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A highly selective fluorescent probe for Cu^{2+} based on rhodamine B derivative

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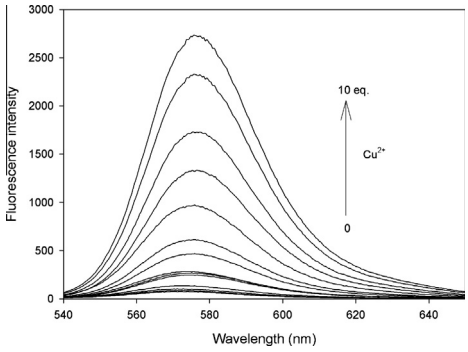
HIGHLIGHTS

- A new rhodamine-based fluorescent probe for Cu^{2+} has been developed.
- Upon the addition of Cu^{2+}, the probe exhibits fluorescence enhancement.
- The probe shows a high sensitivity and selectivity for Cu^{2+}.
- Response of probe to Cu^{2+} is reversible rather than a cation-catalyzed reaction.
- The probe has been used for imaging of Cu^{2+} in living cells.

GRAPHICAL ABSTRACT



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ABSTRACT

A new fluorescent probe **1** for Cu^{2+} based on a rhodamine B derivative was designed and synthesized. Probe **1** displays high sensitivity toward Cu^{2+} and about a 37-fold increase in fluorescence emission intensity is observed upon the addition of 10 equiv. Cu^{2+} in 50% water/ethanol buffered at pH 7.10. Besides, upon binding Cu^{2+} a remarkable color change from colorless to pink was easily observed by the naked eyes. The reversible dual chromo- and fluorogenic response toward Cu^{2+} is likely due to the chelation-induced ring-opening of rhodamine spirolactam. The linear response range covers a concentration range of Cu^{2+} from 8.0×10^{-7} to 1.0×10^{-4} mol/L and the detection limit is 3.0×10^{-7} mol/L. Except Co^{2+}, the probe exhibits high selectivity for Cu^{2+} over a large number of cations such as alkaline, alkaline earth and transitional metal ions. The accuracy and precision of the method were evaluated by the analysis of the standard reference material, copper in water (1.0 mol/L HNO_3). The proposed probe has been used for direct measurement of Cu^{2+} content in river water samples and imaging of Cu^{2+} in living cells with satisfying results, which further demonstrates its value of practical applications in environmental and biological systems.

Introduction

Copper is a transition element essential to plants and animals, including humans. After Fe^{2+} and Zn^{2+}, Cu^{2+} ranks the third in abundance among the essential trace elements in the human body, and plays a pivotal role in various fundamental physiological processes in organisms ranging from bacteria to mammals [1]. However, it is also harmful to biological systems in excessive amounts. Alteration in the cellular homeostasis of copper ions are associated with neurodegenerative disease, including Menkes and Wilson diseases, familial amyotropic lateral sclerosis, Alzheimer's disease, an prion diseases [2]. The Word Health Organisation (WHO) has set the maximum allowable level of copper in drinking

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water at 2 mg/L (31 μM) [3]. Nevertheless, copper contamination and its potential toxic effects on biological systems are still the challenging problems throughout the world because of copper wide use for many industrial, agricultural and domestic purposes. Accordingly, it is very important to monitor copper levels in various samples for environment and human health.

Thus far, various efficient and reproducible methods have been proposed to detect the concentrations of Cu^{2+} including atomic absorption spectrometry (AAS) [4], inductively coupled plasma–atomic emission spectrometry (ICP–AES) [5], inductively coupled plasma–mass spectrometry (ICP–MS) [6], *etc*. Though these techniques are sensitive, selective, and accurate for Cu^{2+} assay, they are usually complicated, time-consuming, and costly. Due to their advantages of high sensitivity, specificity, and ease of operation, a large number of fluorescent probes have been reported for the determination of copper ions so far. However, most of them exhibit fluorescence emission quenching upon binding Cu^{2+} owing to the paramagnetic nature of Cu^{2+} [7–10]. Fluorescence quenching is not only unfavourable for a high signal output upon recognition but also hinders temporal separation of spectrally similar complexes with time-resolved fluorometry [11]. Until now, only a few probes with a Cu^{2+} induced fluorescence enhancement have been proposed [2,12,13]. Thus, searching for Cu^{2+} probes based on fluorescence enhancement is still an active field as well as a challenge for the analytical chemistry research effort.

