# FRET-based Fluorescent and Colorimetric Probe for Selective Detection of Hg(II) and Cu(II) with Dual-mode

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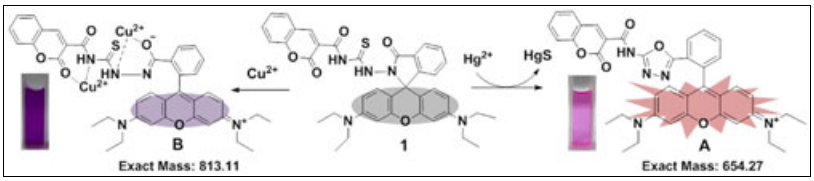


A dual-function fluorescence resonance energy transfer (FRET)-based fluorescent and colorimetric probe was rationally fabricated from an energy donor coumarin moiety and an energy acceptor rhodamine moiety linked by a thiohydrazide arm for selective detection of $Hg^{2+}$ and $Cu^{2+}$. Two distinct mechanisms were used for the selective detection. Results revealed that probe **1** showed high fluorescent selectivity towards $Hg^{2+}$ and evident colorimetric selectivity for $Cu^{2+}$, which was suitable for 'naked-eye' detection.

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## INTRODUCTION

Detecting heavy metal ions has extensively attracted attention because qualitative and quantitative assays of these ions show their direct effect on human health and the environment. Mercury(II) ion is a dangerous and toxic pollutant. Accumulation of this ion can damage the nervous system, kidneys, endocrine system and brain because of its high affinity towards sulphydryl groups [1–3]. Copper(II) is another essential trace element for humans. It is usually present at low concentration levels in biological systems but plays vital roles in different physiological and pathological processes. An elevated level of copper in the liver or kidney is usually associated with Wilson's disease, amyotrophic lateral sclerosis and Menkes syndrome [4,5]. Thus, monitoring the levels of these two metal ions is necessary.

Numerous strategies have been developed to detect the two metals, which have led to significant progress in this field [6–19]. Particularly, chemosensors based on rhodamine, fluorescein, coumarin, or other functional chromophores showed promising applications because of their high selectivity [20], low detection limits [21], real-time detection [22], and potential biological applications [23]. Zhang and co-workers [24] reported a fluorescent "turn-off" sensor towards $Cu^{2+}$ with a detection limit of 4.9 nM in pure water at pH 3.5; this sensor was based on benzimidazo[2,1-a]benz[de]isoquinoline-7-one. Bharadwaj and co-workers developed a fluorescent "turn-on" probe by covalently attaching 5-(benzothiazol-2-yl)-4-hydroxyisophthalaldehyde onto 8-aminoquinoline; this probe exhibited high sensitivity towards $Hg^{2+}$ with a detection limit down to 0.11 μM [25]. Although many elaborate chemosensors with single functions can monitor $Hg^{2+}$ or $Cu^{2+}$ individually [26–31], designing dual-function fluorescent probes for monitoring $Hg^{2+}$ and $Cu^{2+}$ is attractive.

Metal binding changes the molecular structure and/or electronic property of a sensor, which consequently results in changes in fluorescence properties that can be detected through monitoring. A number of intensity-dependent fluorescent sensors have been reported. However, these sensors still exhibit many limitations, including inaccurate measurements of fluorescence intensity, which is influenced by variations in probe concentration, environmental conditions, instrumental efficiency and excitation intensity [32,33]. Fluorescence resonance energy transfer (FRET) strategy, as a promising platform to solve these problems, has been widely explored for designing ratiometric and/or colorimetric fluorescent probes. Zeng and co-workers designed a FRET-based fluorescent probe with the fluorescein analogue as the energy acceptor and the coumarin group as the energy donor. They found that this sensor with a detection limit of as low as 0.39 μM was highly selective and sensitive to $H_2S$ over other biologically relevant species [34]. Han and co-workers employed a phenothiazine donor and a rhodamine acceptor to construct a FRET-directed ratiometric

fluorescent probe with a low detection limit of 9.2 nM for selective response to $Hg^{2+}$ [35]. These studies motivate us to rationally design a FRET-based colorimetric scaffold **1**. In this scaffold, rhodamine moiety served as the energy acceptor because of its high fluorescence quantum yields and large absorption coefficients [36,37]. The coumarin group acted as an energy donor because of its overlaps between its emission spectra and the absorption spectrum of rhodamine [38,39], and thiohydrazide moiety served as the binding sites with metals because of its strong affinity towards $Hg^{2+}$ and $Cu^{2+}$ [40–44]. By combining the donor and the acceptor with a thiohydrazide arm, a FRET system was constructed for selective detection of $Hg^{2+}$ and $Cu^{2+}$ directed by two distinct mechanisms.

