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A Retrievable and Highly Selective Fluorescent Probe for Monitoring Sulfide and Imaging in Living Cells

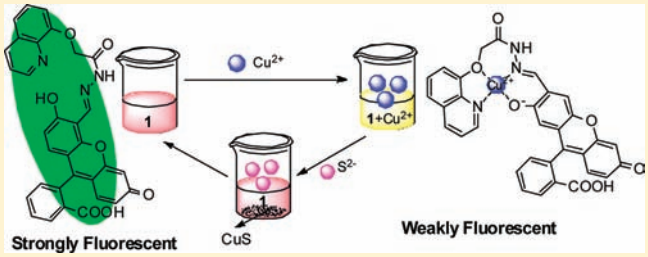
Fengping Hou,[†] Liang Huang,[†] Pinxian Xi,[†] Ju Cheng,[‡] Xuefei Zhao,[†] Guoqiang Xie,[†] Yanjun Shi,[†] Fengjuan Cheng,[†] Xiaojun Yao,[†] Decheng Bai,*[,‡] and Zhengzhi Zeng*[,†]

[†]Key Laboratory of Nonferrous Metals Chemistry and Resources Utilization of Gansu Province, State Key Laboratory of Applied Organic Chemistry and College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, People's Republic of China

[‡]School of Basic Medical Sciences, Lanzhou University, Lanzhou 730000, People's Republic of China

Supporting Information

ABSTRACT: A novel selective fluorescent chemosensor based on an 8-hydroxyquinoline-appended fluorescein derivative (**L1**) was synthesized and characterized. Once combined with Cu^{2+}, it displayed high specificity for sulfide anion. Among the various anions, only sulfide anion induced the revival of fluoresecence of **L1**, which was quenched by Cu^{2+}, resulting in "off−on"-type sensing of sulfide anion. What's more, the sensor was retrievable to indicate sulfide anions with Cu^{2+}, and S^{2-}, in turn, increased. With the addition of Cu^{2+}, compound **L1** could give rise to a visible pink-to-yellow color change and green fluorescence quenching. The resulting yellow solution could change to pink and regenerate to green fluorescence immediately upon the addition of sulfide anion; however, no changes were observed in the presence of other anions, including CN^-, $P_2O_7^{4-}$, and other forms of sulfate, making compound **L1** an extremely selective and efficient sulfide chemosensor. The signal transduction occurs via reversible formation−separation of complex **L1**Cu and CuS. What's more, the biological imaging study has demonstrated that the chemosensor can detect sulfur anions in biological systems at a relatively low concentration.



INTRODUCTION

The development of selective and efficient signaling systems to detect various chemically and biologically pertinent species has attained significant interest.[1] Recently, considerable efforts have been made to develop artificial optical receptors for selective anion recognition for their important roles in biological, industrial, and environmental processes.[1b] As one of the members, sulfide is widely used in different fields, for instance, conversion into sulfur and sulfuric acid, dyes and cosmetic manufacturing, production of wood pulp, etc.[2] As a consequence, the sulfide anion can be widely found in water not only owing to industrial processes but also thanks to microbial reduction of sulfate by anaerobic bacteria or formed from the sulfur-containing amino acids in meat proteins. Sulfide can irritate mucous membranes and even cause unconsciousness and respiratory paralysis.[2,3] Once protonated to produce HS^- or H_2S, sulfide will become even more toxic. At a low concentration, H_2S can produce personal distress, while at a higher concentration, it can result in loss of consciousness, permanent brain damage, or even death through asphyxiation.[4] Therefore, it is important to develop a rapid and sensitive method for immediate sulfide monitoring in aqueous media and in biological systems.

A large quantity of techniques for recognizing sulfide anions have been designed,[5] for example, titration,[6] fluorimetry,[7] chemiluminescence,[8] spectrophotometry,[9] inductively coupled plasma atomic emission spectroscopy,[10] hydride generation atomic fluorescence spectrometry,[11] an electrochemical method,[12] and ion chromatography.[13] Considering practicality and convenience, fluorimetry is acceptable. However, the development of selective chemosensors for the detection of sulfide ions has attracted little interest relative to other widely investigated anions such as fluoride,[14] cyanide,[15] and other forms of sulfate.[16] Utilization of metal-anion affinity has been reported as another method of sensing anions;[17] however, most of them are irreversible. Among the various approaches to sensing sulfide anions, reversible sensors exploiting copper sulfide affinity[17d,18] attracted our special attention. Sulfide is known to react with copper ions to form a very stable CuS species, which has a low-solubility product constant $k_{sp} = 6.3 \times 10^{-36}$. Recently, the Chang and Nagano groups reported new approaches for the detection of sulfide in live biological systems through the development of chemoselective sulfide-responsive fluorescent probes.[19] It is reported that the concentration of sulfide required to elicit physiological responses has been reported to be 10−600 μM, although there remains some controversy as to the intracellular sulfide levels generated in response to physiological stimuli.[19a,c,20] Encouraged by these biologically compatible conditions,

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we envisioned that the inorganic-reaction-based method could be employed as a platform for the construction of fluorescent sulfide probes that may be potentially useful for sulfide detection at physiological pH.

