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Optical Cu^{2+} probe bearing an 8-hydroxyquinoline subunit: High sensitivity and large fluorescence enhancement

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ABSTRACT

A new fluorescent and colorimetric probe **RCu1** with ultra-sensitivity (detection limit is 4.7 nM (3σ)) was developed. It is based on a rhodamine B derivative modified with a functionalized 8-hydroxyquinoline group as a copper-binding site which greatly increases the affinity for Cu^{2+}. Cu^{2+} acts not only as a selective recognizing guest but also a hydrolytic promoter. **RCu1** showed single-selectively sensing Cu^{2+} over other cations and much larger fluorescence enhancement (as high as over 1000-fold) than those based on Cu^{2+}-complexation. The detection mechanism was proved by TOF-MS, FT-IR, ^1H NMR and Gaussian calculations. Based on the outstanding recognition ability of **RCu1** toward Cu^{2+}, an analytical procedure was developed for Cu^{2+}-determination in natural water samples and soil sample.

1. Introduction

Copper, the third most abundant transition metal in human bodies, is vital for both environmental and biological systems. Nevertheless, at high concentrations, copper becomes a toxic and hazardous element to organisms such as many bacteria [1]. Owing to its toxicity to bacteria, elevated concentrations of copper hamper the self-purification capability of the sea or rivers and destroy the biological reprocessing systems in water. Furthermore, alteration in the cellular homeostasis of copper ions can cause neurodegenerative diseases [2], such as Menkes and Wilson's diseases, familial amyotrophic lateral sclerosis, prion disease and Alzheimer's disease, probably by its involvement in the production of reactive oxygen species [3]. Therefore, considerable efforts have been devoted to develop Cu^{2+}-selective fluorescent probes due to the nondestructive, quick, and sensitive advantages of emission signals [4]. Various fluorescent sensors for Cu^{2+} sensing have been reported to date. However, most Cu^{2+} probes showed "on–off" (fluorescence quenching) behavior [5–9] due to paramagnetic nature of Cu^{2+}. Such "on–off" signals might be caused by other quenchers in practical samples, and thereby, the fluorescence "on–off" probes are undesirable for practical analytical application. In terms of sensitivity concerns, probes exhibiting fluorescence enhancement (fluorescence "off–on") are favored over those showing fluorescence quenching (fluorescence "on–off"). In recent years, "off–on" type sensors have also been developed [10–19]. While among the reported Cu^{2+} probes, few of them have nanomolar level sensitivity [17,18,20] and the non-aqueous work conditions [21–23] (due to the strong hydration ability of Cu^{2+}) inhibited the application of these probes in complicated environmental samples. In addition, the fluorescence enhancement signals in most cases are still weak, and it often suffers from a high background fluorescence. Therefore, it is of great challenge and increasing interest to develop a fluorescence probe for Cu^{2+} determination with high sensitivity and large fluorescence enhancement in aqueous media.

Rhodamine dyes, are used extensively as fluorophores by virtue of their excellent photophysical properties, such as long absorption and emission wavelengths, high fluorescence quantum yield, and large absorption coefficient. On the other hand, rhodamine derivatives are nonfluorescent and colorless in the "ring-closed" state, whereas the ring-open form gives rise to strong fluorescence emission and absorption. Based on this mechanism, many fluorimetric and colorimetric chemosensors and chemodosimeters for metal detection have been exploited [13,17,24–28].

Quinoline-based ligands have proved popularly as fluorogenic agents for the chemical assay for Zn^{2+} [29–31]. Whereas in recent years, quinolines and their derivatives have also applied in copper recognition [23,32–34]. Compared to the phenolic ligand in many excellent Cu^{2+} probes [13,14,35,36], as the lone electron pair of N atom at quinoline-based ligands is isolated from the π-electron of the aromatic ring, it is more conducive for quinoline to coordinate with metal ions. Lippard and co-workers

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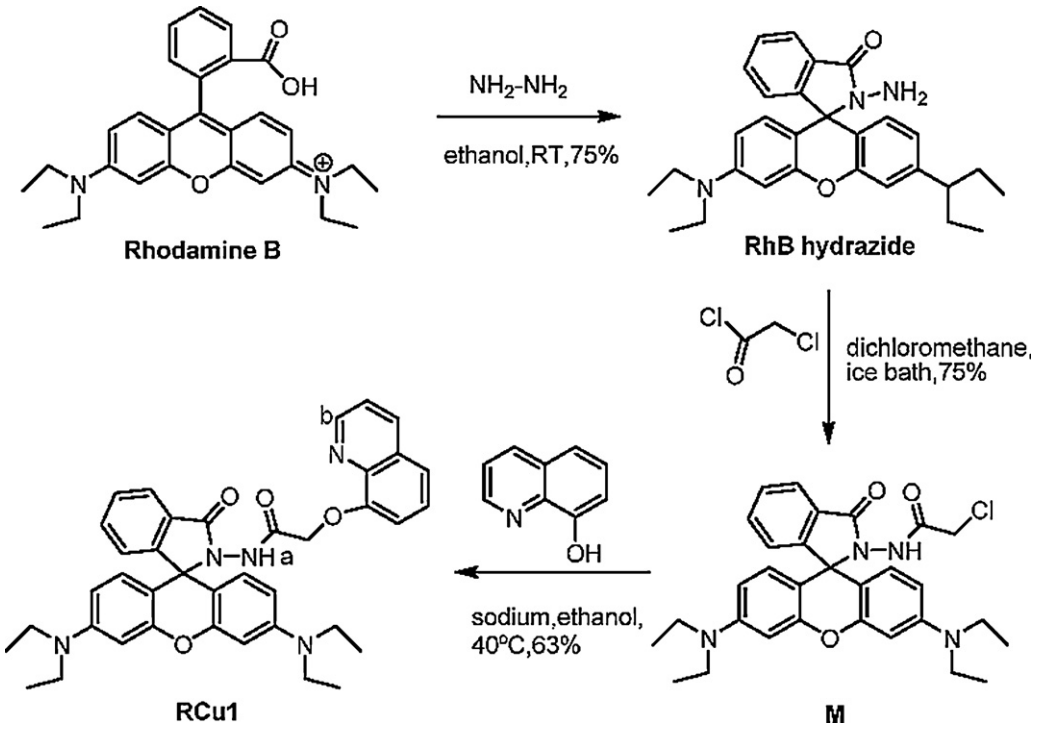