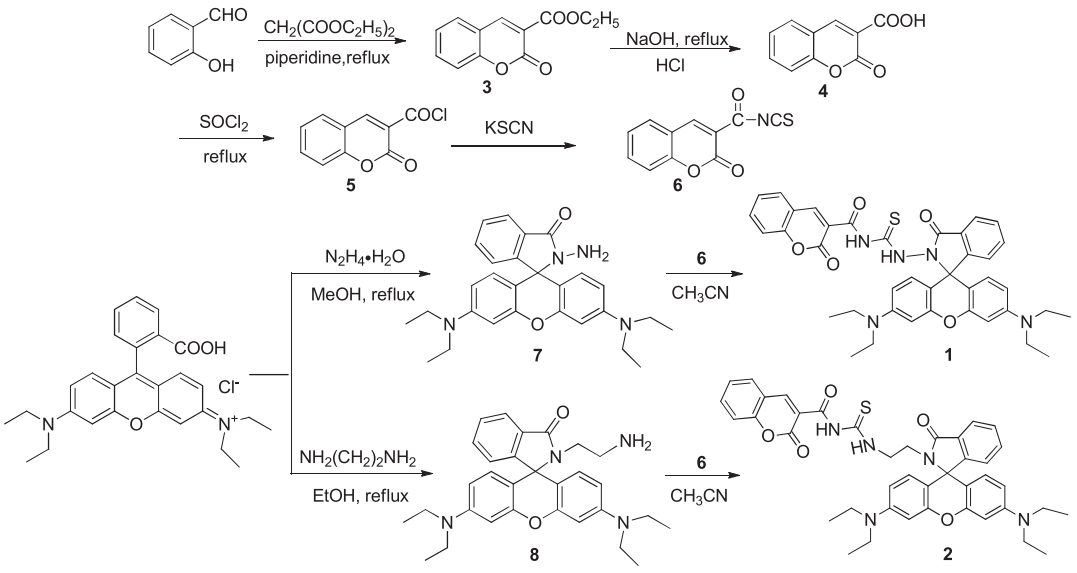
## RESULTS AND DISCUSSION

**Synthesis of probes 1**. As shown in Scheme 1, the starting reagent 2-hydroxybenzaldehyde was treated using cyclisation, hydrolysis, chloroformylation, and substitution reaction to produce an important intermediate **6**. Intermediate **7** was also an important intermediate that was achieved through the condensation reaction between rhodamine B and hydrazine hydrate. By mixing intermediates **6** and **7** in $CH_3CN$ solvent, target compound **1** was obtained and characterised by $^1H$ NMR, $^{13}C$ NMR and mass spectrometry (Figs. S1–S2, supplementary data). After adding $Hg^{2+}$ or $Cu^{2+}$ into the solution (EtOH:$H_2O$ = 9:1, v/v) of probe **1**, the colorless solution was immediately transformed into pink or purple, suggesting that a colorimetric probe for selective detection of $Hg^{2+}$ and $Cu^{2+}$ could be fabricated.