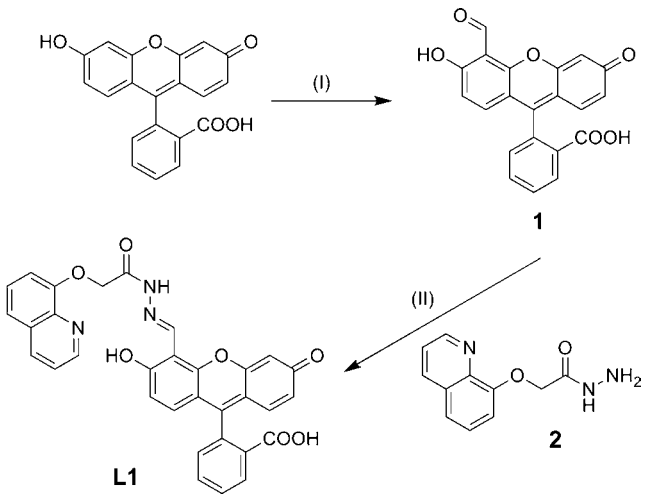
In this paper, we report a novel fluorescent sensor, **L1**, based on fluorescein and 8-hydroxyquinoline as the receptor. The receptor can combine Cu^{2+} to form complex **L1Cu**, which displays high sensitivity and selectivity for S^{2-} over other possible competitive anions on the basis of forming CuS. The 1:1 complexation was characterized by mass spectrometry (MS), elemental analysis, and Job's plot. The photophysical properties and recognition behaviors of the chemsensor have been investigated in detail through UV−vis absorption spectra, fluorescence spectra, MS, theoretical calculations, and fluorescence images in biological cells. These data present a basic concept in intracellular and extracellular for the detection of sulfide anion and other forms of sulfide based on the simple inorganic reaction, accompanying reversible formation−separation of the complex.

EXPERIMENTAL SECTION

General Information and Materials. All of the materials for synthesis were purchased from commercial suppliers and used without further purification. ^{1}H and ^{13}C NMR spectra were taken on a Varian mercury-400 spectrometer with tetramethylsilane (TMS) as an internal standard and dimethyl sulfoxide (DMSO) as the solvent. Absorption spectra were determined on a Varian UV-Cary100 spectrophotometer. Fluorescence spectra measurements were performed on a Hitachi F-4500 spectrofluorimeter. All pH measurements were made with a pH-10C digital pH meter. MS spectra were performed on a HP-5988A spectrometer (EI at 70 eV). Electrospray ionization MS (ESI-MS) spectra were determined on a Bruker Daltonics Esquire 6000 spectrometer.

Preparation of (*E*)-2-[6-Hydroxy-3-oxo-5-[[2-[2-(quinolin-8-yloxy)acetyl]hydrazono]methyl]-3*H*-xanthen-9-yl]benzoic Acid (L1). 2-(5-Formyl-6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoic acid[21] (**1**) and 2-(quinolin-8-yloxy)acetohydrazide (**2**)[22] were prepared by the reported method. Compound **1** (180 mg, 0.5 mmol) was dissolved in 25 mL of ethanol, followed by the addition of compound **2** (130 mg, 0.6 mmol). Then the mixture solution was stirred at 60 °C for 5 h. Cream-colored precipitates formed were filtered and washed with ethanol and acetone and dried under vacuum, and compound **L1** was obtained (Scheme 1). Yield: 74%. ^{1}H NMR (DMSO-d_6, 400 MHz, TMS): δ 12.602 (s, 1H, −COOH), 12.512 (s, 1H, −OH), 10.285 (s, 1H, −(O)CNH−), 9.184 (s, 1H, −CH=N−), 8.429−8.409 (d, J = 8.0 Hz, 1H), 8.029−8.010 (d, J = 7.6 Hz, 1H), 7.833−7.796 (t, J = 7.6 Hz, 1H), 7.757−7.720 (t, J = 7.6 Hz, 1H), 7.673−7.632 (m, J = 4.0 Hz, 2H), 7.601−7.561 (t, J = 8.0 Hz, 1H), 7.371−7.352 (d, J = 7.6 Hz, 1H), 7.333−7.314 (t, J = 7.6 Hz, 1H), 6.756−6.682 (m, 4H, J = 7.6 and 4.0 Hz), 6.613 (s, 1H), 5.050 (s, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz, TMS): δ 168.53, 164.60, 159.82, 158.56, 153.79, 152.15, 151.13, 149.58, 149.21, 144.57, 139.83, 136.25, 135.65, 130.80, 130.18, 129.24, 129.01, 126.82, 126.00, 124.66, 123.98, 122.05, 121.46, 113.26, 112.55, 109.59, 109.38, 105.54, 102.21, 82.35, 68.79. MS (ESI). Calcd for $C_{32}H_{21}N_3O_7$ [$(M + 1)^+$] m/z 559.14. Found: m/z 560.2.

Scheme 1. Synthesis of Compound L1[a]



[a]Conditions: (I) $CHCl_3$, NaOH, methanol, H_2O, 55 °C, 10 h; (II) ethanol, 60 °C, 5 h.

Preparation of L1Cu. The DMSO and ethyl acetate solution of **L1** (22.4 mg, 0.04 mmol) and $CuCl_2{\cdot}2H_2O$ (13.7 mg, 0.08 mmol) were mixed and stirred at room temperature for 5 h. The dark-brown solid formed was filtered, washed with water, and dried under vacuum. MS (ESI). Found: m/z 622.2 [(**L1** + Cu^{2+} − 1) $^+$]. Elem anal. Found (calcd) for $C_{32}H_{20}N_3O_7Cu$: C, 59.88 (60.79); H, 3.42 (3.24); N, 6.56 (6.75).

