

Coumarin Derivative Fluorescent Probe for Highly Selective and Sensitive Sequential Detection of $\text{Cu}^{2+}/\text{S}^{2-}$ and Cell imaging

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Abstract

Rapid detection of copper ion (Cu^{2+}) and sulfide anion (S^{2-}) has become a research priority in view of their crucial roles in human health and environmental monitoring; therefore, there is an intense demand for highly effective detection of their concentrations in biological and environmental samples. In this work, a fluorescent probe **Y** based on the "ON-OFF-ON" strategy for sequential recognition of Cu^{2+} and S^{2-} with high selectivity and anti-interference ability was developed. Probe **Y** can form a complex with Cu^{2+} to realize Cu^{2+} detection due to fluorescence quenching, and the $\text{Y}+\text{Cu}^{2+}$ complex can react with S^{2-} to achieve fluorescent recovery by generation of insoluble copper sulfide (CuS) to release probe **Y**. Probe **Y** toward Cu^{2+} and its complex to S^{2-} detection exhibit good sensitivity with the detection limit (LOD) of 2.40×10^{-8} mol/L and 1.80×10^{-8} mol/L, respectively. Moreover, the detection process can be completed within 30 s to demonstrate rapid response capability over a wide pH range. Test strip, reversibility, and cell imaging experiments were also conducted to confirm their practical application capability in real environments.

1. Introduction

Copper plays a vital role in structure formation and biological processes of various enzymes and proteins, such as DNA synthesis, respiration, redox balance, and energy metabolism [1, 2]. But copper (Cu^{2+}) ion pollution originating from industrial development is also a major factor threatening human health and environmental safety [3, 4]. Excessive accumulation of copper in organisms can easily cause various diseases, including cancer, inflammation, Wilson's, and Alzheimer's disease [5–7]. The appropriate content of Cu^{2+} in blood and water is 15.7–23.6 μM and 31.4 μM . Therefore, it is essentially imperative to establish a highly selective method for detecting Cu^{2+} . Sulfides are widely used in various fields such as leather and battery manufacturing, water treatment, papermaking, organic intermediates, and medicine. The sulfide ion (S^{2-}), as a toxic pollutant, has a widespread impact on people's daily lives [8, 9]. Low concentrations of S^{2-} are extremely important for physiological processes, including vasodilation, hepatic circulation, reduction of inflammation, cellular angiogenesis, and memory formation [10]. But exposure to high levels of S^{2-} can cause various physiological problems, including respiratory paralysis, memory impairment, and irritation in the mucous membrane [11, 12]. According to the report from the World Health Organization (WHO), the maximum allowable level of S^{2-} in drinking water is 14.7 $\mu\text{mol/L}$ [13]. Therefore, strictly control and real-time monitoring of S^{2-} concentration are essential.

Fluorescence analytical methods for $\text{Cu}^{2+}/\text{S}^{2-}$ have attracted increasing attention due to their simple operation, fast response, low detection cost, high sensitivity, and selectivity [14–19]. Generally, fluorescence probes can selectively capture Cu^{2+} to generate an absorptive complex in situ, leading to an obvious quenching signal through an inner filter effect to realize the determination of Cu^{2+} . However, some Lewis acids such as Al^{3+} , Fe^{3+} , and Zn^{2+} may interfere with the determination of Cu^{2+} . The utilization of

Cu^{2+} reaction with fluorophores to form a Cu^{2+} complex usually suffers from strict reaction conditions and long response times. S^{2-} can efficiently capture Cu^{2+} to form stable CuS ($K_{\text{sp}} = 6.3 \times 10^{-36}$) to present excellent responsiveness [20]; thus, the sensor based on Cu^{2+} complex may have good potential for S^{2-} recognition through a displacement strategy. So far, many probes have been used for continuous detection of Cu^{2+} and S^{2-} ions, for example, carbon dots [21–24], nanoparticles [25–27], peptide-based probes [28–31], and other small molecules [32–36]. However, some sensors also have such as multiple synthesis steps, complex structures, and too many recognition sites leading to poor selectivity. Consequently, there is an intense demand to develop fluorescent probes with high efficiency, sensitivity, as well as good solubility, efficient spectroscopy, and outstanding selectivity to monitor Cu^{2+} and S^{2-} in environmental samples and organisms in real time.

Coumarins and their derivatives present excellent UV absorption and fluorescence emission properties, and biocompatibility, as well as highly variable size and chelating ability, commonly used to construct sensors for detecting metal ions [37–42]. Moreover, their optical properties vary greatly with changes in different substituent groups. In this work, a coumarin-based fluorescent probe and its Cu^{2+} complex rapidly realize detection of S^{2-} through an "on-off-on" strategy. Firstly, an intermediate was synthesized using 4-dimethylaminosalicylaldehyde and ethyl acetoacetate as the substrates, which reacts with furan-2-carbohydrazide to achieve probe **Y**. Cu^{2+} can quench the fluorescence of probe **Y** to form a Y-Cu^{2+} complex for detection of Cu^{2+} with high selectivity and anti-interference ability. When the addition of S^{2-} and Cu^{2+} was removed from the Y-Cu^{2+} complex and combined with S^{2-} to generate copper(II) sulfide (CuS) to release free probe **Y** to result in fluorescence recovery, which can efficiently recognize S^{2-} . The Y-Cu^{2+} complex exhibits high selectivity and anti-interference ability in recognizing S^{2-} with a low detection limit. To investigate its practical application capability for probe **Y**, test strip, reversibility, and live cell imaging experiments were also conducted.

2. Experimental

2.1 Materials and apparatus

The main substrates, including 4-diethylaminosalicylaldehyde, ethyl acetoacetate, and furan-2-carbohydrazide, were purchased from Adamas-beta; the other reagents were obtained from Sigma-Aldrich. All reagents used in experiments had no further treatment. F-7000 spectrometer (Baihe Instrument Technology Co., LTD, Shanghai, China) was used to measure the fluorescence spectra. FT-IR spectra were recorded on FTIR-650S Fourier Transform infrared spectrometer (Tianjin Port East). Ultraviolet-visible absorption spectrum was obtained on ATU-1810DPC spectrometer (Tianjin Gangdong). ^1H NMR and ^{13}C NMR were measured on a Bruker avance neo spectrometer at 400 and 100 MHz using $\text{DMSO-}d_6$ as the solvent. Xevo G2-XS mass spectrograph provided the corresponding ESI-MS data. The synthetic process was monitored by thin-layer chromatography (TLC) under 365 nm UV lamp.