Rhodamine and its derivatives are excellent fluorophores and chromophores owing to their very good spectroscopic properties, such as large molar extinction coefficient, relatively long excitation and emission wavelengths elongated to visible region, high light stability, and high fluorescence quantum yield [14]. Rhodamine derivatives with a spirolactam-ring moiety are non-fluorescent and colorless. In the presence of a proton or metal cation, they can be converted to the open-ring forms via a reversible coordination or an irreversible chemical reaction and give rise to strong fluorescence emission and a pink color [15]. Thus, the rhodamine framework is an ideal model to fabricate the turn-on fluorescent probe. In the past few years, several rhodamine-modified probes have been developed for various cations such as Cu^{2+} [16–18], Hg^{2+} [19–21], Fe^{3+} [22–24], Pb^{2+} [25], Cr^{3+} [26], Ag^{+} [27], Zn^{2+} [28], and Cr^{5+} [29]. According to the Soft–Hard Acid–Base theory, the probes attached the recognition moiety with N and O atoms could display good affinity to Cu^{2+}. Recently, several probes based on rhodamine and salicylaldehyde derivatives have been widely used for detecting Cu^{2+} [30–39]. However, these probes contain hydrazone group which makes them unstable for the long term storage neither in the air nor in the solvent [40]. Herein, a Cu^{2+} fluorescent probe (probe **1**) based on the rhodamine hydrazone derivative has been developed. It exhibits fluorescence enhancement upon the addition of Cu^{2+} in 50% water/ethanol buffered at pH 7.10 and its high selectivity for Cu^{2+} in the presence of many other metal cations. Furthermore, the proposed probe has been used for direct measurement of Cu^{2+} content in river water samples and imaging of Cu^{2+} in living cells with satisfying results.

Experimental

Reagents

Rhodamine B, hydrazine hydrate (85%), salicylaldehyde, sodium borohydride and ethylenediaminetetraacetic acid disodium salt (EDTA-2Na) were obtained from Shanghai Chemical Reagents and used as received. Before being used, dichloromethane was distilled at atmospheric pressure from CaH_2 and stored over 4 Å molecular sieves. Other chemicals were of analytical reagent grade and used without further purification except when specified. Doubly distilled water was used throughout all experiments. Thin layer chromatography (TLC) was carried out using silica gel 60 F254, and column chromatography was conducted over silica gel (200–300 mesh), both of which were obtained from the Qingdao Ocean Chemicals (Qingdao, China). The Cu standard solution, copper in water (1.0 mol/L HNO_3) (GSB 04-1725-2004) was obtained from Analysis and Testing Center of National Nonferrous Metals and Electronic Materials (Beijing, China).

Syntheses

The synthetic route for fluorescence probe **1** was shown in Scheme 1.

Compound **2** was synthesized according to Xiang's method [16] by the reaction of rhodamine B and hydrazine hydrate (85%). Compound **3** was prepared starting from compound **2** and salicylaldehyde by the reported literature [16].

*Synthesis of compound **1***

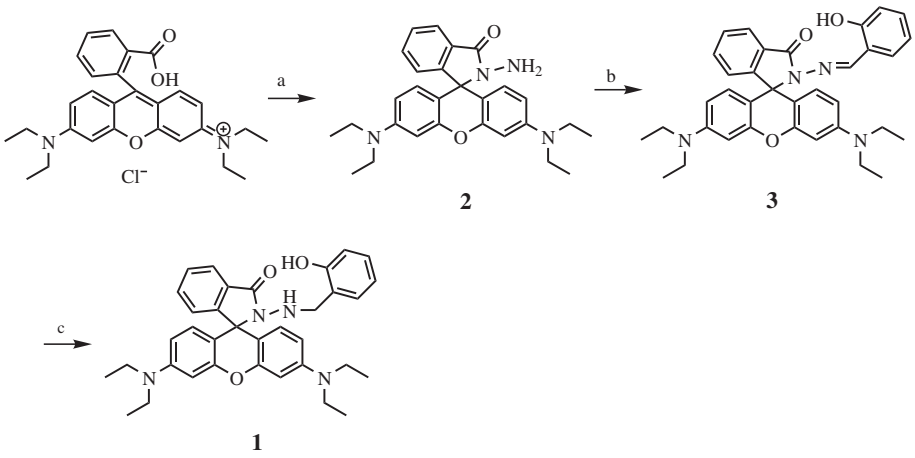
To a stirred solution of compound **3** (0.50 g, 1 mmol) in anhydrous CH_2Cl_2 (20 mL) cooled to 0 °C was carefully added solid sodium borohydride (0.19 g, 5 mmol) in portions. The resulting mixture was stirred at room temperature for 48 h. After reaction, the reaction mixture was filtrated and the filtrate was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using petroleum ether/$CH_3COOCH_2CH_3$ (7:1, V/V) as eluent to afford compound **1** (0.23 g, 40%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$), δ(ppm): 1.16 (t, 12H, $NCH_2C\underline{H}_3$, J = 7.2 Hz), 3.33 (q, 8H, $NC\underline{H}_2CH_3$, J = 7.2 Hz), 3.86 (d, 2H, $NHC\underline{H}_2$, J = 6.8 Hz), 4.22 (t, 1H, $N\underline{H}CH_2$, J = 6.8 Hz), 6.24 (dd, 2H, Xanthene-H, J = 8.8 Hz, 2.8 Hz), 6.43 (m, 2H, Xanthene-H), 6.45 (m, 2H, Xanthene-H), 6.68 (m, 1H, Phen-H), 6.81 (d, 1H, Phen-H, J = 8.0 Hz), 6.85 (dd, 1H, Phen-H, J = 7.4 Hz, 1.6 Hz), 7.10 (m, 1H, Phen-H), 7.14 (dd, 1H, Ar-H, J = 7.2 Hz, 1.6 Hz), 7.50 (m, 2H, Ar-H), 7.95 (m, 1H, Ar-H), 8.80 (bs, 1H, Phen-OH).