Scheme 1. Synthesis of **RCu1**.

incorporated an 8-aminoquinoline group as a copper-binding moiety into a fluorescein, which could coordinate well with Cu^{2+}, and the copper (II)-fluorescein complex could detect nitric oxide in aqueous solution [34]. Inspired by their successful work, we equipped rhodamine fluorophore with a quinoline moiety, serving as a Cu^{2+}-chelator to increase the affinity for Cu^{2+}, which might improve the sensitivity of the probe **RCu1** (Scheme 1). Among the various metal ions, **RCu1** presented highly selective fluorimetric and colorimetric changes toward Cu^{2+} in aqueous solution. The weakly fluorescent probe displayed a large enhancement ($F/F_0 > 1000$) upon binding with Cu^{2+}. As expected, the quinoline moiety in the compound **RCu1** indeed played an indispensable role in the course of binding with Cu^{2+} and the detection limit is as sensitive as 4.7 nM (3σ). In addition, the probe **RCu1** displayed the potential application for trace Cu^{2+} analysis in four real environmental samples: pool-, tap-, sea-water and soil.

2. Experimental

2.1. Materials and methods

All the solvents were of analytic grade. The solutions of metal ions were prepared from KCl, NaCl, $CaCl_2$, $MgCl_2{\cdot}6H_2O$, $ZnCl_2$, $CdCl_2$, $BaCl_2{\cdot}2H_2O$, $FeCl_3{\cdot}6H_2O$, $CrCl_3$, $PbCl_2$, $HgCl_2$, $AgNO_3$, $MnCl_2{\cdot}5H_2O$, $NiCl_2{\cdot}6H_2O$, $CoCl_2{\cdot}6H_2O$, NH_4Cl, $LaCl_3{\cdot}7H_2O$, $CuCl_2{\cdot}2H_2O$, KI, $Na_2HPO_4{\cdot}12H_2O$, KBr, $Na_2CO_3{\cdot}10H_2O$, CH_3COONa, NaCl, $NaNO_3$, $NaClO_4{\cdot}H_2O$, respectively, and were dissolved in distilled water: 5.0 mM for $HgCl_2$, 25 mM for the other ions. For the determination of Cu^{2+} at low concentrations, Cu^{2+} stock solutions of 0.5 mM and 5 mM were prepared by diluting the original stock solution (25 mM), and then used in the analytical procedure. Cu^{+} was delivered in the form of $[Cu(MeCN)_4[PF_6]]$ from an acetonitrile stock solution (5 mM). **RCu1** and **M** were dissolved in DMSO in concentration of 5 mM as mother samples. Test solutions were obtained by adding appropriate volumes of Cu^{2+} or other cations stock solution (25 mM) and 6 μl **RCu1** stock solution (5 mM) subsequently into 3 ml aqueous acetonitrile (acetonitrile/water = 7/3, v/v, pH 7.2). The solutions were well mixed. Measurements were done 7 h later at room temperature. ^{1}H NMR and ^{13}C NMR spectra were recorded on a VARIAN INOVA-400 spectrometer chemical shifts (δ) reported as ppm (in CD_3SOCD_3 or CD_3CN, TMS as internal standard). Mass spectrometry data were obtained with a HP1100LC/MSD mass spectrometer and a LC/Q-TOF MS spectrometer. The IR spectrum was taken in KBr disks on a Bruker EQUINOX 55 Fourier Transform Infrared Spectrometer. Fluorescence measurements were performed on a VAEIAN CARY Eclipse Fluorescence Spectrophotometer (Serial No. FL0812-M018). Absorption spectra were measured on Lambda 35 UV/vis spectrophotometer of PerkinElmer. All pH measurements were made with a Model PHS-3C meter.

2.1.1. Water-sample analysis by ***RCu1***

The water samples (pool, tap and sea water) and pure acetonitrile were mixed intensively to prepare the acetonitrile–water solutions (acetonitrile/water = 7/3 for pool- and tap-water samples, 5/5 for sea water sample, v/v), respectively. After filtering insoluble materials, these solutions were spiked with different concentrations of $CuCl_2$ (0–10 μM). Finally, 10 μM **RCu1** was added to the different Cu^{2+}-contaminated acetonitrile–water samples. Then fluorescence measurements were performed after equilibration.

2.1.2. Soil-sample analysis by ***RCu1***

Soil was heated in an oven at 133 °C for 24 h prior to use. Then it was suspended in 70% acetonitrile–water solution to prepare a 10 mg/ml soil sample and spiked with different concentrations of $CuCl_2$ (0–10 μM). After filtering to remove insoluble materials, **RCu1** solution (10 μM) was added to the different Cu^{2+}-contaminated soil samples. Then fluorescence measurements were performed after equilibration.

3. Synthetic routes of RCu1

3.1. Synthesis of *RhB hydrazide*

Rhodamine hydrazide was synthesized from rhodamine B by the procedure published in literature.