UV−Vis and Fluorescence Spectral Studies. Stock solutions of various ions (1.0×10^{-3} mol·L^{-1}) were prepared in deionized water. A stock solution of **L1** (1×10^{-3} mol·L^{-1}) was prepared in *N*,*N*-dimethylformamide/CH_3CN (1:1, v/v). The solution of **L1** was then diluted to 1×10^{-5} mol·L^{-1} with phosphate-buffered saline (PBS)/CH_3CN (1:1, v/v, pH = 7.2). In titration experiments, each time a 2×10^{-3} L solution of **L1** (1×10^{-5} mol·L^{-1}) was filled in a quartz optical cell of 1 cm optical path length, and the ion stock solutions were added into the quartz optical cell gradually by using a micropipet. Spectral data were recorded at 1 min after the addition of the ions. In selectivity experiments, the test samples were prepared by placing appropriate amounts of the anion stock into 2 mL of solution of **L1** (2×10^{-5} mol·L^{-1}). For fluorescence measurements, excitation was provided at 495 nm, and emission was collected from 500 to 700 nm.

Cell Culture. The Hela cell lines were provided by the Institute of Biochemistry and Cell Biology (China). Cells were grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% air at 37 °C humidified air for 24 h. Then the cells were treated with the relative compound using fluorescence microscopic imaging.

Fluorescence Microscopic Imaging. The cultured cells were treated with **L1** and incubated for 30 min. Subsequently, the cells were treated with $CuCl_2$. Then different concentrations of Na_2S were added. After incubation for the corresponding time, the cells were washed with PBS three times to remove free compound and ions before analysis. Then fluorescence microscopic images were acquired. Excitation of loaded cells at 495 nm was carried out with a He−Ne laser. The confocal microscopic optical setup was in multichannel mode. All confocal images were collected with a Zeiss Leica inverted epifluorescence/reflectance laser scanning confocal microscope.

Assessment of the Biocompatibility. The biocompatibility was determined in L929 mouse fibroblast cell lines. The cell viability was evaluated using a modified MTT assay. Basically, cells were plated at a density of 1×10^5 in 96-well plates 24 h prior to exposure to the sensor. Different concentrations of **L1**, **L1Cu** [via interactions with DMEM (<0.25% DMSO) before use], and Na_2S were added to the wells, and the cells were further incubated for 24 h. After treatment, 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg·mL^{-1} in PBS) was added into each well. After 4 h of incubation, culture supernatants were aspirated, and a purple insoluble MTT product was redissolved in 150 μL of DMSO in 10 min. The concentration of the reduced MTT in each well was determined spectrophotometrically by subtraction of the absorbance reading at 630 nm from that measured at 570 nm using a microplate reader. All MTT experiments were performed in a set of five copies; the maximum and minimum were deleted. The results were expressed as the mean relative to standard deviation.

RESULTS AND DISCUSSION

Synthesis of Complexes. Fluorescent sensor **L1** was prepared from a fluorescein derivative[21] and 2-(quinolin-8-yloxy)acetohydrazide,[22] and the structure of **L1** was confirmed

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by ^{1}H and ^{13}C NMR, ESI-MS, emission, and UV−vis spectra (see the Supporting Information). In addition, complex **L1**Cu was also obtained. The 1:1 stoichiometry was confirmed by ESI-MS, element analysis, Job's plot, and UV−vis titration, which means that the sensors bearing only one copper site are candidates for sensing S^{2-} in a 50% aqueous solution.

Absorption Spectroscopy of L1 and Cu^{2+}. The coordination of **L1** with Cu^{2+} was investigated by spectrophotometric titration in PBS/CH_3CN (1:1, v/v) at pH = 7.2. The UV−vis absorption spectrum of **L1** in the media revealed a strong band with the maximum absorbance peak at about 502 nm, which is characteristic of fluorescein under this condition. Upon an increase in the concentration of Cu^{2+} (0−1 equiv of Cu^{2+}), the maximum absorbance intensity gradually decreased and shifted to 493 nm (1 equiv of Cu^{2+}) and a new absorbance about 468 nm appeared (Figure 1a), which further indicated