Apparatus

All fluorescence measurements were made on a Hitachi F-4500 Fluorescence Spectrometer (Tokyo, Japan) in 1 cm × 1 cm quartz cell. UV–Vis absorption spectra were recorded on a Shimadzu UV-2450 UV–Vis spectrophotometer (Tokyo, Japan) in 1 cm path length quartz cuvettes with a volume of 4 mL. ^{1}H NMR was acquired in $CDCl_3$ on Varian INOVA-400 MHz NMR spectrometer using TMS as an internal standard. The measurements of pH were carried out on a Mettler-Toledo Delta 320 pH meter (Shanghai, China) with a Mettler combination glass electrode (No. 4140230002). The electrode was calibrated for pH using commercial pH reference solutions (pH 4.00, pH 6.86 and pH 9.18 standard solutions). Data processing was performed on an Inter Core i5 computer with software of SigmaPlot.

Measurement procedures

A stock solution of 2.0×10^{-5} mol/L compound **1** was obtained by dissolving the requisite amount of **1** in absolute ethanol. A standard stock solution of 1.0×10^{-2} mol/L Cu^{2+} was prepared by dissolving an appropriate amount of copper chloride in water and adjusting the volume to 100 mL in a volumetric flask. Working solutions of copper chloride were freshly prepared by serial dilution of the stock solution with 0.05 M Tris–HCl buffer solution. The complex solution of Cu^{2+} and compound **1** was obtained by mixing 12.5 mL of the stock solution of compound **1** and 2.5 mL of Cu^{2+} solution of the different concentrations in a 25 mL volumetric flask. Then the mixture was diluted to 25 mL with Tris–HCl buffer solution. In the solution thus obtained, the concentrations were



Scheme 1. Synthesis of fluorescent probe **1**. (a) hydrazine hydrate(85%), ethanol, reflux, 12 h, 73%; (b) salicylaldehyde, ethanol, reflux, 5 h, 55%; (c) $NaBH_4$, anhydrous CH_2Cl_2, 48 h, 40%.

1.0×10^{-5} mol/L in compound **1** and 8.0×10^{-7}–1.0×10^{-3} mol/L in Cu^{2+}. Blank solution of compound **1** was prepared under the same conditions without Cu^{2+}. The salts used in other stock solutions of metal ions were LiCl, NaCl, KCl, $MgSO_4$, $AlCl_3$, $CaCl_2$, $MnCl_2$, $AgNO_3$, $Pb(NO_3)_2$, $Hg(NO_3)_2{\cdot}0.5H_2O$, $(CH_3CO_2)_2Cd{\cdot}2H_2O$, $Co(CH_3CO_2)_2{\cdot}H_2O$, $NiSO_4{\cdot}6H_2O$, $ZnSO_47H_2O$, $Fe_2(SO_4)_3$. The wide pH range solutions were prepared by adjustment of 0.05 mol/L Tris–HCl solution with HCl or NaOH solution. For all measurements of fluorescence spectra, excitation was fixed at 520 nm with excitation slit set at 10.0 nm and emission at 10.0 nm. The voltage of the photomultiplier tube (PMT) detector is 700 V.

Cell incubations

H292 lung cancer cells were cultured in RPMI Medium 1640 (Gibco) containing 10% calf bovine serum (HyClone) at 37 °C in humidified air and 5% CO_2. H292 lung cancer cells grown on coverslips were washed with phosphate-buffered saline (PBS), followed by incubating with 10 μM of probe **1** in DMSO–RPMI Medium 1640 (1:99, V/V) for 30 min at 37 °C, and then washed with PBS three times. After incubating with 10 μM of $CuCl_2$ in PBS for 1 h at 37 °C, the cells were washed with PBS three times again.

Fluorescence imaging

Confocal fluorescence imaging was performed with a Zeiss LSM 700 laser scanning microscope with 20× objective lens. Excitation of **1**-loaded cells at 555 nm was carried out with a solid laser and emission was collected at 560–1000 nm.