3.2. Synthesis of *M*

To a 100 ml flask, **RhB hydrazide** (912 mg, 2.00 mmol) was dissolved in 30 ml dichloromethane, and then cooled with ice bath. Then 0.1 ml (a little excess) chloroacetyl chloride was dissolved in 10 ml dichloromethane and added dropwise to the above 100 ml flask with vigorous stirring. All reagents and glass apparatuses should be dried before use. After the addition, the stirred mixture was allowed to stand in ice bath for about 2 h, then the solvent was removed under reduced pressure. 0.1 M HCl (about 40 ml) was added to the solid in the flask to generate a clear purple solution. After that, 0.1 M NaOH was added slowly with stirring until the pH of the solution reached about 7, the purple precipitate generated was collected by the filtration, and evaporated to dryness. The crude compound was purified by flash column chromatography on silica gel (2:1 petroleum ether-ethyl acetate) to afford a white solid (799 mg, 75%). ^{1}H NMR (400 MHz, CD_3SOCD_3), δ: 1.08 (*t*, *J* = 16 Hz, 12H), 3.31 (m, 8H), 3.98 (s, 2H), 6.33 (d, *J* = 4 Hz, 4H), 6.50 (*t*, *J* = 8 Hz, 2H), 7.02 (d, *J* = 8 Hz, 1H), 7.56 (m, 2H), 7.83 (d, *J* = 8 Hz, 1H); ^{13}C NMR (100 MHz, CD_3SOCD_3), δ: 12.90, 41.01, 44.11, 65.55, 97.55, 104.50, 108.13, 123.13, 124.32, 128.95, 133.83, 148.87, 152.29, 153.48, 163.90, 165.13 ppm; TOF MS: *m*/*z* calcd. for $C_{30}H_{34}N_4O_3^+$ $[M+H]^+$: 533.2319, found: 533.2322.

3.3. Synthesis of *RCu1*

Sodium (40 mg, 1.75 mmol) was added to 5 ml absolute ethanol in a 25 ml flask, stirred until the sodium dissolved completely. 8-hydroxyquinoline was added and stirred for 2 h at 40 °C. **M** (500 mg, 1.0 mmol) was added to the mixture and stirred for another 5 h. After removal of ethanol under vacuum, the residue was dissolved in 0.1 M HCl aqueous solution (40 ml), and then 0.1 M NaOH aqueous solution was added slowly with stirring until the pH of the solution reached about 7, and generated the purple precipitate, filtered, evaporated to dryness. The crude compound was purified by flash column chromatography on silica gel (1:2 petroleum ether-ethyl acetate) to afford a white solid (405 mg, 63%). ^{1}H NMR (400 MHz, CD_3CN), δ: 1.11 (*t*, *J* = 16 Hz, 12H), 3.34 (m, 8H), 4.65 (s, 2H), 6.24 (d, *J* = 4 Hz, 2H), 6.37 (d, *J* = 12 Hz, 2H), 6.61 (d, *J* = 8 Hz, 2H), 7.04 (d, *J* = 8 Hz, 1H), 7.23 (d, *J* = 8 Hz, 1H), 7.35 (m, 1H), 7.53 (m, 4H), 7.84 (d, *J* = 8 Hz, 1H), 8.21 (d, *J* = 8 Hz, 1H), 8.32 (s, 1H), 10.59 (s, 1H); ^{13}C NMR (100 MHz, CD_3CN), δ: 11.87, 43.96, 65.57, 71.14 97.18, 104.48, 107.81, 121.89, 122.49, 122.77, 123.79, 126.96, 128.50, 129.26, 129.55, 133.22, 136.38, 149.00, 149.52, 151.99, 153.69, 154.44, 163.82, 167.21 ppm; TOF MS: *m*/*z* calcd. for $C_{39}H_{40}N_5O_4^+$ $[M+H]^+$: 642.3080, found: 642.3074.

4. Determination of the detection limit

The detection limit was calculated based on the method used in the previous literatures [37]. Fluorescence titrations of **RCu1** (10 μM) in the presence of Cu^{2+} (0–40 nM) were measured by three times and σ and *k* were achieved. The detection limit was calculated as 4.7 nM with following equation:

$$\text{Detection limit} = \frac{3\sigma}{k} = 3 \times \frac{0.03}{0.019} = 4.7$$

where σ is the standard deviation of blank measurement, *k* is the slope between the fluorescence intensity versus Cu^{2+} concentration.

4.1. Theoretical calculations

The structures of **RCu1**, **M** and **RCu1–Cu^{2+}** complex were optimized using density functional theory (DFT) by the B3LYP method with the 6-31G basis set. The DFT calculations were performed using the Gaussian 09 program.

4.2. Fluorescence quantum yields

The fluorescence quantum yields of **RCu1–Cu^{2+}** and **M–Cu^{2+}** were determined according to the method below.

$$\varphi_u = \frac{(\varphi_s)(FA_u)(A_s)(\lambda_{exs})(\eta_u^2)}{(FA_s)(A_u)(\lambda_{exu})(\eta_s^2)}$$

where φ is fluorescence quantum yield; FA is integrated area under the corrected emission spectra; *A* is the absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the subscripts u and s refer to the unknown and the standard, respectively. We chose rhodamine B as standard, which has the fluorescence quantum yield of 0.49 in ethanol [38].