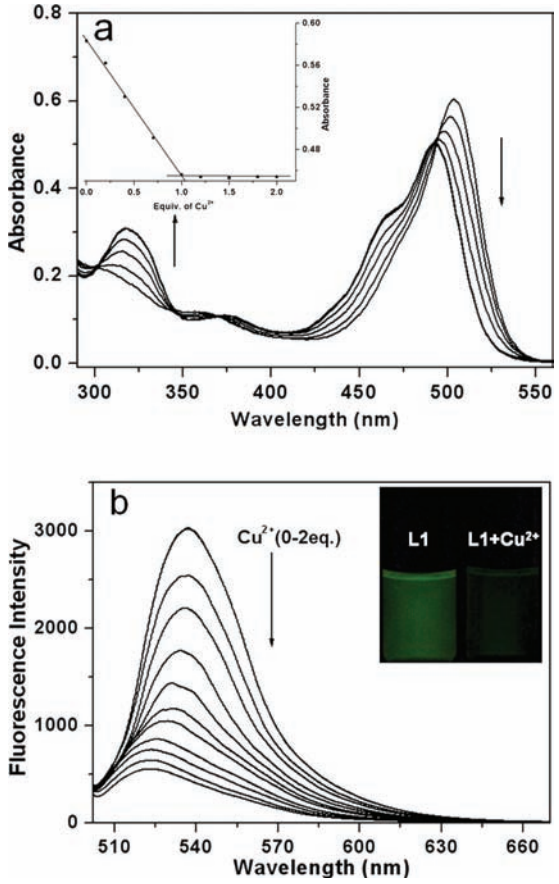

Figure 1. (a) UV−vis absorption and (b) fluorescence response of **L1** (10 μM) upon the addition of different concentrations of Cu^{2+} (0−2 equiv) in PBS/CH_3CN (1:1, v/v, pH = 7.2). Excitation wavelength: 495 nm. Inset in part a: Absorption intensity at 498 nm of **L1** as a function of the Cu^{2+} concentration. Inset in part b: Photograph of **L1** and **L1** + Cu^{2+} (20 μM).

that the coordination of fluorescein to a paramagnetic Cu^{2+} center formed. In addition, a new absorbance about 318 nm, which is the characteristic peak of quinoline, gradually appeared and increased with an increase of the concentration of Cu^{2+}. This explained that Cu^{2+} coordinated with quinoline. The absorbance peak was no longer changed when the concentration of Cu^{2+} increased from 1 to 2 equiv (Figure 1a, inset), implicating a 1:1 complexation of the ligand with the Cu^{2+} ion.

Fluorescence Spectroscopy of L1 and Cu^{2+}. The fluorescence spectrum of **L1** in PBS/CH_3CN (1:1, v/v) at pH = 7.2 was characterized by a strong emission at 534 nm (λ_{ex} = 495 nm; Φ_1 = 0.123). Upon interaction with Cu^{2+} (2 equiv), the system exhibited significant quenching in the fluorescence intensity (Φ_2 = 0.061) with minor shifts to 523 nm in the maximum emission (Figure 1b). The fluorescence quench of **L1** was due to the coordination to a paramagnetic Cu^{2+} center.[23] The 1:1 stoichiometry of the complexation was confirmed with a Job's plot (Figure 2) and a linear fitting of the absorption titration curve

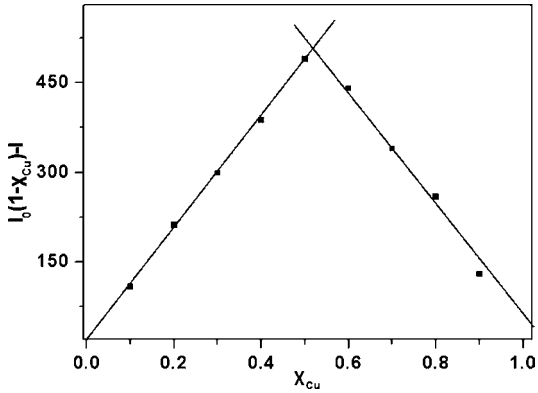

Figure 2. Job's plot for determining the stoichiometry of **L1** and Cu^{2+} in PBS/CH_3CN [1:1, v/v, pH = 7.2; χ_{Cu} = $[Cu^{2+}]/([Cu^{2+}] + [\mathbf{L1}])$; the total concentration of **L1** and Cu^{2+} was 5 μM]. Excitation wavelength: 495 nm.

(Figure 1a, inset). When 2 equiv of Cu^{2+} was added to the solution of **L1**, a more than 5-fold decrease in the fluorescence intensity at 534 nm was observed. The association constant for Cu^{2+} was estimated to be 5.0×10^4 M^{-1} in the media (Figure S1 in the Supporting Information) on the basis of a linear fitting of the titration curve, assuming a 1:1 stoichiometry.

Density Functional Theory (DFT) Calculations. To further verify the configuration of **L1**Cu, we carried out DFT calculations with B3LYP by using the *Gaussian09* package. The 6-31G basis set was used for the H, C, N, and O atoms; the exception was for the Cu atom, where the LANL2DZ effective core potential was employed. The optimized configuration is shown in Figure 3, which shows that the Cu^{2+} ion binds to **L1**

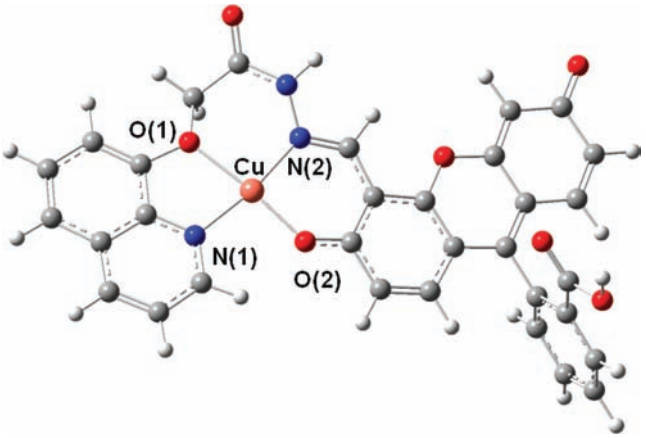

Figure 3. Calculated energy-minimized structure of **L1** with Cu^{2+}.