Results and discussion

Spectral characteristics

Fig. 1 exhibits fluorescence emission changes of 10 μM probe **1** in buffered (Tris–HCl, pH = 7.10) ethanol/water (1:1, v/v) upon addition of different Cu^{2+} concentrations. As can be seen from Fig. 1, free **1** shows very slight fluorescence in the 540–650 nm range and the fluorescence intensities of probe **1** increase obviously at 576 nm with increasing concentration of Cu^{2+}. When the concentration of Cu^{2+} is up to 10 equiv. of compound **1**, an approximately 37-fold enhancement in the fluorescence emission intensity was observed. These results constitute the basis for the determination of Cu^{2+} concentration with probe **1** proposed in this work.

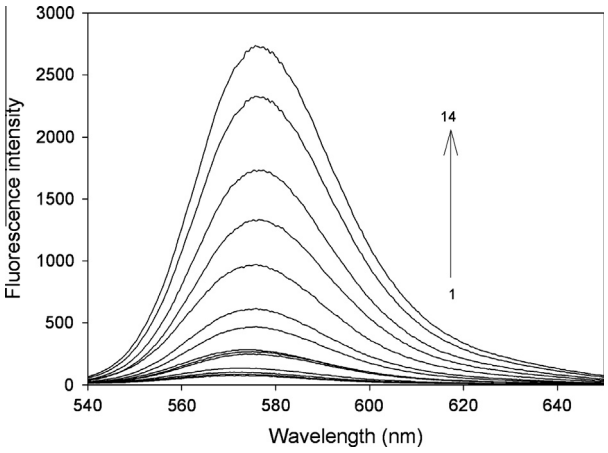


Fig. 1. Changes of the fluorescence spectra of probe **1** (10 μM) in the prescence of various concentrations of Cu^{2+}: 0, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0, 10, 20, 30, 40, 60, 80, 100 μM from 1 to 14 (λ_{ex} = 520 nm). These spectra were measured in 0.05 M Tris–HCl buffer (ethanol/water = 1:1, v/v, pH 7.10).

In order to obtain a better insight into the response mechanism of **1** toward Cu^{2+}, the absorption spectra of **1** in the absence and presence of Cu^{2+} were recorded (Fig. 2). The colorless free **1** (10 μM) in ethanol/water (1:1, v/v) exhibited almost no absorption near 560 nm, which could be ascribed to the closed spirolactam form of probe **1** in the absence of Cu^{2+}. Upon addition of 1.0×10^{-3} M Cu^{2+} to the solution of probe **1**, a new absorption peak at 559 nm ($\varepsilon = 1.20 \times 10^3$ L mol^{-1} cm^{-1}) emerged and the solution displayed a clear change from colorless to pink, indicating the formation of the ring-opened amide form of probe **1** in the presence of Cu^{2+} [15]. Job's method for the absorbance was applied to determine the stoichiometry between **1** and Cu^{2+}, by keeping the sum of the initial concentration of copper ion and at 1.0×10^{-4} mol/L (Fig. 3) [41]. As depicted in Fig. 3, a maximum absorbance was showed when the molecular fraction of Cu^{2+} was close to 0.5, which indicated the 1:1 complex formation between **1** and Cu^{2+}. Thus, a possible coordination mode for probe **1** with Cu^{2+} was proposed (Scheme 2).

Principle of operation

On the basis of 1:1 stoichiometric relationship mentioned above, the linear response of the fluorescence emission intensity

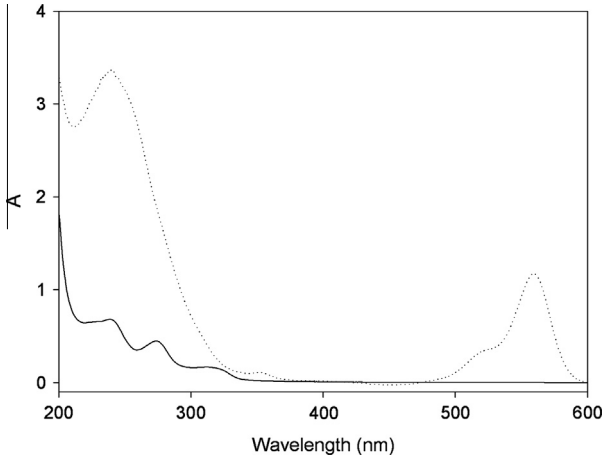


Fig. 2. UV-spectra of probe **1** (10 μM) before (—) and after (···) the addition of Cu^{2+} (1.0×10^{-3} mol/L) in pH 7.10 tris–HCl buffer (ethanol/water, 1:1, v/v).