2.2 Synthesis and characterization of probe **Y**

The synthetic pathway of probe **Y** was described in Fig. 1. Firstly, 4-diethylaminosalicylaldehyde (1.93 g, 10.0 mmol) and ethyl acetoacetate (1.96, 15 mmol) were added to a round-bottom flask with 30 mL of anhydrous ethanol followed 10 drops of piperidine to be added into the system. The reaction mixture was performed for 8 h at 80°C, and TLC used to monitor the process. After completion the mixture cooled to room temperature to yield intermediate **M** as a yellow solid (Figs. S1-S3). The compound **M** (0.259, 1 mmol) and furan-2-carbohydrazide (0.126 g, 1 mmol) was added to a flask with 15 mL anhydrous ethanol and a few drops of acetic acid, and the reaction mixture was refluxed for 6 h. After completion, the reaction mixture was cooled to room temperature to generate precipitate, which was collected by vacuum filtration, washed with anhydrous ethanol (3×10 mL), dried, and recrystallized in DMF/EtOH to obtain red solid **Y** with 84% yield. The corresponding analytical spectra of **Y** were shown in Figs. S4-S6.

2.3 Fluorescence and UV spectroscopy tests

All probe **Y** solutions used in tests were prepared with DMSO and stored under a dry atmosphere at a concentration of 2×10^{-5} mol/L for later use. The cationic nitrates (Na^+ , K^+ , Zn^{2+} , Al^{3+} , Mg^{2+} , Co^{2+} , Cd^{2+} , Mn^{2+} , Hg^{2+} , Ca^{2+} , Fe^{3+} , Cu^{2+} , Pb^{2+} , Ni^{2+} , Ag^+ , Cr^{3+}) and sodium salts of anions (HPO_4^{2-} , F^- , H_2PO_4^- , Cl^- , NO_3^- , NO_2^- , HSO_3^- , Br^- , HSO_4^- , SO_3^{2-} , SO_4^{2-} , HCO_3^- , $\text{S}_2\text{O}_3^{2-}$, AcO^- , S^{2-}) used in experiments were prepared with pure water to form a concentration of 4×10^{-4} mol/L. 0.5 mL of probe solution was taken and diluted with an equal amount of ion to 5 mL to generate a DMSO/H₂O solution (volume ratio = 2:3) with a concentration of 2×10^{-5} mol/L for fluorescence and UV testing. The fluorescence spectrum of **Y** was used 495 nm excitation and 536 nm emission wavelength with 10 nm slits.

2.4. Cell toxicity assays and imaging methods

The possible cytotoxicity of probe **Y** was evaluated with HeLa cells through the CCK8 assay [43]. After incubation with different concentrations of **Y** (0, 20, 40, and 80 μM) for 24 h, the cell viability was above 85% at various concentrations, indicating that the biological toxicity of **Y** was negligible. Confocal microscopy was used to observe live HeLa cells imaging upon the addition of exogenous Cu^{2+} and S^{2-} . First, 2.5×10^5 cells/well were plated in a 6-well cell culture plate for 24 hours. Then, the **Y** solution (10 μM) was added to the 6-well plate's discarded medium containing HeLa cells to culture for 30 min at 37°C and imaged. Next, 10 and 30 μM concentrations of Cu^{2+} were added to the cell culture medium containing 10 μM concentration of **Y**, and the cells were imaged after 30 min at 37°C. Finally, different concentrations of S^{2-} (10 and 30 μM) were added to the culture cells including 10 μM of **Y** and Cu^{2+} , and then were again imaged after 30 min at 37°C.

3. Results and Discussion

3.1 Determination of water content

To determine suitable water contents of probe to Cu^{2+} and its complex to S^{2-} detection in real environments, the water content experiments were performed, and the results are shown in Figs. 2a. The probe solution without Cu^{2+} exhibited strong fluorescence emission as the water content varied from 20% to 90%. After adding Cu^{2+} , the fluorescence of the Y-Cu^{2+} complex remained quenched at different water contents. When S^{2-} was added to its Y-Cu^{2+} complex, it could be observed that as the water content increased, the fluorescence intensity of $\text{Y-Cu}^{2+}\text{-S}^{2-}$ first increased and then decreased, reaching a maximum at 60%, which was similar to the probe Y solution. Then, a 60% water content ($\text{DMSO/H}_2\text{O} = 2:3$) solution was selected for subsequent experiments.

3.2 pH effect on Y , Y-Cu^{2+} , and $\text{Y-Cu}^{2+}\text{+S}^{2-}$

The pH effects on the fluorescence intensity of Y , Y-Cu^{2+} and $\text{Y-Cu}^{2+}\text{+S}^{2-}$ systems were conducted as shown in Figs. 2b. Obviously, pH value effects on the fluorescence properties of the Y+Cu^{2+} system were limited in view of its fluorescence quenching. Thus, the fluorescence performances of Y , $\text{Y-Cu}^{2+}\text{+S}^{2-}$ systems were investigated to evaluate appropriate pH ranges for Cu^{2+} and S^{2-} recognition. The Y and $\text{Y-Cu}^{2+}\text{+S}^{2-}$ systems exhibit good fluorescence emission at $\text{pH} = 4.0\text{--}9.0$ due to diethylamino group in Y molecular structure is easily protonated at stronger acidity unfavorable to intramolecular electron transfer to result in weaker fluorescence emission or quenching. A rapid decrease can be observed when pH values are more than 9.0. Possible reason may ascribe to strong alkalinity ($\text{pH} > 12$) promptly reduce the stability of Y and Y-Cu^{2+} complex to cause fluorescence lowering. Therefore, all subsequent testing generally adjusted pH value to $\text{pH} = 7.4$ using HEPES buffer solution.

