+}$ in comparison with a tren/dansyl-appended rhodamine based FRET probe for $Cu^{2+}$ (with a $K$ value of $7 \times 10^3$ $M^{-1}$),[6d] or a rhodamine spirolactam derivative-based probe for $Cu^{2+}$ (with a $K$ value of $2.08 \times 10^4$ $M^{-1}$).[6n]

To validate the selectivity of **1** in practice, some other metal ions, such as alkali or alkaline-earth metals ($Na^+$, $Mg^{2+}$ and $Ca^{2+}$) and heavy and transition metal ions ($Pb^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Ag^+$, $Hg^{2+}$, $Cr^{3+}$ and $Mn^{2+}$) were added to the solution of **1** under the same conditions (Fig. 3). The various metal ions did not induce any obvious color change and fluorescent enhancement, only $Fe^{3+}$ caused the ignored absorption and fluorescence change. For $Cu^{2+}$, the $F/F_0$ value was almost 100-fold, while the values for other metal ions were less than 10-fold. From the above experimental results, it suggested that **1** was a $Cu^{2+}$-selective probe in aqueous

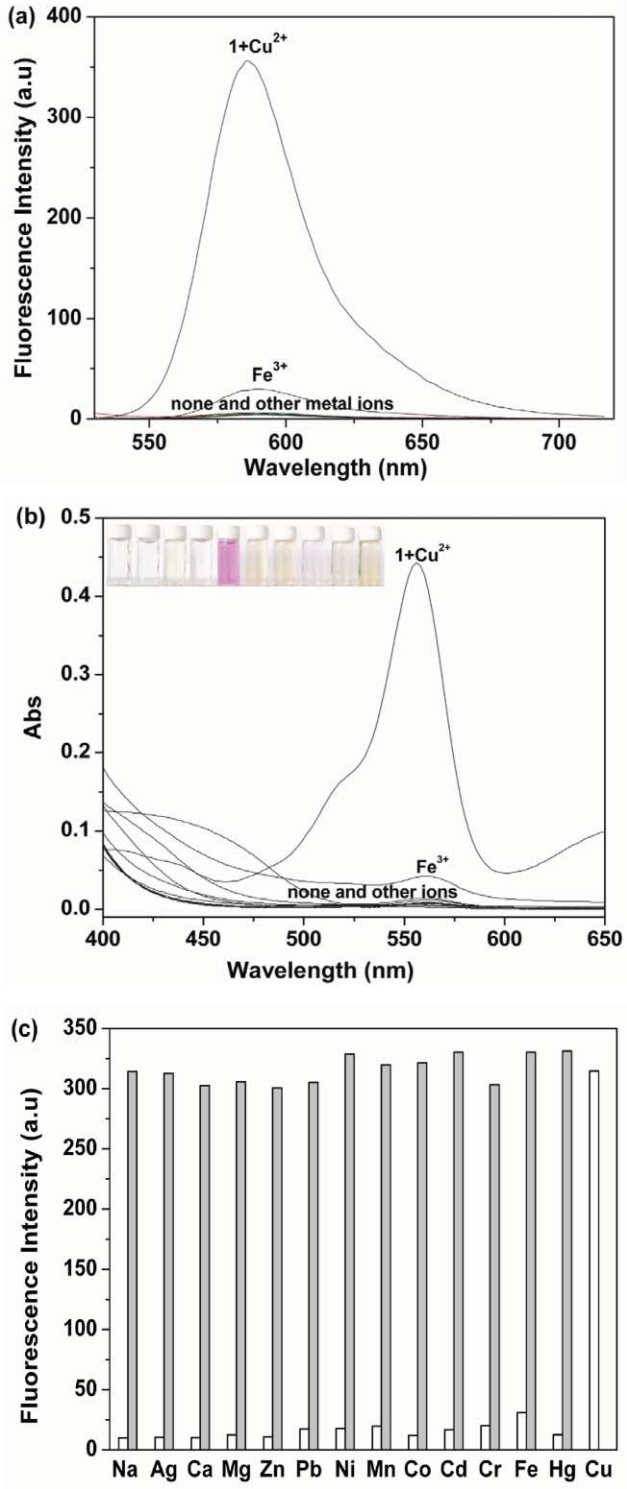


**Fig. 3** (a) Fluorescent emission changes of **1** (1.0 μM) in the presence of different metal ions (50 μM) in 20% (v/v) water–methanol solution (0.02 M HEPES, pH 6.0). (b) The absorption spectra of **1** (10 μM) in the absence and presence of different metal ions (50 μM). Inset: Change in color of **1** (20 μM) with metal ions (100 μM) (from left to right): blank, $Na^+$, $Ag^+$, $Hg^{2+}$, $Cu^{2+}$, $Pb^{2+}$, $Zn^{2+}$, $Fe^{3+}$, $Cd^{2+}$ and $Ni^{2+}$. (c) Fluorescence response of **1** (1.0 μM) to 10 μM of $Cu^{2+}$ or 50 μM of other metal ions (the gray bar portion) and to the mixture of 50 μM of other metal ions with 10 μM of $Cu^{2+}$ (the black bar portion).

 

condition. Finally, the competition experiments were also carried by adding $Cu^{2+}$ to the solution of **1** in the presence of 5 equiv. of other metal ions, and the results revealed that $Cu^{2+}$-induced fluorescence response was unaffected in the background of metal ions mentioned above (Fig. 3c).

The EDTA-adding experiments were conducted to examine the reversibility of this reaction. Addition of EDTA to the solution containing **1** and $Cu^{2+}$ diminishes the absorbance significantly, whereas readdition of excess $Cu^{2+}$ could recover the absorbance signal (Fig. 2 (top)). The $Cu^{2+}$-induced coloration and emission of **1** with $Cu^{2+}$ leading to spirocycle opening of **1**, as is the case for related rhodamine-based probes.[6]

Similar to many reported rhodamine spirolactam-based fluorescent probes,[6] the fluorescence enhancement response of **1** toward $Cu^{2+}$ is most likely the result of the spiro ring-opening mechanism rather than an ion-catalyzed hydrolysis reaction. The above-mentioned EDTA experiment could serve as experimental evidence to support this reversible spiro ring-opening mechanism. The proposed binding mechanism of **1** with $Cu^{2+}$ was shown in the bottom of Fig. 2.

In summary, a new rhodamine derivative used as selective and sensitive probe was developed, which could specifically recognize $Cu^{2+}$ in the aqueous buffer solution by the "naked eye", UV/vis and fluorescent responses. Furthermore, it also showed a "turn-on" type of absorption and fluorescence response. The quenching effect of water will limit the probes applicability in biological milieu at some extent. However, by simple modified with hydrophilic groups, we believed that this kind probe can be used for many practical applications, including biological systems.

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