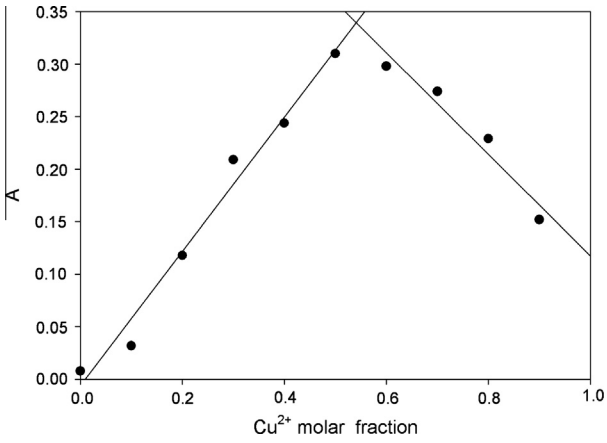
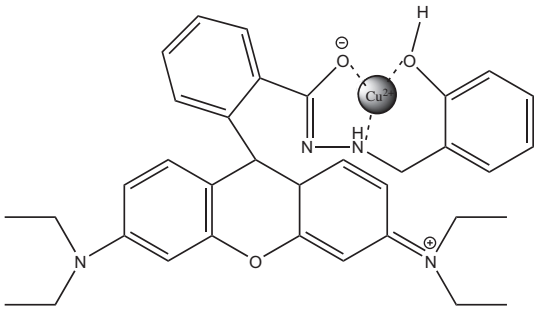


Fig. 3. Job's plot for probe **1** in 0.05 M tris–HCl buffer (ethanol/water = 1:1, v/v, pH 7.10). The total concentration of 1 and Cu^{2+} was 100 μM.



Scheme 2. Possible coordination mode for probe **1** with Cu^{2+}.

of probe **1** toward amounts of Cu^{2+} added was obtained in Cu^{2+} concentration range of 8.0×10^{-7} to 1.0×10^{-4} mol L^{-1} (Fig. 4). The linear response can be expressed by the following Eq. (1) of the calibration line:

$$F = 113.3242 + 27.0467 \times 10^{6} C_{Cu}^{2+} \quad (R = 0.9979) \qquad (1)$$

Here F is the fluorescence emission intensity of probe **1** actually measured at a given metal concentration, and C_{Cu}^{2+} represents the concentration of Cu^{2+} added. The detection limit attained for Cu^{2+}, calculated by 3 s_B/m (where s_B is the standard deviation of

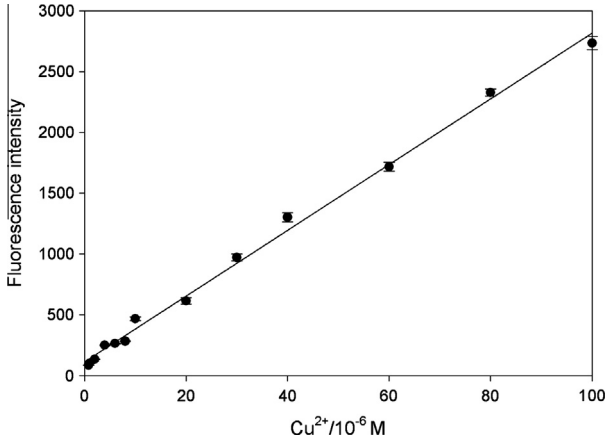


Fig. 4. Plot of fluorescence intensity of the proposed probe **1** as a function of C_{Cu}^{2+}. These data were measured in pH 7.10 Tris–HCl buffer (ethanol/water = 1:1, v/v). The excitation wavelength was 520 nm. Measurements were carried out in triplicate.

10 measurements of the blank and m is the slope of the calibration line) was 3.0×10^{-7} mol/L [42]. Thus, our proposed probe **1** could sensitively detect environmentally relevant levels of Cu^{2+}.

Effect of pH

To study the practical applicability, the effects of pH on the fluorescence intensity of probe **1** in the absence Cu^{2+} were investigated in the pH range from 3.00 to 13.00 (Fig. 5). As shown in Fig. 5, probe **1** did not show any obvious and characteristic fluorescence (excitation at 520 nm) in the pH range of 6.00–12.00, suggesting that it was insensitive to pH and that the spirolactam form is still preferred in this condition. When pH was lower than 6.00, the fluorescence intensity increased with decreasing pH values, which might be caused by the ring-opened form of **1** in the presence of proton. From the view of sensitivity and the speed time, the Tris–HCl buffer solution at pH 7.10 (ethanol/water, 1:1, v/v) was used as an ideal experimental media.