very well through four coordination sites, and the whole molecular system forms a nearly planar structure. The Cu−O

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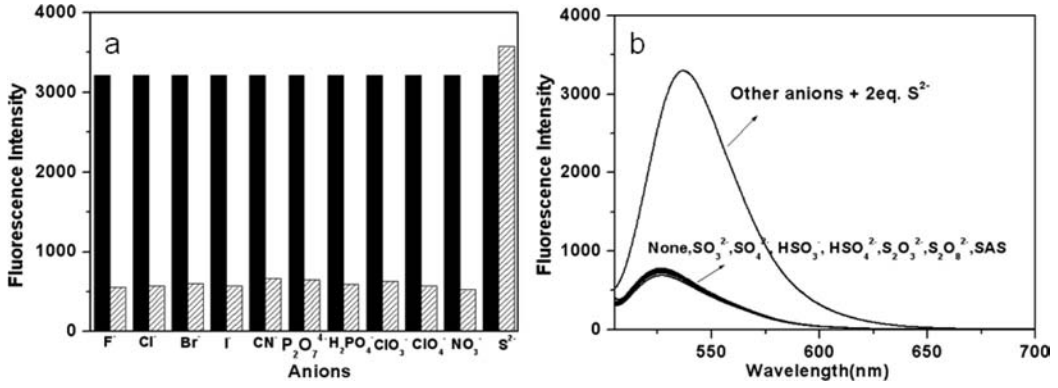

Figure 4. (a) Fluorescence emission spectra of **L1** (10 μM) and Cu^{2+} (20 μM) in PBS/CH_3CN (1:1, v/v, pH = 7.2) in the presence of different anions (30 μM). The black bars represent the emission intensities of **L1**, and the light-gray bars represent the emission intensities of **L1** and Cu^{2+} (20 μM) after the subsequent addition of 30 μM different anions (λ_{ex} = 495 nm; λ_{em} = 534 nm). (b) Emission of 100 μM other forms of sulfates and after the addition of 40 μM S^{2-} in the same media (SAS: sulfoacetic acid disodium salt).

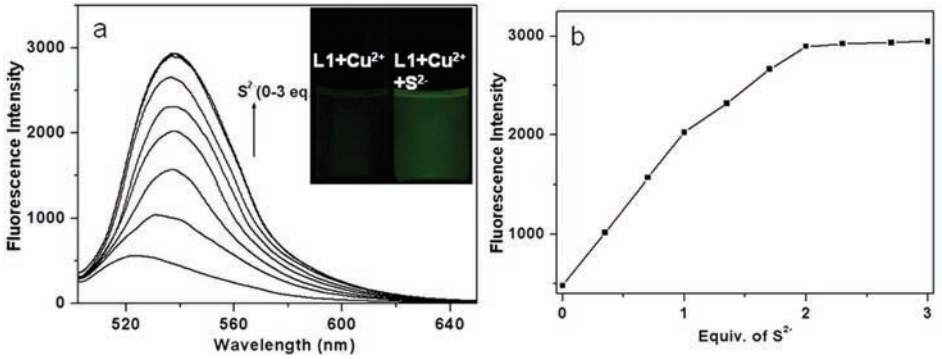

Figure 5. (a) Fluorescence titration of **L1** (10 μM) and Cu^{2+} (20 μM) upon the addition of different concentrations of S^{2-} (0–30 μM) in PBS/CH_3CN (1:1, v/v, pH = 7.2). Excitation wavelength: 495 nm. Inset: Photograph of **L1** + Cu^{2+} and **L1** + Cu^{2+} + S^{2-} (20 μM). (b) Fluorescence intensity at 534 nm of **L1** as a function of the Cu^{2+} concentration.

bond lengths are 2.07 Å ($Cu-O_{ether-oxygen}$) and 1.91 Å ($Cu-O_{hydroxy}$), and the Cu–N bond lengths are 1.99 Å ($Cu-N_{quinoline-nitrogen}$) and 1.96 Å ($Cu-N_{schiff\ base-nitrogen}$). These data indicate that **L1** could provide suitable space to better accommodate corresponding ions.

Selective Optical Response of Complex L1Cu to Various Anions. An important property of the chemosensor is high selectivity toward the analyte. It is very exciting and noteworthy that compound **L1** could be regenerated only by adding S^{2-} to the solution containing **L1**Cu. Commom anions, such as F^-, Cl^-, Br^-, I^-, CN^-, $H_2PO_4^-$, $P_2O_7^{4-}$, NO_3^-, ClO_3^-, ClO_4^-, SO_4^{2-}, and PO_4^{3-}, and other forms of sulfate did not generate the same results (Figure 4a). The **L1**Cu system revealed remarkably selective fluorescence "on" behavior exclusively with S^{2-}. As shown in Figure 5a, when 3 equiv of sulfide ion is added to the **L1**Cu system, the fluorescence intensity and maximum emission peak were totally regenerated; however, there were nearly no differences in the presence and absence of other anions. Thus, the results strongly proved that **L1**Cu can selectively detect S^{2-}. Besides, the S^{2-} selective probe was not affected by the presence of other types of sulfates, such as SO_3^{2-}, SO_4^{2-}, HSO_4^{2-}, HSO_3^{2-}, $S_2O_3^{2-}$, $S_2O_8^{2-}$, and sulfoacetic acid disodium salt (Figure 4b).