5. Results and discussion

RCu1 was facilely synthesized from rhodamine B by a three-step procedure (Scheme 1). The fluorescence and absorption spectra of **RCu1** in the presence of different concentration of Cu^{2+} in 70% acetonitrile–water solution (pH 7.2) were recorded in Fig. 1. The free **RCu1** is colorless and non-fluorescent due to its stable "spirolactam-form". Upon gradual addition of Cu^{2+} to the solution of **RCu1**, a new absorption peak at 559 nm emerged with increasing intensity, and the solution displayed a clear change from colorless to brilliant pink. Simultaneously, the fluorescence intensities increased in a Cu^{2+}-dependent way, forming a strong fluorescence band centered at 587 nm, which indicated the rhodamine derivative mainly stayed in the "ring-open" form. Moreover, the fluorescence and absorption intensities increased linearly with the Cu^{2+} concentration in the range of 0–10 μM (Fig. S1, SI). A time course study revealed the recognizing event could complete in 30 min (Fig. S2, SI), and the fluorescence enhancement (F/F_0) of the probe **RCu1** to Cu^{2+} was as high as over 1000-fold when it reached the maximum (Fig. 4). The colorimetric and fluorometric behaviors of **RCu1–Cu^{2+}** complex could be conveniently distinguished from those of the free **RCu1** by the naked eye (Fig. 3b).

To test sensitivity, the kinetics of fluorescence increase at 587 nm in the presence of varied concentrations of Cu^{2+} was recorded, as shown in Fig. 2. The fluorescence intensity of **RCu1** was also linearly proportional ($R = 0.98582$) to the amount of Cu^{2+} in the range of 0–40 nM in 70% acetonitrile–water solutions (pH 7.2), confirming that **RCu1** has the capability of detecting Cu^{2+} both qualitatively and quantitatively. The detection limit is 4.7 nM (3σ) [37], which is sensitive enough to detect Cu^{2+} in medical diagnosis [39,40]. **RCu1** could be considered as one of the most sensitive fluorescence "off–on"-type Cu^{2+} sensors [17,18,20].

High selectivity toward the analyte over the potentially competing species is an essential feather for a probe. Changes of absorption and fluorescence spectra caused by **RCu1** in the presence of Cu^{2+} and other competitive ions were recorded in Figs. 3a and 4, respectively. **RCu1** did not exhibit obvious fluorescence changes or significant absorption in the presence of other cations, such as alkali or alkaline-earth metals (Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}), heavy and transition metal ions (Mn^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Fe^{2+}, Cr^{3+},

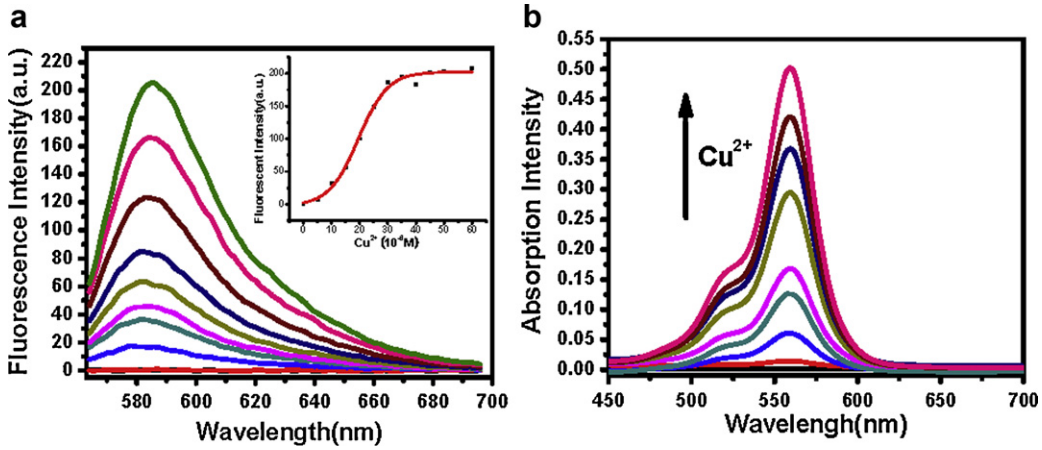


Fig. 1. Emission spectra (a) and absorption spectra (b) of **RCu1** (10 μM) upon addition of Cu^{2+} (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 μM) in aqueous solution (acetonitrile/water = 7/3, v/v, pH 7.2). Inset: the changes of fluorescence intensity at 587 nm. Excitation wavelength is 555 nm. Slit: 2.5 nm/2.5 nm.

Hg^{2+}, Zn^{2+}, Pb^{2+}, La^{3+}). Moreover, Cu^{+} did not cause any fluorescence and color changes, showing the high selectivity of **RCu1** to divalent copper ion. The selective recognition of **RCu1** to Cu^{2+} could be detected by naked eye both for colorimetric and fluorometric methods, as shown in Fig. 3b. Although a slight fluorescence and absorption enhancement occurred with Fe^{3+} and Ag^{+}, in the presence of miscellaneous competing cations (mentioned above), **RCu1** maintained the fluorescence enhancement toward Cu^{2+} as high as approximately 1000-fold, which indicated that the Cu^{2+} detection by **RCu1** was little interfered from the other common cations (Fig. 4). Also, various common anions have little interference on **RCu1** function (Fig. S3, SI). However, EDTA, the well known strong chelator for metal ions showed some fluorescence quenching to **RCu1**–**Cu^{2+}** system (Fig. S4, SI).

It is well known that the spirolactam ring of the rhodamine derivative is open in acidic media and then exhibits the strong fluorescence of rhodamine. Therefore, it is necessary to evaluate the effect of pH on the fluorescence of **RCu1**. As shown in Fig. 5, the acid-base titration experiments elucidated that **RCu1** did not show any detectable fluorescence in the pH range of 5.0–12.0. The sigmoidal curve gave a pK_a of 3.03 ± 0.03. Furthermore, the fluorescence behavior of **RCu1** in the presence of Cu^{2+} in different pH values was also evaluated. In the pH range of 5.0–7.5, **RCu1** can recognize Cu^{2+} without any interference by protons.