Selectivity

Selectivity is a very important parameter to evaluate the performance of a fluorescence probe. The selectivity experiments were carried out by fixing the concentration of ion at 1.0×10^{-4} mol/L,

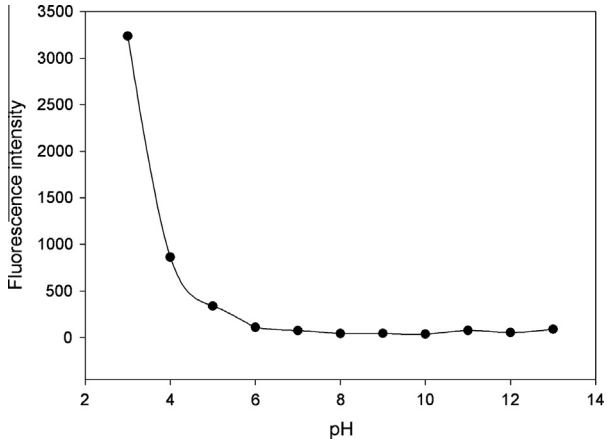


Fig. 5. pH dependence of the fluorescence intensity of 10 μM probe **1** in the absence of Cu^{2+}. All data were obtained at various pH values (pH 3.00–13.00) and the excitation wavelength was 520 nm.

and various cations were added as chlorides, nitrates, acetate and sulfates. Fig. 6 (the black bar portion) illustrated the fluorescence responses of probe **1** to various cations. As can be seen from the black bars in Fig. 6, only Co^{2+} cause a mild fluorescence intensity enhancement, while the other metal ions not induce any obvious fluorescence interferences, indicating that our proposed probe exhibits high selectivity for the Cu^{2+} ion over other metal ions. To test practical applicability of our fluorescent probe for Cu^{2+}, competition experiments were performed in which the fluorescent probe was added to a solution of Cu^{2+} in the presence of other metal ions (white bars in Fig. 6). Experimental results indicate that the response of the probe to Cu^{2+} is unaffected by the presence of the other possible contaminating metal ions. It can be found that except Co^{2+} the relative error of common interference such as alkali, alkaline earth and transitional metal ions was less than ±5%, which is considered as tolerated. Fortunately, Co^{2+} could be effectively circumvented by adding masking reagent F^-. All these selective results indicate that our proposed probe could meet the selective requirements for practical applications.

Reversibility and long-term stability

As is well known, the reversibility is important for the fabrication of devices to sense copper ion. Because of the high stability constant of the EDTA-Cu, the EDTA-adding experiments were conducted to examine the reversibility of the probe **1** in Fig. 7. Firstly, the addition of Cu^{2+} to the free **1** solution makes the fluorescence increase significantly. Secondly, the pink color and fluorescence of the solution containing **1** and Cu^{2+} disappeared instantly upon the addition of excess EDTA. The above results elicit that the spectral response of probe **1** to Cu^{2+} is reversible rather than a cation-catalyzed reaction [43].

In order to examine the long-term stability of the prepared probe, a stock solution of 2.0×10^{-5} mol/L compound **1** in absolute ethanol and a standard stock solution of 1.0×10^{-2} mol/L Cu^{2+} in water were stored in a refrigerator when not in use. The probe works well and no detectable change in working concentration range is found for one month, which implies that the proposed probe is stable in absolute ethanol.

Preliminary analytical application

In order to test the practical application of the proposed probe, the probe was first applied in the determination of Cu^{2+} in the river water samples obtained from different locations of Jinshui River, Zhengzhou and simply filtrated. In order to reduce pH influence on the Cu^{2+} detection, the pH values of sample solutions were buffered to pH 7.10. The results were summarized in Table 1, which agreed well with the atomic absorption spectrometry reference method with a relative deviation less than 3%. These results demonstrated that our proposed fluorescent probe could meet the sensitivity as well as selectivity requirements for environmental water samples monitoring applications. In order to evaluate the accuracy using the proposed method, the determination of copper in standard reference materials, copper in water (1.0 mol/L HNO_3) (GSB 04-1725-2004) was carried out. The copper mass concentration in the Cu standard solution (GSB 04-1725-2004) was referenced to be 1000 μg/mL. The measured copper mass concentration in the Cu standard solution (GSB 04-1725-2004) was 999.5 ± 7.9 μg/mL, which was in good agreement with the certified value. Thus, the proposed sensor seems useful for the determination of iron ions in real samples.

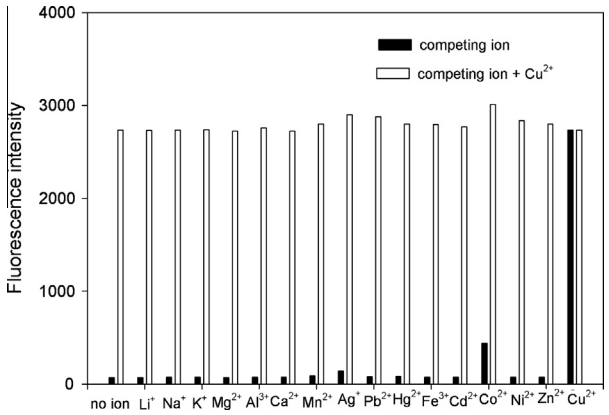


Fig. 6. Metal ion selectivity of probe **1** (10 μM). All data were obtained at pH 7.10 Tris–HCl buffer (ethanol/water = 1:1, v/v). The concentration of ions added to probe 1 was 1.0×10^{-4} mol/L for all metal ions. The excitation wavelength was 520 nm. Black bars: different metal ions were added. White bars: different metal ions in the presence of Cu^{2+} were added.