3.3 Selectivity experiments of Y towards Cu^{2+} detection

The sensing performance of probe Y towards various metallic cations was investigated. As shown in Figs. 2c. When various metal cations were added to the EY solution, only Cu^{2+} caused an obvious decrease in fluorescence intensity, changing from bright green to colorless. These changes may be due to the addition of Cu^{2+} to promote the isomerization of the $\text{C} = \text{N}$ bond, which facilitates the PET process and thus decreases the fluorescence emission. In contrast, the addition of other metal cations has no significant effect on the fluorescence intensity, indicating that Y exhibits good selectivity towards Cu^{2+} detection. In the UV-visible absorption spectrum (Fig. 2d), the absorption peak only with the addition of Cu^{2+} undergoes a red shift, which also demonstrates that Y shows good selectivity towards Cu^{2+} . To investigate the anti-interference ability of Y toward Cu^{2+} identification, Cu^{2+} was added to the Y solution containing other metal cations. As shown in Figs. 2e, the presence of other ions has no effect on the detection of Cu^{2+} . A similar result can also be observed in the UV absorption bar chart (Fig. 2f), indicating that the presence of other metal ions almost has no interference on the detection of Cu^{2+} .

3.4 Titration experiments of Cu^{2+} toward Y

To investigate the sensitivity of Y to Cu^{2+} recognition, a fluorescence titration experiment was carried out as depicted in Figs. 2g. With the continuous addition of Cu^{2+} , the fluorescence intensity of the emission

peak at 536 nm continuously decreased. When the addition of Cu^{2+} concentration reaches $15.2 \mu\text{mol/L}$, the fluorescence of the system is completely quenched and presents a minimum value. In addition, within the concentration range from $1.06\text{--}6.43 \mu\text{mol/L}$, the changes in fluorescence intensity showed a certain linear relationship with the concentration of Cu^{2+} (Figs. 2h). The linear fitting equation and correlation coefficient are $Y = 1120.9067 - 162.5563X$ and $R^2 = 0.9806$. According to Formula I ($\text{LOD} = 3\delta/K$), where δ represents the standard deviation of the blank sample measured 10 times and K is the slope obtained from the calibration curve [43, 44]. The detection limit for Y toward Cu^{2+} is calculated to be $2.40 \times 10^{-8} \text{ mol/L}$, which is far lower than the $1.3 \mu\text{mol/L}$ allowed by the Environmental Protection Agency (EPA) [45]. Moreover, the detection limit is lower than most of the reported literature (Table S1).

3.5 Selectivity experiments of $Y\text{-Cu}^{2+}$ towards S^{2-} detection

Based on the strong binding affinity of Cu^{2+} towards S^{2-} , the $Y\text{-Cu}^{2+}$ complex could serve as an excellent "turn-on" fluorescent probe for further detection of S^{2-} . Then, the fluorescence emission and ultraviolet absorption of the $Y\text{-Cu}^{2+}$ complex towards S^{2-} were measured, and selective experiments of $Y\text{-Cu}^{2+}$ towards S^{2-} and other various anions (HPO_4^{2-} , F^- , H_2PO_4^- , Cl^- , NO_3^- , NO_2^- , HSO_3^- , Br^- , HSO_4^- , SO_4^{2-} , HCO_3^- , I^- , $\text{S}_2\text{O}_3^{2-}$, AcO^-) were conducted. As exhibited fluorescence spectrum Figs. 3a, there were no significant changes in the fluorescence intensity of the $Y\text{-Cu}^{2+}$ complex with the addition of other anions, whereas when S^{2-} was added to the $Y\text{-Cu}^{2+}$ complex, the fluorescence intensity at 536 nm significantly increased, indicating that it can serve as a fluorescent probe to specifically detect S^{2-} . S^{2-} can combine with Cu^{2+} to remove Cu^{2+} from $Y\text{-Cu}^{2+}$ complex to form CuS , thereby releasing free ligand Y to cause fluorescence recovery. Similarly, compared with the addition of other anions, UV-visible spectrum Figs. 3b can clearly observe a similar phenomenon. Moreover, the action of $Y\text{-Cu}^{2+}$ with S^{2-} causes the UV absorption peak to shift from 485 nm to 430 nm, indicating that $Y\text{-Cu}^{2+}$ has a specific selectivity towards S^{2-} under the UV-visible spectrum. Overall results demonstrate that the $Y\text{-Cu}^{2+}$ complex can serve as an excellent fluorescent probe to recognize S^{2-} through their interaction.

3.6 Anti-interference studies of $Y\text{-Cu}^{2+}$ against S^{2-} detection

To further investigate the recognition ability of $Y\text{-Cu}^{2+}$ complex towards S^{2-} in complex environments, competitive experiments with various anions were performed, and the corresponding results are shown in Figs. 3c. In the presence of other coexisting anions, the fluorescence emission of the $Y\text{-Cu}^{2+}$ complex can be restored by S^{2-} due to the formation of CuS and the generation of free Y , indicating that the $Y\text{-Cu}^{2+}$ complex shows great potential for selectively distinguishing S^{2-} .

3.7 S^{2-} to $Y\text{-Cu}^{2+}$ titration experiments

Fluorescence titration was used to investigate the relationship between Y-Cu²⁺ complex and S²⁻ concentration. As shown in Figs. 3d, in the DMSO/H₂O (2:3, v/v) solution used by HEPES adjusted to pH = 7.4, it can be observed that the fluorescence intensity at 536 nm gradually increases with the addition of S²⁻. When the concentration reaches 24.0 μmol/L, the fluorescence intensity no longer changes and reaches a maximum value. Meanwhile, there is a good linear relationship in the 0.24–22.0 μmol/L concentration range with the linear equation of $Y = 72.0264X + 17.9335$ and correlation coefficient $R^2 = 0.9868$ (Figs. 3e). According to formula I, the detection limit of Y-Cu²⁺ to S²⁻ detection was calculated to be 1.80×10^{-8} mol/L, which is relatively low compared to the reported data (Table S1). Obviously, the probe **Y** can achieve Y-Cu²⁺ complex to continuously detect S²⁻ within the concentration range from 0.24–22.0 μmol/L.