Fluorescence Spectroscopy of L1Cu and S^{2-}. The "off–on" property of the sensor was researched further. Figure 5a explains the fluorescence enhancement upon the addition of various amounts of sulfide ions in the presence of 2 equiv of Cu^{2+}. Both the intensity and shape of the system's emission spectrum closely match those of compound **L1** (Figure 1b), that is to say, not only the fluorescence intensity but also the maximum emission peak were totally revived. This showed that Cu^{2+} was released from complex **L1**Cu, and CuS formed. The calibration curve of the fluorescence intensity at 534 nm of **L1** (Figure 5b) elucidated that sulfide anion interacted with copper. Besides, the fluorescence spectrum was recorded within 1 min after sulfide anion was added, and the intensity barely changed for more than 2 h (Figure S2 in the Supporting Information), indicating that the monitoring system is a real-time and stable one.

Absorption Spectroscopy of L1Cu and S^{2-}. Besides, it is almost matched between the UV–vis absorption spectrum of sulfide-ion titration in the presence of **L1** with 2 equiv of Cu^{2+} (Figure 6a) and that of copper-ion titration (Figure 1a). The intrinsic UV–vis absorption is, consequently, in favor of the recovery of compound **L1**. What's more, the sensor was recyclable to indicate sulfide anions with Cu^{2+}, and S^{2-}, in turn, increased. In the system of **L1**Cu, the absorption peak and intensity changes were similar to the formers, that is, Figures 6a and 1a; under these conditions, S^{2-} restored the UV–vis spectra. This signifies that signal transduction is reversible; therefore, the chemosensor would be recyclable, which is a highly efficient one. As illustrated in Figure 6, the color of **L1** in the media is pink, turns yellow after the addition of 2 equiv of Cu^{2+}, and then returns to pink with the addition of 2 equiv of S^{2-}.

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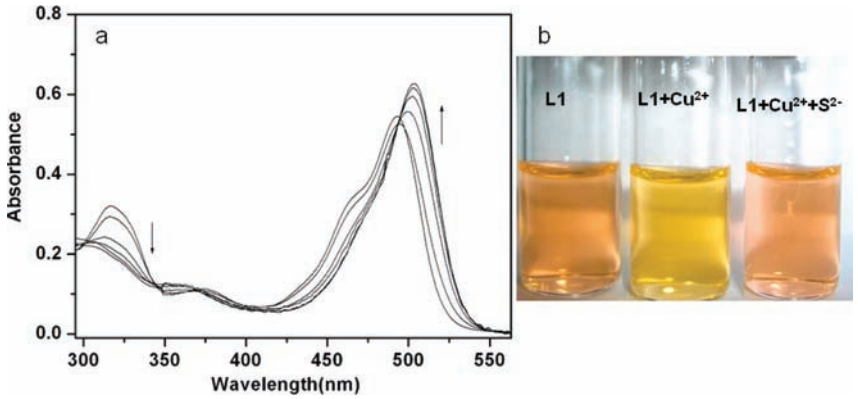
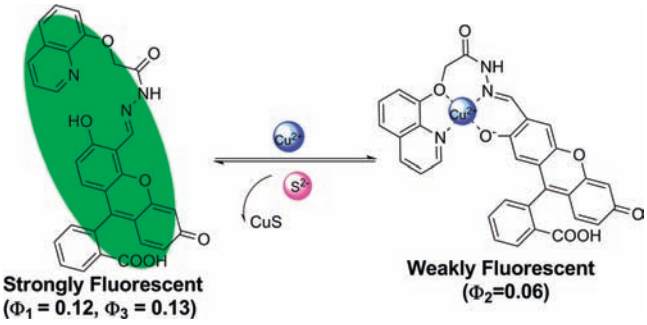


Figure 6. (a) UV−vis response of **L1** (10 μM) and Cu^{2+} (20 μM) upon the addition of different concentrations of S^{2-} (0−30 μM) in PBS/CH_3CN (1:1, v/v, pH = 7.2). (b) Photographs of **L1**, **L1** + Cu^{2+}, and **L1** + Cu^{2+} + S^{2-} in color changes.

Mechanism of the Sensing of Sulfide. As illustrated in Scheme 2, the addition of copper ion can cause fluorescence quenching (Φ_2 = 0.061), simultaneously inducing a color change from light pink to light yellow. Also, the added sulfide can capture copper ion, resulting in fluorescence revival (Φ_3 = 0.132) and inducing a yellow to pink colorimetric change (Figure 6b). This fluorescence quenching can be attributed to complex **L1**Cu formation upon the addition of Cu^{2+}, as shown in Scheme 2, and then the copper ions were captured by sulfide ions, resulting in the revival of **L1**, which is accompanied by fluorescence and color regeneration. Therefore, the addition of sulfide can be monitored via naked eyes.