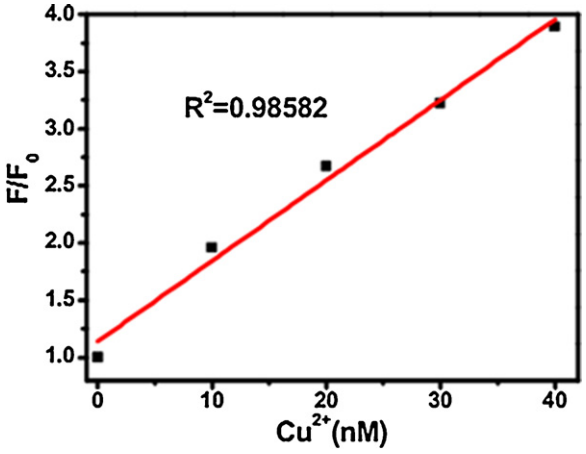


Fig. 2. Linear fluorescence enhancement (F/F_0) of **RCu1** (10 μM) upon addition of Cu^{2+} (0–40 nM). Conditions: acetonitrile/water = 7/3, v/v, pH 7.2, excitation wavelength is 555 nm.

To explore the mechanism, **RCu1**–**Cu^{2+}** complex was detected by the TOF mass spectrum analysis. A peak at *m*/*z* 703.2224 corresponding to intermediate **[RCu1 + Cu–H]$^{+}$** was observed (Fig. S8, SI) after the addition of Cu^{2+} to the organic solution of **RCu1** as illustrated in Scheme 2. Simultaneously, the complex emitted evident fluorescence and color changes, which could be ascribed to variation from a spirocyclic form to a ring-opened amide form. While after addition of water (acetonitrile/water = 7/3, v/v, pH 7.2), the peak at *m*/*z* 703.2224 disappeared, and a new peak at 443.1376

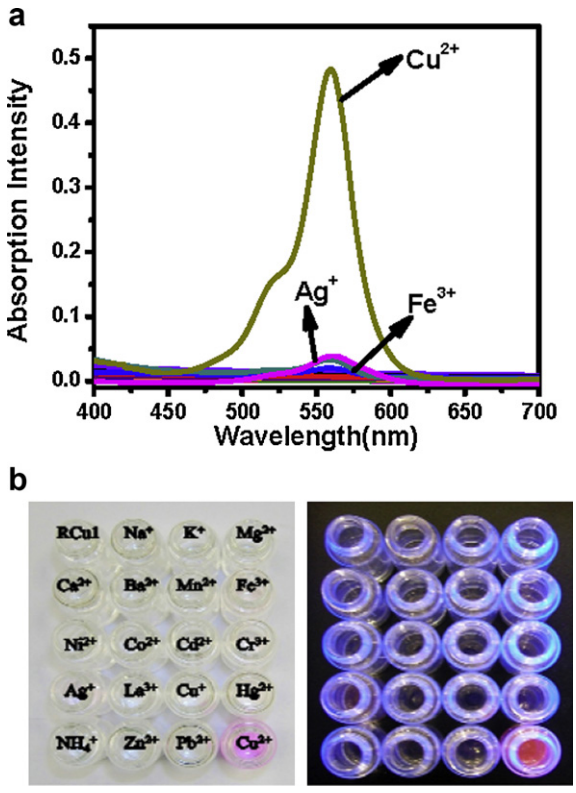
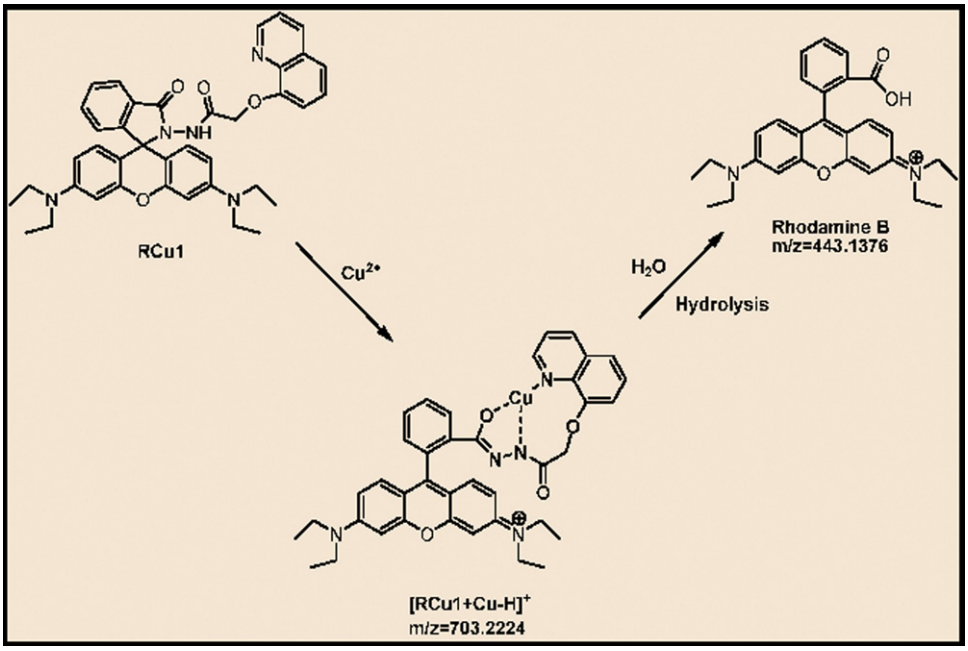