**Fluorescence spectra of probe 1 with various metal ions.** To examine the selectivity of probe **1** for metal ions, we employed fluorescence microscopy and UV light. As shown in Figure 1a, a strong emission peak at 586 nm was observed upon the addition of $Hg^{2+}$. This observation indicates that $Hg^{2+}$ promoted the spiro-opening of rhodamine, which occurred after the interactions between the thiourea moiety and metal ions. No evident emission peak was observed after adding a variety of metal ions, including $Li^+$, $Na^+$, $K^+$, $Ag^+$, $Mg^{2+}$, $Ca^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Ba^{2+}$, $Pb^{2+}$, $Cr^{3+}$, $Al^{3+}$ and $Fe^{3+}$, suggesting that $Hg^{2+}$ can be selectively detected using a fluorescence microscope at an excitation wavelength of 350 nm (excitation wavelength of coumarin group). In addition, When $Hg^{2+}$ (one equivalent) was added into the mixture of probe **1** and $Cu^{2+}$ (molar ratio 1:1), the fluorescence of the solution was significantly enhanced, suggesting that $Hg^{2+}$ also can be detected in the presence of $Cu^{2+}$ (Fig. S3, supplementary data). Under UV light, only an orange solution of **1**/$Hg^{2+}$ was observed. This observation further confirmed that a colorimetric probe for detecting $Hg^{2+}$ was developed. The titration of **1** with $Hg^{2+}$ showed that the emission intensity at 586 nm was gradually enhanced with increasing $Hg^{2+}$ concentration, reaching a maximum value in the presence of 20 μM $Hg^{2+}$ (Fig. 1b). This result shows a good linear detection range from 2 μM to 10 μM. A 1:2 binding mode of **1** and $Hg^{2+}$ was revealed by the Job's plot method (Fig. S4, supplementary data).

**UV–vis spectra of probe 1 with various metal ions.** Evident colour changes of probe **1** appeared after adding $Hg^{2+}$ or $Cu^{2+}$. Hence, UV–vis spectrometry was conducted to monitor the variations. Interestingly, not only $Hg^{2+}$ induced obvious absorption at 562 nm but also

**Scheme 1.** Synthesis route of probes **1** and **2**.



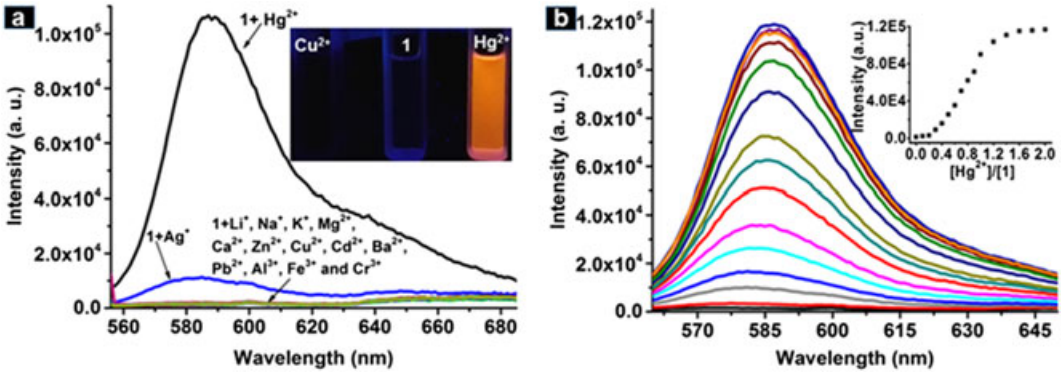



**Figure 1.** (a) Fluorescence spectrum of **1** (10 μM) in the presence of $Hg^{2+}$ and various competitive ions (10 μM). Inset photograph shows the fluorescent detection of $Hg^{2+}$. (b) Fluorescent titration of **1** (10 μM) with $Hg^{2+}$ (from 0 to 20 μμ). Inset shows the fluorescence intensity (at 586 nm) as a function of $Hg^{2+}$ concentration ($\lambda_{ex}$ = 350 nm, slit = 2, EtOH:$H_2O$ = 9:1, v/v). [Color figure can be viewed at wileyonlinelibrary.com]

$Cu^{2+}$ showed evident absorption at 558 nm (Fig. 2a). In the presence of $Cu^{2+}$, the color of the mixture changed from colorless to purple, which was different from the pink color of **1**/$Hg^{2+}$ or the achromatic color of **1** or **1**/other metals. Therefore, probe **1** was suitable for 'naked-eye' detection of $Cu^{2+}$ (purple colour without fluorescence). Moreover, the same phenomenon and results could be observed after treating the probe **1** with $Cu^{2+}$ bearing different anions such as $SO_4^{2-}$, $Cl^-$, $CH_3COO^-$, $NO_3^-$, indicating that anions could not affect the detection of $Cu^{2+}$ (Fig. S5, supplementary data). The titration of **1** with $Cu^{2+}$ showed that the absorption at 558 nm was gradually enhanced with increasing $Cu^{2+}$ concentration, in which a good linear detection range from 2 μM to 10 μM was observed (Fig. 2b). Moreover, the stoichiometry between probe **1** and $Cu^{2+}$ was determined to be 1:2 by the Job's plot method (Fig. S6, supplementary data).