3.8 Time response of Y-Cu²⁺ to S²⁻ identification

Response time is an important parameter for accurate and rapid analysis of targets. Thus, the time response of Y-Cu²⁺ complex before and after the addition of S²⁻ was investigated to evaluate the sensitivity of Y-Cu²⁺ to S²⁻. As depicted in Figs. 3f, after the addition of Cu²⁺, the fluorescence intensity of **Y** rapidly decreased to the minimum value within 30 seconds, and it remained stable within 240 seconds. When S²⁻ was added to Y-Cu²⁺, the maximum value in fluorescence intensity was observed within 3 seconds, then gradually reached a steady state.

3.9 Possible mechanism on **Y** to Cu²⁺ and its complex to S²⁻ detection

To determine the binding mode between **Y** and Cu²⁺, The solution was prepared by varying volume difference between probe **Y** (1 – 0 mL) and Cu²⁺ (0–1 mL), Jobs' work plot was conducted to determine the combination ratio between probe **Y** with Cu²⁺. The results shown in Figs. 2i indicates that the binding ratio of **Y** to Cu²⁺ is 1:1. The binding constants of probe **Y** to Cu²⁺ is calculated according to a modified Benesi-Hildebrand equation II ($K_c = (F_x - F_0) / (F_{max} - F_0) \times [M]$), where F_{max} , F_0 and F_x are fluorescence intensities of **Y** solutions at Cu²⁺ saturation, Cu²⁺ blank and at any intermediate concentration, respectively, $[M]$ is molar concentration of Cu²⁺ [46–48]. The complexation of **Y** with Cu²⁺ was calculated to be 2.81×10^6 L/mol, which is relatively large to demonstrate good stability of Y+Cu²⁺ complex. By the comparison infrared spectrum of **Y** and Y+Cu²⁺, the peak at 1733cm^{-1} and 1658cm^{-1} belonged to C = O bands stretching vibration nearly disappears to indicate that it may be link with Cu²⁺ [49].

The possible mechanism of **Y** to recognize Cu²⁺ can be inferred as shown in Figs. 4a. To verify this mechanism, DFT calculations of **Y** and Y+Cu²⁺ were performed on the Gaussian 09W program with the B3LYP functional for ground-state simulations. Geometry optimization and frequency calculations for all atoms of the molecules were carried out at the 6–31g (d, p) basis set, considering ethanol as the solvent. As shown in Figs. 4b, the energy levels of HOMO and LUMO of **Y** and Y+Cu²⁺ are – 3.22, –7.12 eV

and -2.23 , -4.16 eV, and the corresponding energy gaps are 3.90 and 1.93 eV. Obviously, the formation of $\mathbf{Y}+\text{Cu}^{2+}$ complex can effectively reduce the energy gap, indicating that $\mathbf{Y}$ has an intense tendency for the formation of $\mathbf{Y}+\text{Cu}^{2+}$ coordination. However, Cu^{2+} , as a paramagnetic ion whose empty d -shell exhibits high thermodynamic affinity to ligands, may efficiently suppress the fluorescence emission of a fluorophore based on a photo-induced metal-to-fluorophore electron or energy transfer process to strongly result in fluorescence quenching [50]. In addition, in view of highly insoluble CuS , the $\mathbf{Y}+\text{Cu}^{2+}$ coordination easily reacts with S^{2-} to generate CuS and release free $\mathbf{Y}$ to lead to recovery of the $\mathbf{Y}+\text{Cu}^{2+}$ system.

4. Practical application of $\mathbf{Y}$

4.1 Test strip experiment

A test strip experiment was conducted to facilitate rapid and sensitive detection of S^{2-} using the $\mathbf{Y}+\text{Cu}^{2+}$ system. The filter paper strips were immersed in $\mathbf{Y}$ solution with a concentration of 2×10^{-5} mol/L in $\text{DMSO}/\text{H}_2\text{O}$ (2:3, v/v, pH = 7.4) for 24 h, then taken out and naturally dried in air. A clear green fluorescence emission could be observed under a UV lamp. Subsequently, Cu^{2+} was added dropwise to the test strip, and the disappearance of green light was observed. After adding S^{2-} to the filter paper, green fluorescence was again observed, as shown in Figs. 5b. The results indicate that the $\mathbf{Y}+\text{Cu}^{2+}$ complex can be used for the on-site detection of S^{2-} , making the detection of S^{2-} in real samples to become simpler, more efficient, and faster. Reversibility experiment was used to further investigate the practicality of probe $\mathbf{Y}$. As shown in Figs. 5c, the $\mathbf{Y}$ solution presents regular fluorescence quenching and recovery after the alternating addition of Cu^{2+} and S^{2-} , the reaction can be cycled at least 6 times and low fluorescence efficiency loss were observed. These results demonstrated that probe $\mathbf{Y}$ has good reversible and switchable sensor characteristics, also means that S^{2-} can use for the regeneration of $\mathbf{Y}+\text{Cu}^{2+}$ complex.

4.2. Cell imaging for probe $\mathbf{Y}$, $\mathbf{Y}+\text{Cu}^{2+}$ and $\mathbf{Y}+\text{Cu}^{2+}+\text{S}^{2-}$

Fluorescent molecules must be non- or low-cytotoxic in applicants for imaging of living cells and organisms. Thus, HeLa cells were used to evaluate the cytotoxicity of probe $\mathbf{Y}$ through a proliferation inhibition test. The cell viability of HeLa cells in different concentrations of $\mathbf{Y}$ solution for cultivation for 24 h was determined according to the CCK8 assay, and the results confirmed that probe $\mathbf{Y}$ toward HeLa cells has no obvious cytotoxicity. As depicted in Fig. 5d, the blank solution has no fluorescence, and the cultured HeLa cells in $\mathbf{Y}$ solution showed stronger fluorescence under white or green light channels. When the Cu^{2+} solution was added to the cultured solution, the fluorescence of HeLa cells significantly became weaker. As the concentration of Cu^{2+} was increased to $30 \mu\text{mol/L}$, the fluorescence of HeLa cells was completely quenched. Conversely, for the $\mathbf{Y}+\text{Cu}^{2+}$ system, the fluorescence of HeLa cells became brighter as the concentration of S^{2-} solution increased. These results suggest that probe $\mathbf{Y}$ can

be applied for imaging of living cells and detection of trace Cu^{2+} and S^{2-} , indicating that probe **Y** may have good application potential in biomedicine.