Fig. 3. (a) The absorption spectra of **RCu1** (10 μM) in the presence of different metal ions. (50 μM for all the cations: Cu^{2+}, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cd^{2+}, Ba^{2+}, Fe^{3+}, Fe^{2+}, Cr^{3+}, NH_4^{+}, Pb^{2+}, Hg^{2+}, Ag^{+}, Mn^{2+}, Cu^{+}, La^{3+}, Ni^{2+}, Co^{2+}). (b) The colorimetric (left) and fluorometric (right) changes of **RCu1** (10 μM) upon addition of Cu^{2+} and other cations in aqueous solution, 50 μM for Cu^{2+} and other cations. The two photos were obtained in sunlight (left) and upon at 365 nm using UV lamp (right), respectively. Conditions: acetonitrile/water = 7/3, v/v, pH 7.2, excitation wavelength is 555 nm, slit: 2.5 nm/2.5 nm.



Scheme 2. Cu^{2+}-promoted ring-opening of the spirolactam and hydrolysis into rhodamine B in aqueous media.

was detected which revealed the final product was rhodamine B in aqueous solution.

To determine the binding mode of **RCu1**–Cu^{2+} complex, the infrared spectra of **RCu1** and **RCu1**–Cu^{2+} were depicted in Fig. S5 in Supporting information. For compound **RCu1**, the 1616 cm^{-1} and 1724 cm^{-1} bands correspond to the conjugated C=O group of spirolactam and the amide carbonyl group, respectively. While in the case of **RCu1** + Cu^{2+}, the peak at 1616 cm^{-1} shifted to 1590 cm^{-1} which indicated the carbonyl group of spirolactam was involved in Cu^{2+} coordination. In addition, the infrared spectra of **RCu1**–Cu^{2+} also showed a shift of the amide C=O frequency from 1724 cm^{-1} to 1732 cm^{-1}, which might be put down to the binding of Cu^{2+} with the nitrogen of amide group. The affinity of carbonyl and amido of **RCu1** with Cu^{2+} was also proved by Czarnik and co-workers [41].

^1H NMR spectrum of **RCu1** in the presence of different concentration of Cu^{2+} provided further structural information on the complex (Fig. 6). On addition of 0.5 equiv. of Cu^{2+} to **RCu1**, the peak corresponding to the proton (H_a) of the amide carbonyl [42] group disappeared, which was in agreement with the MS results (intermediate [**RCu1** + **Cu**–**H**]$^+$). The proton (H_b) near the nitrogen of the quinoline group was downfield shifted from δ = 8.32 ppm to δ = 8.45 ppm in the presence of 1 equiv. of Cu^{2+}. Such a downshift is expected when Cu^{2+} coordinates with the nitrogen of the quinoline group of probe **RCu1**. There was no appreciable change in the signal positions on addition another 0.5 equiv. of Cu^{2+} to **RCu1**, confirming a 1:1 binding stoichiometry for **RCu1** and Cu^{2+} in organic solutions.

To study the role of the quinoline group in probe **RCu1** for Cu^{2+} detection, we checked the different fluorescence and absorption responses to Cu^{2+} of **RCu1** and **M** which did not contain the quinoline group. As shown in Fig. 7 and Fig. S6 in Supporting information, the free **RCu1** and **M** scarcely displayed fluorescence (560–700 nm) and absorption (450–700 nm),

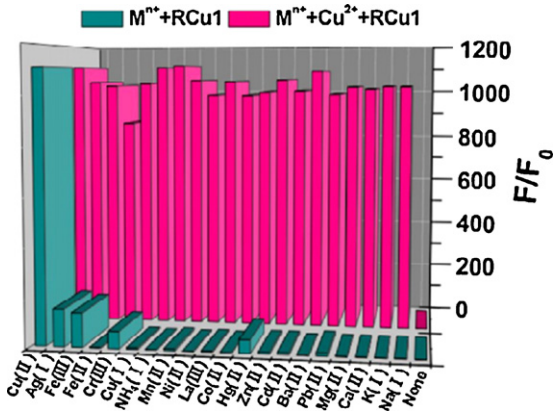


Fig. 4. Fluorescence (at 587 nm) responses of **RCu1** (10 μM) to miscellaneous cations. Green bars represent **RCu1** + cations; pink bars represent **RCu1** + Cu^{2+} in the presence of other cations (50 μM for all the cations). Conditions: acetonitrile/water = 7/3, v/v, pH 7.2, excitation wavelength is 555 nm, slit: 2.5 nm/2.5 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

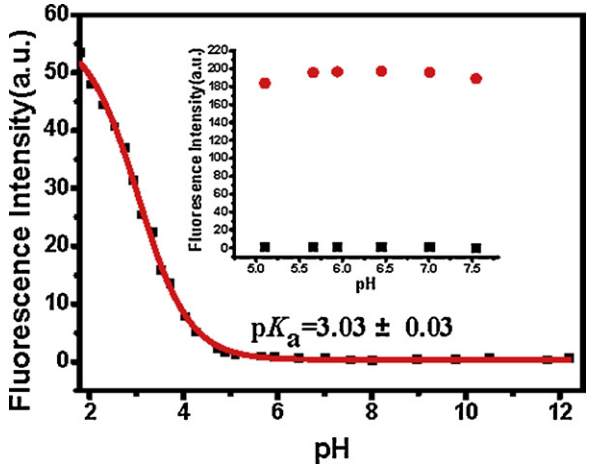


Fig. 5. Effect of pH on the fluorescence intensity of **RCu1** (10 μM) at 587 nm in the aqueous solution (acetonitrile/water = 7/3, v/v, pH 7.2), excitation wavelength is 555 nm. The pH of solution was adjusted by aqueous solution of NaOH (1 M) or HCl (1 M). Slit: 2.5 nm/2.5 nm.