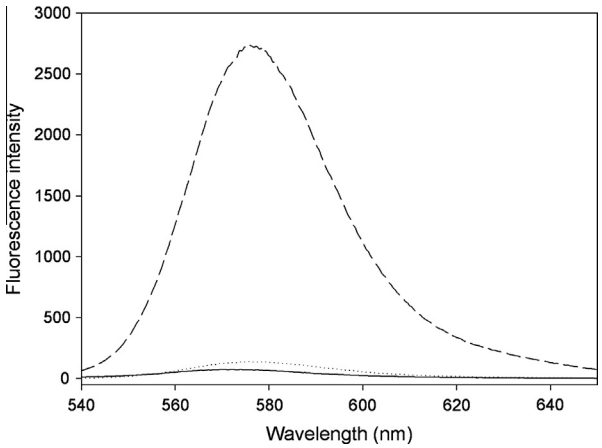


Fig. 7. Reversible fluorescence response of **1** to Cu^{2+} in pH 7.10 Tris–HCl buffer (ethanol/water, 1:1, v/v). —: 10 μM probe **1**; ----: 10 μM probe **1** with 100 μM Cu^{2+}; ···: 10 μM probe **1** with 100 μM Cu^{2+} and then addition of 400 μM EDTA-2Na. Excitation was performed at 520 nm.

To further demonstrate the practical applicability of the proposed probe in biological samples, fluorescence imaging experiments were carried out in living cells (Fig. 8). Incubation of H292 lung cancer cells with 10 μM of probe **1** for 30 min at 37 °C gave very weak fluorescence (Fig. 8A). When H292 lung cancer cells were incubated with 10 μM Cu^{2+}, the same treatment with probe **1** generated much brighter fluorescence (Fig. 8D). The results suggested that probe can penetrate the cell membrane and can be applied for in vitro imaging of Cu^{2+} in living cells and potentially in vivo.

Method performance comparison

The performance of the proposed probe toward Cu^{2+} was compared with some reported fluorescent probes based on either rhodamine or salicylaldehyde derivative for Cu^{2+} determination, as

Table 1
Results of Cu^{2+} determination in river water samples.

Sample	Concentration of copper (M)		Relative error (%)
	The proposed sensor	Atomic absorption spectrometry	
River water 1	$(11.81^a \pm 0.24^b) \times 10^{-6}$	$(11.68^a \pm 0.18^b) \times 10^{-6}$	1.11
River water 2	$(8.86^a \pm 0.13^b) \times 10^{-6}$	$(8.64^a \pm 0.15^b) \times 10^{-6}$	2.55
River water 3	$(6.43^a \pm 0.07^b) \times 10^{-6}$	$(6.57^a \pm 0.12^b) \times 10^{-6}$	−2.13

[a] Mean value of the three determinations.
[b] Standard deviation.