4.3 Detection of Cu^{2+} and S^{2-} in water samples

As common water pollutants, it is very necessary to rapidly detect trace Cu^{2+} and S^{2-} in water samples. To evaluate the practical applicability of probe **Y**, spiked recovery experiments were conducted for Cu^{2+} and S^{2-} in pure, tap, and river water samples obtained from Guiyang city. The mother liquor of probe **Y** was diluted to 20 $\mu\text{mol/L}$ with DMSO/HEPES solution (2/3, v/v, pH 7.4), then various concentrations of Cu^{2+} (2.0, 4.0, and 6.0 $\mu\text{mol/L}$) or S^{2-} (10.0, 15.0, and 20.0 $\mu\text{mol/L}$) standard solutions were respectively added to water samples, and their concentrations were determined based on the measured fluorescence intensity. As shown in Table 1, probe **Y** and **Y**+ Cu^{2+} exhibit good performance across increasing concentrations of Cu^{2+} or S^{2-} . The spiked recovery rates are 96.0%–104.3% for Cu^{2+} and 96.2%–104.4% for S^{2-} , and relative standard deviations (RSD, $n = 3$) do not exceed 5%, demonstrating that probe **Y** and **Y**+ Cu^{2+} complex can serve as an effective analytical tool for tracing Cu^{2+} or S^{2-} in water samples.

Table 1
Determination of Cu^{2+} or S^{2-} in water samples

Sample (μM)	Spiked (μM)		Recovery (μM)		Recovery (%)		RSD (%, $n = 3$)	
	Cu^{2+}	S^{2-}	Cu^{2+}	S^{2-}	Cu^{2+}	S^{2-}	Cu^{2+}	S^{2-}
Pure water	2.00	10.00	2.08	9.73	104.0	97.3	4.0	3.7
	4.00	15.00	3.90	14.55	97.5	97.0	2.5	3.0
	6.00	20.00	6.14	20.88	102.3	104.4	2.3	4.4
Tap water	2.00	10.00	1.92	9.71	96.0	97.1	4.0	2.9
	4.00	15.00	3.88	15.45	97.3	103.0	2.7	3.0
	6.00	20.00	6.25	19.36	104.2	96.8	4.2	3.2
River water	2.00	10.00	1.96	10.32	98.0	103.2	2.0	3.2
	4.00	15.00	4.17	14.47	104.3	96.2	4.3	3.8
	6.00	20.00	6.23	20.79	103.8	104.0	3.8	4.0

4. Conclusion

In summary, novel probe **Y** was designed and synthesized via a simple synthetic pathway from 4-dimethylaminosalicylaldehyde and ethyl acetoacetate via Knoevenagel condensation reaction to generate an intermediate, followed by a Schiff base reaction with furan-2-carbohydrazide. Utilizing the

reaction of Cu^{2+} with probe **Y** to form the Y-Cu^{2+} complex to load fluorescence quenching to recognition of Cu^{2+} , and which further reacts with S^{2-} to generate CuS and release free **Y** to cause fluorescence recovery, thereby achieving a "turn-on recognition of S^{2-} ." Probe **Y** exhibits good sensitivity for sequential monitoring of Cu^{2+} and S^{2-} in DMSO/HEPES (2/3, v/v) with low detection limits of 2.40×10^{-8} mol/L and 1.80×10^{-8} mol/L. The combination mode between **Y** and Cu^{2+} has been investigated via FTIR and DFT calculations. Time-response experiments demonstrated that the Y-Cu^{2+} complex can rapidly respond to S^{2-} in 30 seconds. Test strip experiments demonstrated that the Y-Cu^{2+} probe can rapidly detect S^{2-} in real environments, and cell imaging experiments validated probe **Y** for continuous detection of Cu^{2+} and S^{2-} in live cells. Therefore, this work provides a crucial strategy for advancing studies on ion monitoring in environmental and biological fields.

Declarations

Author Contribution

Lu-yu Tian: Data curation, Software, Writing-original draft, Visualization. Bo-wen An: Investigation, Conceptualization. Rui-yong Jiang: Software, Visualization. Tao Zhu: Methodology, Resources. Chan Huang: Formal analysis, Investigation. Ye Wang: Project administration, Formal analysis. Xiao-hua Cai: Writing-review & editing, Funding acquisition, Project administration, Supervision.