**Fluorescence and UV–vis spectra of probe 2 with various metal ions.** To study the role of the thiohydrazide moiety towards the detection of $Hg^{2+}$ and $Cu^{2+}$, we designed probe **2** by embedding two methylene groups to disrupt the structure of thiohydrazide unit (Scheme 1; Figs. S7–8, supplementary data). The selectivity of probe **2** for $Hg^{2+}$ and $Cu^{2+}$ was evaluated by fluorescence and UV–vis spectra under the same conditions as that of probe **1**. The result showed no change in the fluorescent and UV–vis intensity (Fig. S9, supplementary data), as well as in the solution color. These results verified the crucial role of the thiohydrazide unit.

**Proposed detection mechanisms of probe 1 for $Hg^{2+}$ and $Cu^{2+}$.** Two distinct mechanisms were proposed for selective detection of $Hg^{2+}$ and $Cu^{2+}$. In the presence of $Hg^{2+}$, ring-opening reaction of the rhodamine B moiety is induced because the sulphur atom of thiohydrazide moiety will be deprived, resulting in the formation of a new oxadiazole moiety. In this irreversible process, the energy can effectively transfer from the coumarin moiety to the rhodamine B moiety and consequently gives an orange fluorescence (Fig. 1a). However, in the presence of $Cu^{2+}$, the sulphur atom of thiohydrazide moiety cannot be deprived. In comparison, the $Cu^{2+}$-complex forms between the carbonyl oxygen atom and

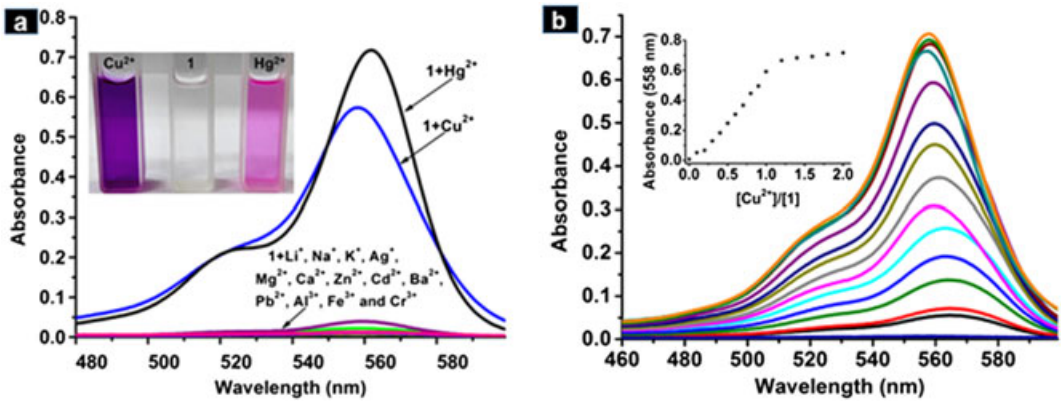



**Figure 2.** (a) Absorption spectrum of **1** (10 μM) in the presence of $Cu^{2+}$ and various competitive ions (10 μM). Inset photograph shows the visual detection of $Cu^{2+}$. (b) Absorbance obtained during the titration of **1** (10 μM) with $Cu^{2+}$ (from 0 to 20 μμ). Inset shows the absorbance (at 558 nm) as a function of $Cu^{2+}$ concentration (EtOH:$H_2O$ = 9:1, v/v). [Color figure can be viewed at wileyonlinelibrary.com]

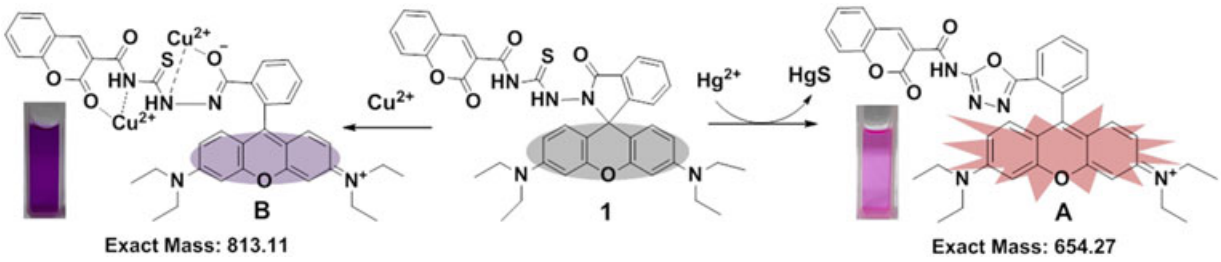



**Figure 3.** Proposed detection mechanisms of probe **1** for $Hg^{2+}$ and $Cu^{2+}$. [Color figure can be viewed at wileyonlinelibrary.com]