Scheme 2. Graphic of the Proposed Mechanism of the Sensing of Sulfide



The result was also confirmed through ESI-MS analysis. ESI-MS of **L1** displayed a molecular-ion peak $[M + 1]^+$ at m/z 560.2 (Figure S6 in the Supporting Information). To have an idea about the quenching in the presence of Cu^{2+}, the MS spectrum of the **L1**Cu system was acquired. The most prominent peak at m/z 622.2 (Figure S7 in the Supporting Information) in the MS spectrum is assignable to $[\mathbf{L1} + Cu - 1]^+$. Furthermore, in order to investigate the "off−on" property, the MS spectrum of the **L1**Cu/S^{2-} system was obtained also. The parent peak $[M + 1]^+$ at m/z 560.2 (Figure S8 in the Supporting Information) is equivalent to that of compound **L1**. Therefore, it elucidated the mechanism of the sensing of sulfide anions.

Fluorescence Images. First of all, we investigated the staining capacity of the sensor **L1** in cells. Hela cells were incubated with **L1** (10 μM) in DMEM for 30 min at 37 °C, and then rhodamine 6G (2 μM) was added for incubation for 30 min. Then the confocal fluorescence images of the Hela cells were observed, and it was found that the sensor has excellent staining capacity. Relative to a rhodamine 6G staining cytoplasm (Figure 7b), the sensor can enter karyon (Figure 7a).

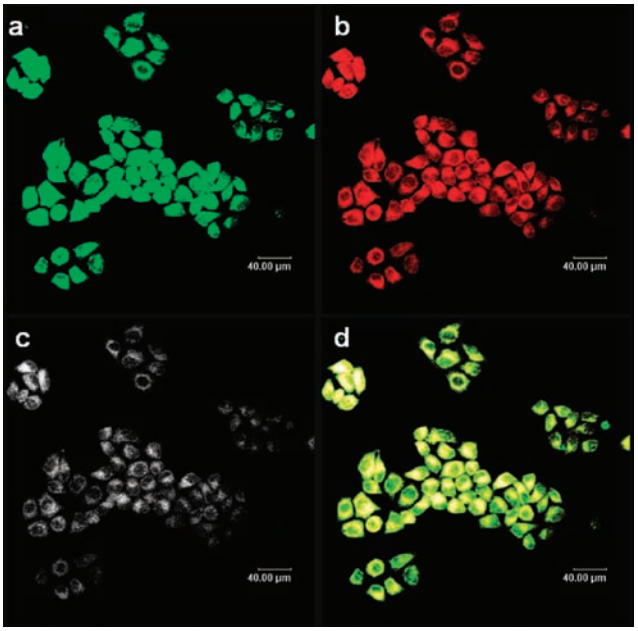


Figure 7. Fluorescence images of probe **L1** in Hela cells. Probe **L1** (10 μM, green, a) was incubated in media for 30 min and counterstained with rhodamine 6G (2 μM, red, b), black and white (c), and an overlay (d). All images were acquired with a 40× objective lens.

We studied bioimaging applications of **L1**Cu for monitoring of S^{2-} ions in biological systems. Hela cells were incubated with **L1** (1, 2, and 10 μM) in DMEM for 30 min at 37 °C, and then Cu^{2+} (1, 2, 10, and 15 μM) was added for incubation for 30 min. Also, various concentrations of Na_2S (1, 2, 10, 20, 50, and 100 μM) were added to the media. The treated cells were incubated in a culture medium for 30 min at 37 °C. After being incubated under these conditions, the cells were washed with PBS and imaged using a fluorescence microscope. Hela cells incubated with **L1** initially displayed a strong fluorescence image (Figure 7a), but the fluorescence image became black in the presence of Cu^{2+} (Figure 8a). However, after incubation with sulfide, the fluorescence gradually recovered. As shown in Figure 8, no fluorescence was observed in cells incubated with Na_2S (100 μM). The cells incubated with **L1**Cu and Na_2S (1 and 2 μM) have weak fluorescence (Figure 8c,d), suggesting

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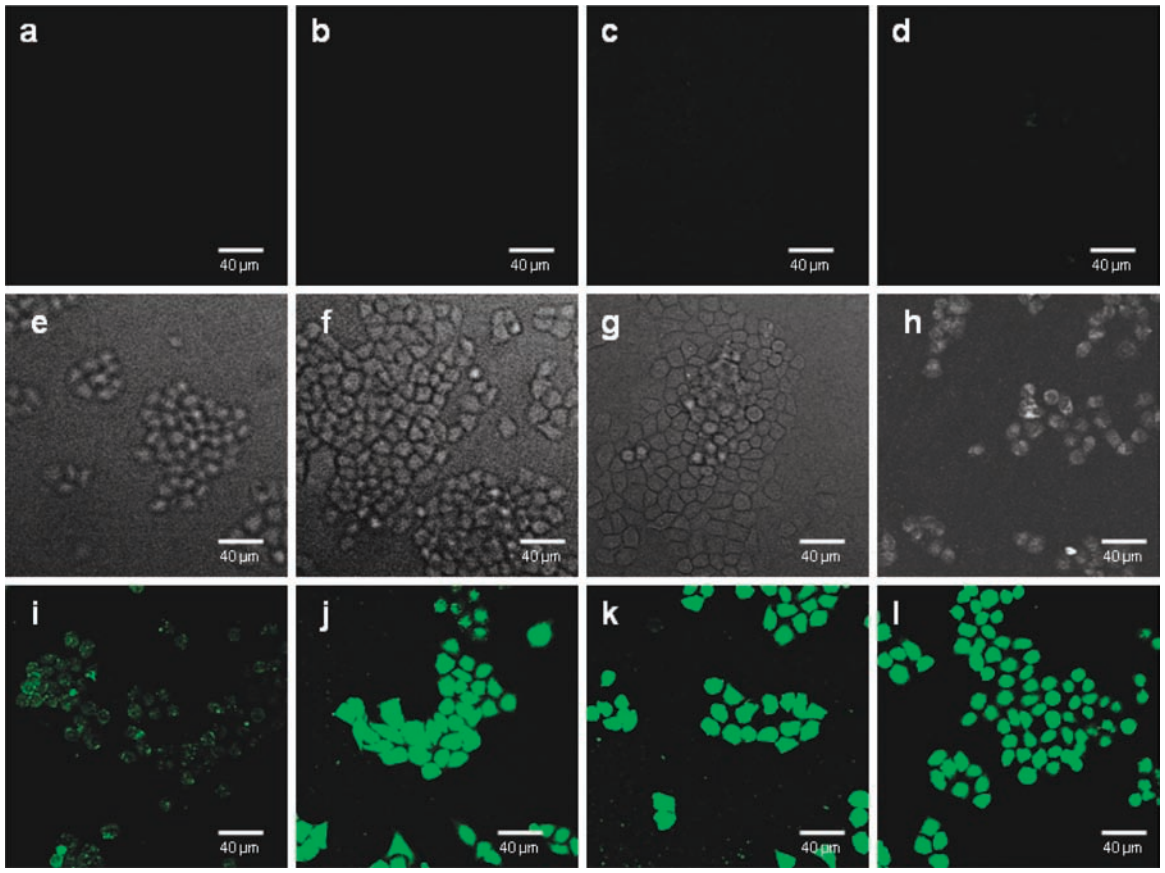