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Figures

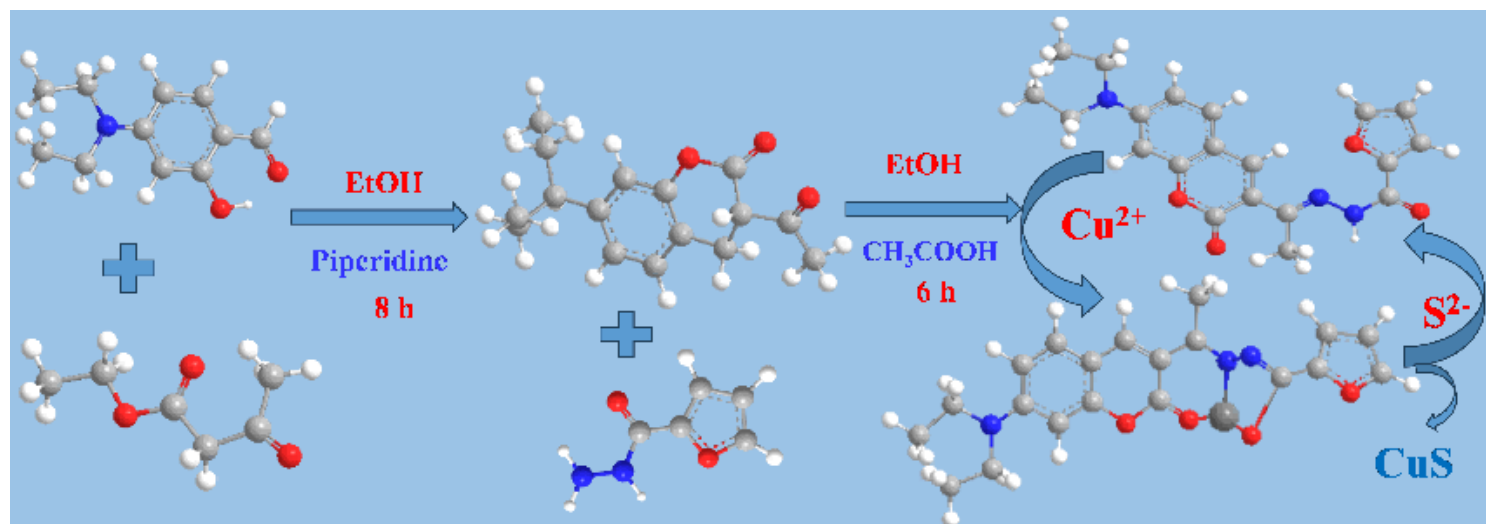


Figure 1

Synthetic route of probe **Y** and detection of $\text{Cu}^{2+}/\text{S}^{2-}$

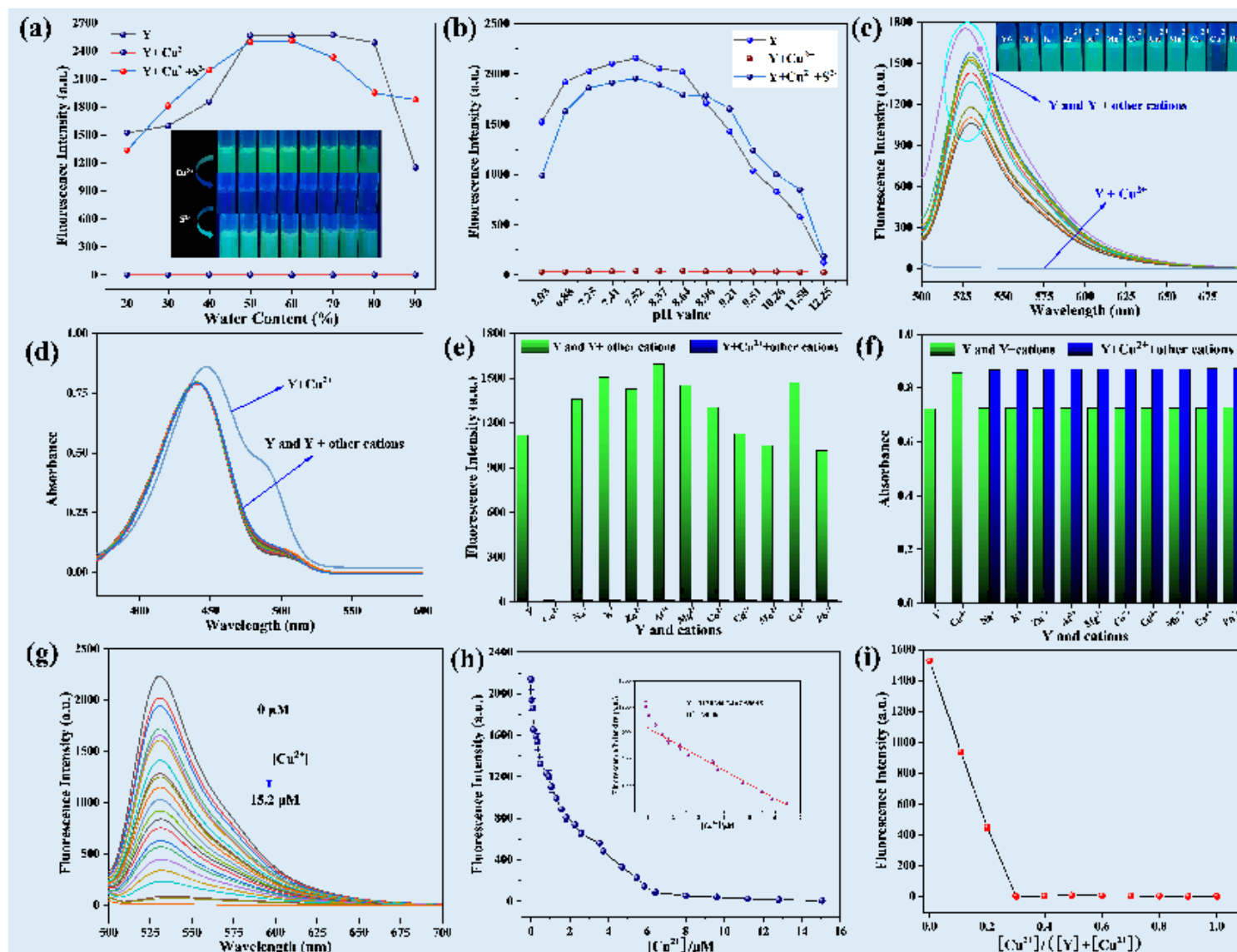


Figure 2

(a) Water content effect on **Y**, **Y**+Cu²⁺, and **Y**+Cu²⁺+S²⁻; (b) Fluorescence intensity of **Y**, **Y**+Cu²⁺, and **Y**+Cu²⁺+S²⁻ systems at different pH values; (c) Selective fluorescence experiment for **Y** to Cu²⁺ detection; (d) Selective UV-visible absorption experiment for **Y** to Cu²⁺ detection; (e) Anti-interference fluorescence experiment for **Y** to Cu²⁺ detection; (f) Anti-interference experiment for **Y** to Cu²⁺ detection under ultraviolet spectrum; (g) Fluorescence titration for Cu²⁺ to **Y** concentration; (h) Scatter linear fitting plot of fluorescence titration for Cu²⁺ to **Y** concentration; (i) Work curve for Cu²⁺ to **Y** concentration.