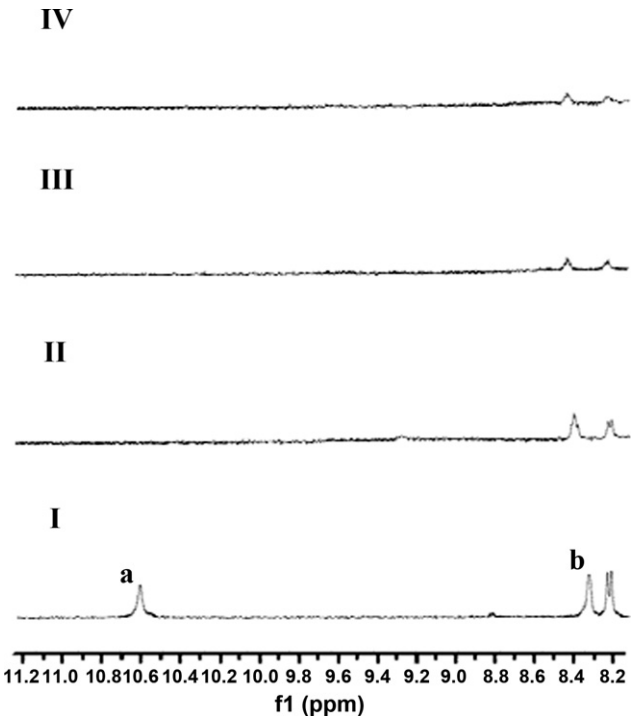


Fig. 6. ^{1}H NMR spectra of **RCu1** (5 mM) in the presence of different amounts of $Cu(ClO_4)_2$ in CD_3CN: I 0, II 0.5, III 1.0, IV 1.5 equiv. For proton labeling of **RCu1**, see Scheme 1.

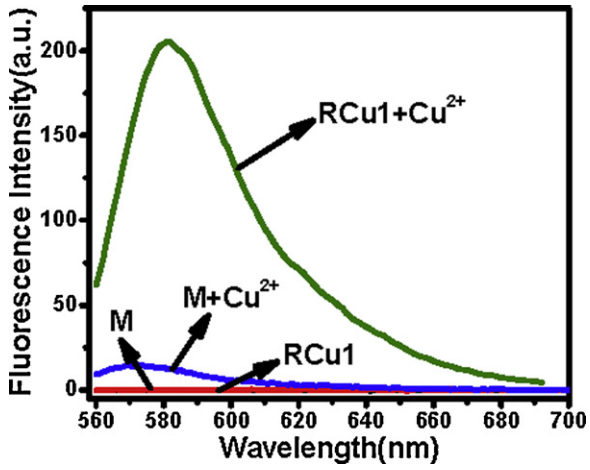


Fig. 7. Fluorescence spectra of **RCu1** and **M** in the presence and absence of Cu^{2+} in aqueous solution (acetonitrile/water = 7/3, v/v, pH 7.2). Conditions: 10 μM for **RCu1** and **M**, 50 μM for Cu^{2+}, excitation wavelength is 555 nm, slit: 2.5 nm/2.5 nm.

resulting from their spirocycle-closed forms. The addition of Cu^{2+} to the aqueous solution (acetonitrile/water = 7/3, v/v, pH 7.2) of **RCu1** caused evident color and fluorescence changes (Φ_F 0.26) as mentioned above. However, as expected, the response of **M** to Cu^{2+} was slight (Φ_F 0.04), which indicated that extra coordination site of 8-hydroxyquinoline can increase the affinity for Cu^{2+}. Furthermore,

Table 1
Results of determination of Cu ions in natural water samples and soil sample.

Sample	ICP-MS (mg/l)[a]	Proposed method (mg/l)[a]
Pool	0.243 ± 0.014	0.258 ± 0.035
Soil	0.325 ± 0.030	0.336 ± 0.030
Sea	0.228 ± 0.028	0.221 ± 0.062
Tap	0.324 ± 0.044	0.327 ± 0.034

[a] Mean of three replicates.

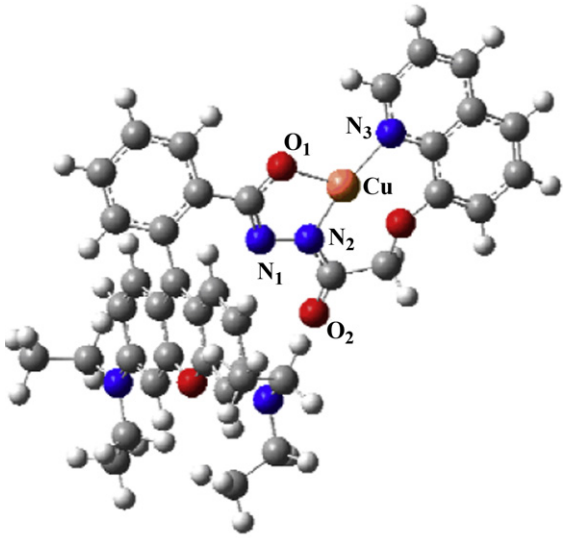


Fig. 8. Lowest energy structure for the **RCu1**–Cu^{2+} complex.