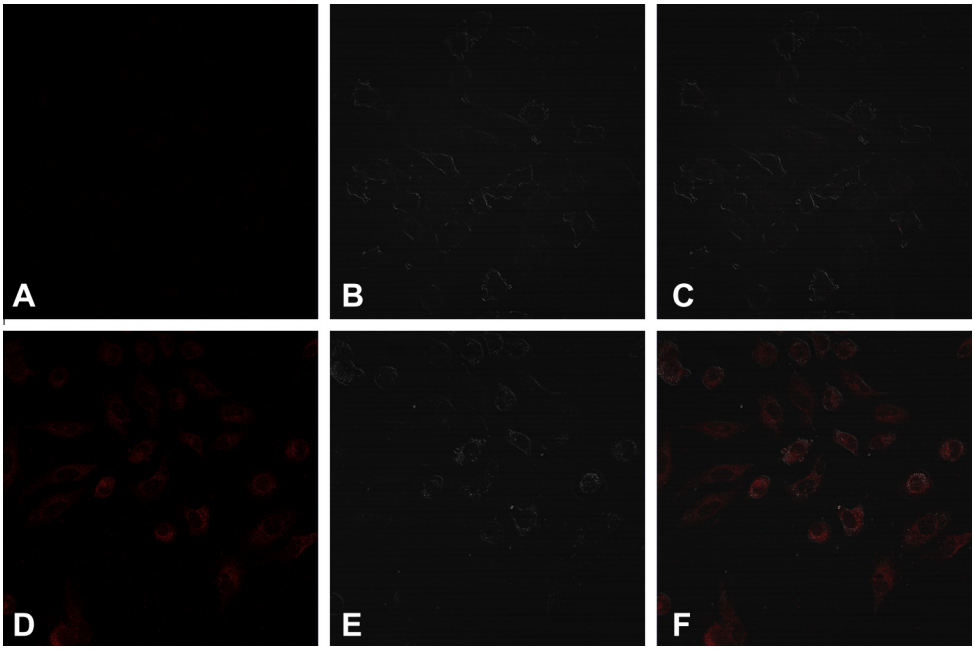


Fig. 8. Images of H292 lung cancer cells treated with the presented probe **1**. (A) Fluorescence image of H292 lung cancer cells incubated with **1** (10 μM) for 30 min. (B) Bright-field transmission image of cells shown in panel (A). (C) Overlay image of (A) and (B). (D) Fluorescence image of H292 lung cancer cells incubated with **1** (10 μM) for 30 min, washed three times, and then further incubated with 10 μM Cu^{2+} for 1 h. (E) Bright-field transmission image of cells shown in panel (D). (F) Overlay image of (D) and (E).

Table 2
The comparison of the proposed probe with some other fluorescence probes for the measurement of Cu^{2+}.

Modes	Reagents	Linear range (M)	Limit of detection (M)	Working media	Interference of other metal ions	Applications	Refs.
Quenching λ_{ex}/em = 529/575 nm	The mixture of Rhodamine B and rhodamine derivative	1×10^{-7}–3.6×10^{-6}	2.5×10^{-8}	pH 7.0 buffer EtOH/H_2O (1:1, V/V) solution	No	Water samples	[30]
Enhancement λ_{ex}/em = 440/510 nm	Salicylaldehyde derivative	2×10^{-6}–4.8×10^{-6}	4.477×10^{-7}	pH 7.0 buffer EtOH/H_2O (9:1, V/V) solution	Co^{2+}, Hg^{2+}	Fluorescent imaging in Hela cells	[39]
Quenching λ_{ex}/em = 365/512 nm	Salicylaldehyde derivative	Not discussed	6×10^{-7}	CH_2Cl_2	Co^{2+}	Molecular logic switch	[44]
Enhancement λ_{ex}/em = 480/530 nm	Rhodamine derivative	Not discussed	Not discussed	CH_3CN	Hg^{2+}	No	[45]
Enhancement λ_{ex}/em = 552/580 nm	Rhodamine derivative	2×10^{-6}–2.0×10^{-5}	2×10^{-6}	pH 7.0 buffer MeOH/H_2O (1:1, V/V) solution	Zn^{2+}	No	[46]
Enhancement λ_{ex}/em = 550/576 nm	Rhodamine derivative	Not discussed	3.0×10^{-6}	pH 7.4 buffer MeOH/H_2O (1:1, V/V) solution	No	Fluorescent imaging in Hela cells	[47]
Enhancement λ_{ex}/em = 530/571 nm	Rhodamine derivative	Not discussed	2.0×10^{-7}	pH 7.04 buffer CH_3CN/H_2O (2:8, V/V) solution	No	No	[41]
Enhancement λ_{ex}/em = 520/576 nm	Rhodamine derivative	8.0×10^{-7}–1.0×10^{-4}	3.0×10^{-7}	pH 7.10 buffer EtOH/H_2O (1:1, V/V) solution	Co^{2+}	Fluorescent imaging in H292 lung cancer cells and water samples	This work

shown in Table 2. It can be found that reported fluorescence probe for Cu^{2+} usually have interference problems caused by other transition metals cations such as Hg^{2+}, Zn^{2+} and Co^{2+} [39,44–46]. But the proposed sensor in this work exhibits good selectivity and has no interference of other transition metals cations except Co^{2+}. Moreover, the proposed probe in this paper displays wide linear span and low detection limit compared with those probes in Refs. [39,46,47]. The two probes in Refs. [44,45] need more rigorous testing media and the applicability in living cells are not investigated. The applications in living cells in Refs. [30,44–46,41] are also not validated. Therefore, from Table 2 it was not difficult to find that our newly developed sensor for detecting of Cu^{2+} featured with high selectivity, a wide linear range and wide applicability.

Conclusion

In summary, we have designed and synthesized a new rhodamine-based fluorescent probe for copper ions. Our proposed probe displays a reversible absorption and fluorescence enhancement response to Cu^{2+} via a 1:1 binding mode. Moreover, probe **1** shows

a high sensitivity and selectivity for Cu^{2+} sensing in comparison to other cations in 50% water/ethanol buffered at pH 7.10. It could work over a wide pH range from 6.00 to 13.00, which is important for possible use in practical view to selective requirements for biomedical and environmental application. The proposed probe can be applied to the quantification of Cu^{2+} with a linear range covering from 8.0×10^{-7} to 1.0×10^{-4} mol/L and the detection limit is 3.0×10^{-7} mol/L. All of its merits make it adequate for practical application of Cu^{2+} array. The present probe has been used for direct measurement of Cu^{2+} content in river water samples and imaging of Cu^{2+} in living cells with satisfying results, which further demonstrates its value of practical applications in environmental and biological systems.

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