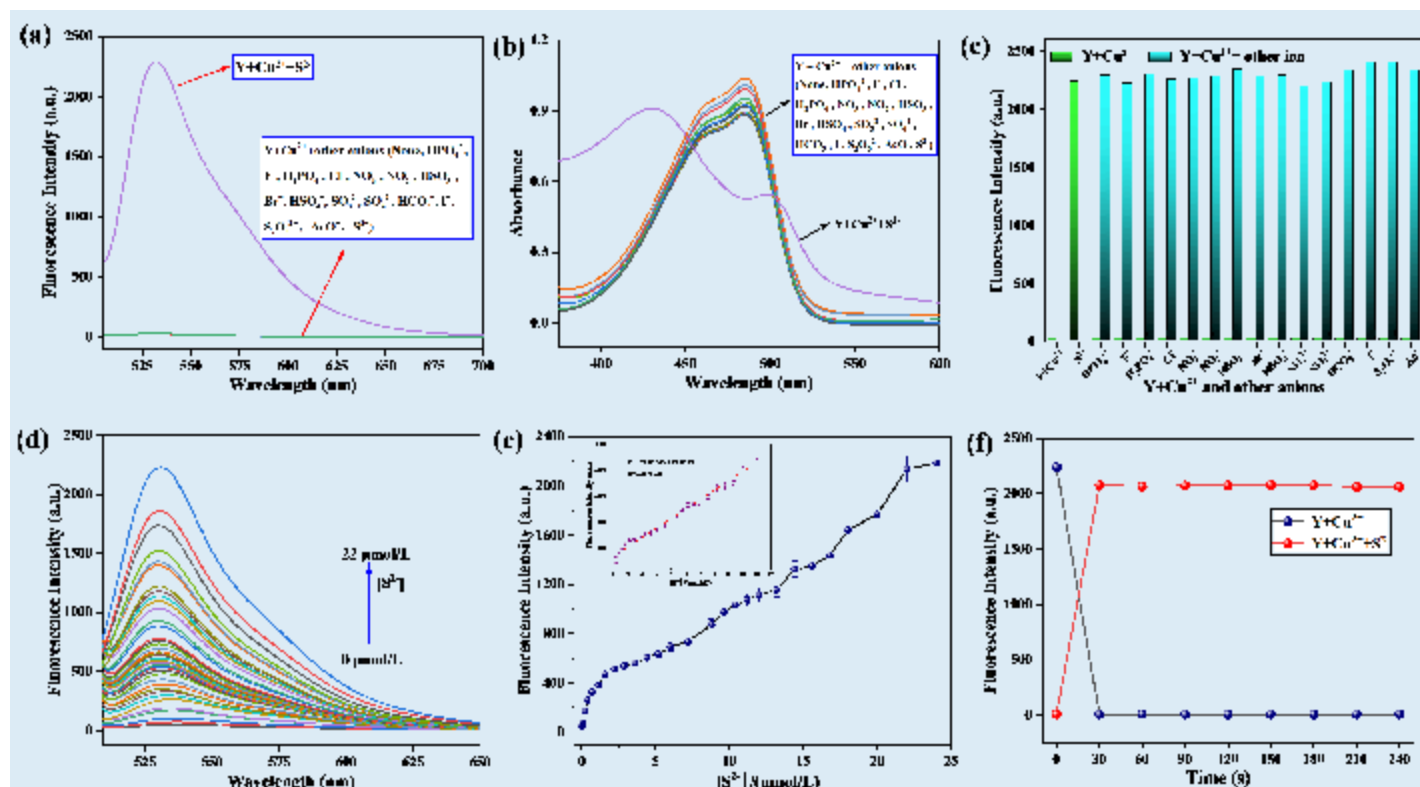


Figure 3

(a) Selective fluorescence experiments for **Y**-Cu²⁺ to S²⁻ detection; (b) Selective experiment for **Y**-Cu²⁺ to S²⁻ detection under ultraviolet spectrum; (c) Anti-interference experiments for **Y** +Cu²⁺ to S²⁻ detection; (d) Fluorescence titration for S²⁻ concentration to **Y** +Cu²⁺; (e) Scatter and linear fitting plot of fluorescence titration for S²⁻ concentration to **Y** +Cu²⁺; (f) Work curve for S²⁻ concentration to **Y** +Cu²⁺.