Figure 8. Confocal fluorescence images of Hela cells: (a) cells after incubation with **L1** (10 μM) and Cu^{2+} (15 μM); (b) cells after incubation with 100 μM Na_2S; cells after incubation with **L1**Cu and (c) 1 μM and (d) 2 μM Na_2S for 30 min; (e–h) cells corresponding to parts a–d , respectively; (i–l) cells after incubation with **L1**(10 μM) and Cu^{2+} and 10, 20, 50, and 100 μM Na_2S for 30 min, respectively.

that **L1**Cu interacted with sulfide in the cells. When the concentration of Na_2S was raised to 10, 20, 50, and 100 μM, a large intracellular fluorescence enhancement was observed (Figure 8i–l). Therefore, the sensor can detect sulfide in living cells at micromolar concentration. In addition, we studied fluorescence imaging at different times. The results reveal that the imaging finished in 10 min and there is almost no difference between that in 10 min and that in 30 min (Figure S3 in the Supporting Information).

Besides, the cell viability was evaluated using a modified MTT assay. Figures 9 and S4 in the Supporting Information show the viability of cells respectively treated with **L1** (5–80 μM) and **L1**Cu (12.5–200 μM) over a range of concentrations for 24 h. It can be seen that the two compounds do not negatively affect the cell viability over the full range of concentrations measured, indicating that they exhibit no cytotoxicity and could reasonably be used for intracellular detection. Also, Na_2S does not affect the cell viability either, even in 400 μM (Figure S5 in the Supporting Information). Therefore, the chemsensor can detect sulfide in biological systems.

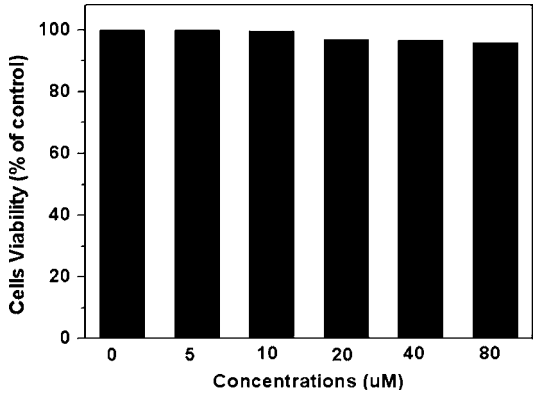

Figure 9. MTT assay of **L1**.

CONCLUSION

In conclusion, we have devised a novel fluorescence probe for sulfide anion based on the Cu^{2+} complex **L1**Cu. The chemsensor exhibited high selectivity for sulfide anion over other common ions and other forms of sulfate. After interaction with sulfide anion, the quenched green fluorescence recovered, and the color changed from yellow to pink. The paper described an inorganic reaction resulting in dosimetric anion determination, which based on a complex **L1**Cu formation–separation process and CuS formation. This work demonstrated that the fluorescent and colorimetric chemosensor **L1**Cu could be used to detect sulfide anion by utilizing this displacement method. Thus, these results are significant and interesting for a new generation of molecular recognition systems. Besides, the sensor can enter living cells and indicate intracellular sulfide anion at micromolar concentration, yet it shows no cytotoxicity. Therefore, the chemsensor has potential applications in biological systems for sulfide anion detection.

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■ ASSOCIATED CONTENT

Supporting Information

MTT assays, binding constant, fluorescence titration curve, intensity, and images, and ^{1}H and ^{13}C NMR and ESI-MS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: zengzhzh@lzu.edu.cn or zengzhzh@yahoo.com.cn (Z.Z.), bdc@lzu.edu.cn (D.B.). Tel.: +86 931 8610877. Fax: +86 931 8912582.

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