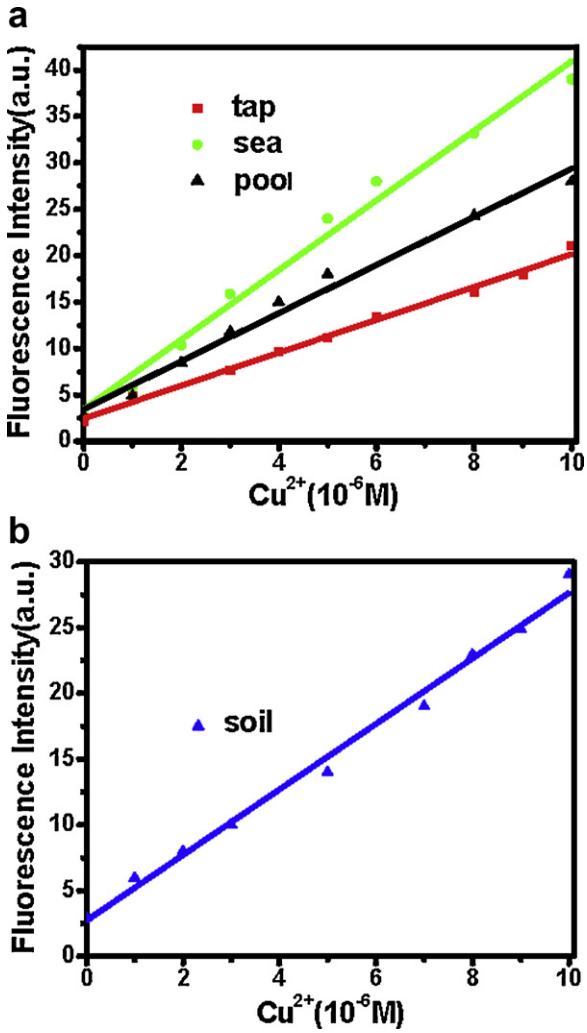


Fig. 9. Linear fluorescence intensities of **RCu1** (10 μM) upon addition of Cu^{2+} (0–10 μM) in three natural water samples (a) and soil sample (b). Data was acquired in the acetonitrile–water solutions (7/3 for pool-water, tap-water and soil samples, 5/5 for sea-water sample, v/v) with excitation at 555 nm, and the emission intensities were collected at 587 nm. Slit: 5.0 nm/5.0 nm.

the charge density of the atoms of **RCu1** and **M** were determined by density functional theory (DFT) calculations. The ligand that contains quinoline moiety in **RCu1** is more electronegative than that of **M**, which was revealed by charge on O_1 and N_2 (Fig. S7, SI), maybe owing to the electron donating effect of quinoline group. In addition, the electron density of N_3 (contained in quinoline group of **RCu1**) is greatly high, suggesting that N_3 can provide a nice binding pocket for Cu^{2+}.

Moreover, the binding pattern of **RCu1**–$\mathbf{Cu^{2+}}$ complex was further confirmed by DFT calculations at B3LYP/6-31G (d, p) level. The lowest energy structure of **RCu1**–$\mathbf{Cu^{2+}}$ complex without imaginary frequency was depicted in Fig. 8, the Cu^{2+} ion was coordinated to O_1, N_2 and N_3, which was in excellent agreement with the obtained MS, IR and ^{1}H NMR results.

Based on the above discussion, the proposed mechanism of Cu^{2+}-induced fluorescence enhancement could be described as follows (Scheme 2): firstly, Cu^{2+} promoted the spirolactam ring-opening of **RCu1**, owing to the strong binding ability of O_1, N_2, N_3 atoms toward Cu^{2+}. Subsequently, a further hydrolysis occurred, resulting in the formation of the final product rhodamine B, which gave rise to the dramatic increases in both fluorescence and absorption.

We further checked the potential utilities of **RCu1** by proof-of-concept experiments [43]. Pool, tap and sea water were collected from the pool of Qinyuan Garden in Dalian University of Technology, laboratory and Yellow Sea (Dalian, area of China), respectively, then prepared as the acetonitrile–water solutions (acetonitrile/water = 7/3 for pool and tap water samples, 5/5 for sea water sample, v/v). Cu^{2+} (0–10 μM) were spiked into these samples before **RCu1** (10 μM) was added. The fluorescence intensities were also proportional to the concentrations of Cu^{2+} in the range of 0–10 μM (lower than the limit of copper (~15.7 μM) in drinking water according to Standardization Administration of the People's Republic of China) in all the three natural water samples (Fig. 9a). According to the test procedures for soil contamination in Ref. [44], similarly, there was a good linear correlation between relative fluorescence intensity and Cu^{2+} concentration in the range of 0–10 μM in soil sample (Fig. 9b). Moreover, the probe was treated with the water samples or soil sample with addition of Cu^{2+} ions at random concentrations, and the results obtained by the proposed fluorimetric method were in good agreement with the ICP-MS method (Table 1). Therefore, the present probe **RCu1** could be used for trace Cu^{2+} analyses in these four complicated environmental systems qualitatively and quantitatively.

6. Conclusion

We have developed a novel rhodamine-based fluorescent probe **RCu1** for the determination of Cu^{2+} in aqueous solution, with nanomolar level sensitivity. After adding Cu^{2+} into the **RCu1** solution, the preliminary reaction relies on a Cu^{2+}-promoted spirolactam ring-opening of **RCu1**, subsequently, the complex hydrolyzes to rhodamine B, in which the quinoline moiety played an important role. It showed a "off–on" type of fluorescence (enhancement as high as over 1000-fold) and absorption response, single-selectively sensing Cu^{2+} over other ions. Furthermore, the present probe was successfully applied to natural water samples and soil sample analysis, we believe that this kind probe can be used for many practical applications, especially environmental systems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2012.01.024.

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