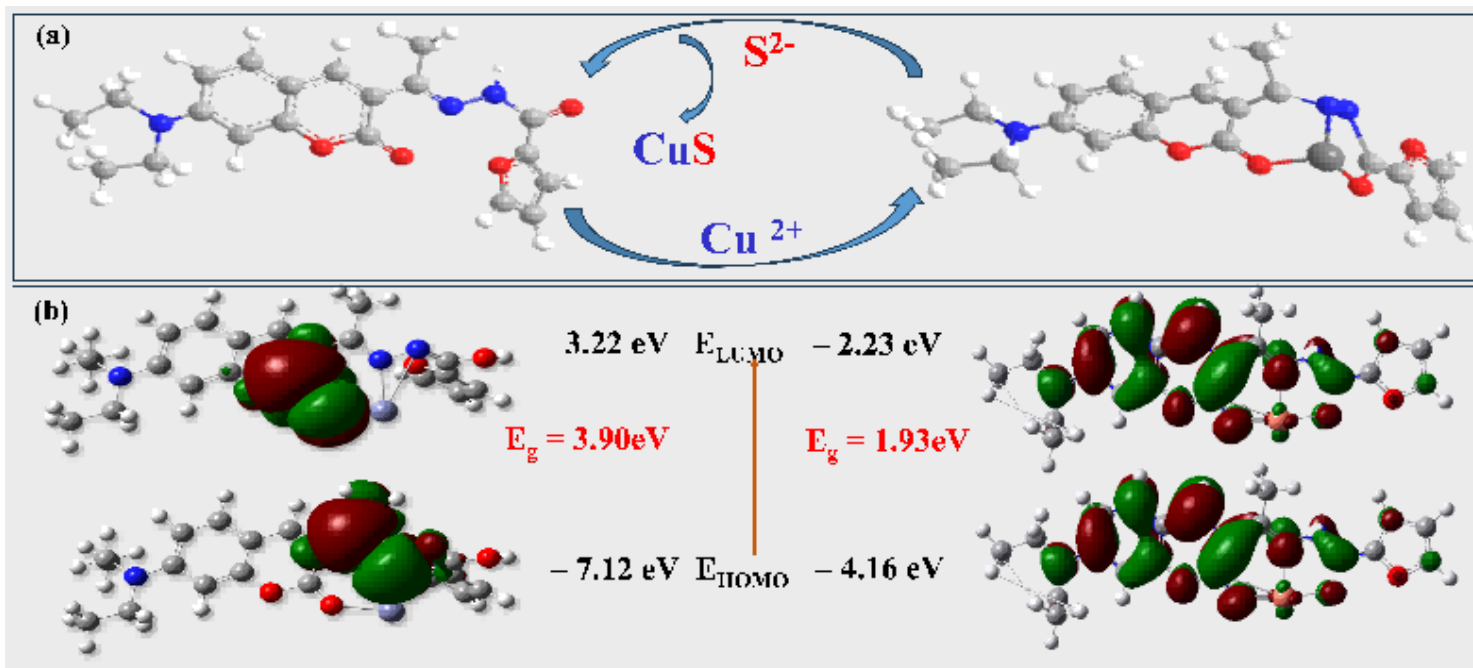


Figure 4

(a) Possible mechanism of **Y** to Cu^{2+} and its complex to S^{2-} -detection; (b) The energy levels of HOMO and LUMO and the corresponding energy gap of **Y** and **Y+Cu²⁺**.

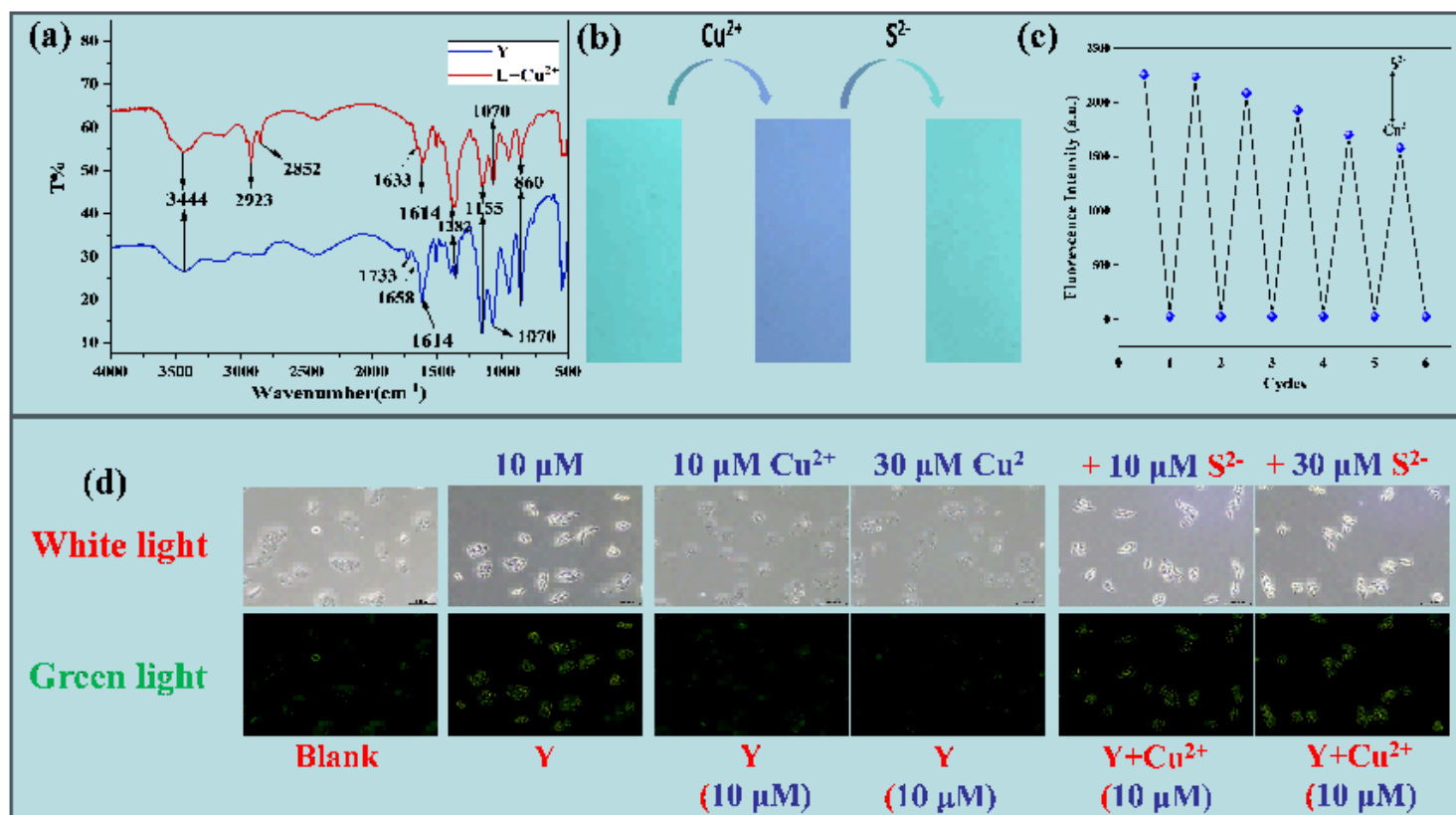


Figure 5

(a) IR spectrum comparison of **Y** and its complex **Y**+Cu²⁺; (b) The photograph after alternate addition of Cu²⁺ and S²⁻; (c) The fluorescence changes after alternate addition of Cu²⁺ and S²⁻; (d) Fluorescent imaging of HeLa cells at different concentrations of **L**, **L**+Cu²⁺, and **L**+Cu²⁺ S²⁻ solutions.