thiohydrazide moiety. This condition induces the spiro-opening of rhodamine and consequently provides a purple colour without fluorescence (Fig. 3). Using electrospray ionisation mass spectrometry, oxadiazole-containing compound **A** (found: 654.4) and copper complex $[\mathbf{B}+H]^+$ (found: 814.7) were observed separately, confirming the above hypothesis (Figs. S10–11, supplementary data).

## CONCLUSIONS

In summary, a dual-function FRET-based colorimetric probe **1** was developed for selective detection of $Hg^{2+}$ and $Cu^{2+}$. Moreover, two different detection mechanisms were proposed. First, $Hg^{2+}$ monitoring was conducted by removing the sulphur atom from the thiohydrazide moiety and consequently resulted in the formation of a new oxadiazole moiety. Second, examination of $Cu^{2+}$ was directed by constructing a coordination complex. We expect that our results can offer more applicable and alternative opportunities for the prediction of these heavy metal ions.

## EXPERIMENTAL

**Materials.** All chemicals were purchased from commercial suppliers and used without further purification. All the solvents were of analytic grade. Deionized distilled water was used throughout. All the metal cations were used as their nitrate salts, respectively. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) using Spectrochem GF254 silica gel coated plates. $^1H$ and $^{13}C$ NMR spectra were measured on a JEOL-ECX 500 NMR spectrometer at room temperature using TMS as an internal standard. Mass spectra were recorded by an Agilent LC/MSD Trap VL mass spectrometer. Elemental analysis was recorded on an Elementar Vario-III CHN analyzer. Fluorescence spectra measurements were performed on a Fluoromax-4 spectrofluorimeter (Horiba Trading CO., LTD). UV–vis spectra were performed on a TU-1900 spectrophotometer (Beijing Pgeneral Instrument Co., China). The following abbreviations are used to describe spin multiplicities in $^1H$ NMR spectra: s = singlet, d = doublet, t = triplet, m = multiplet, q = quartet.

**Experimental section.** ***Synthesis of coumarin-3-carboxylic acid ethyl ester (3).*** To a 50 mL flask, 10 g salicylaldehyde (81.89 mmol), 14.43 g diethyl malonate (90.07 mmol), and 0.5 mL piperidine were added and heated at reflux for 10 h. Upon completion, the cooled reaction solution was transferred into a beaker containing 30 mL distilled water under ice bath. The generated white solid was filtered and further evaporated to afford the compound coumarin-3-carboxylic acid ethyl ester (**3**) (10.8 g, yield of 60%). $^1H$ NMR (500 MHz, DMSO-$d_6$, ppm) $\delta$ 8.76 (s, 1H, CH), 7.93 (d, $J$ = 7.5 Hz, 1H, Ar-H), 7.75 (t, $J$ = 17 Hz, 1H, Ar-H), 7.43 (dd, $J$ = 8.6, 17.0 Hz, 2H, Ar-H), 4.31 (dd, $J$ = 7.5, 14.0 Hz, 2H, $CH_2$), 1.33 (t, $J$ = 14.0 Hz, 3H, $CH_3$); $^{13}C$ NMR (125 MHz, DMSO-$d_6$, ppm) $\delta$ 163.1, 156.5, 155.1, 149.2, 135.0, 130.8, 125.4, 118.3, 118.2, 116.7, 61.8, 14.6; *Anal.* Calcd for $C_{12}H_{10}O_4$: C, 66.05; H, 4.62. Found: C, 66.02; H, 4.60. ESI-MS calcd for m/z = 218.1, found $[M+H]^+$ = 219.2.

***Synthesis of coumarin-3-carboxylic acid (4).*** Compound (**3**) (4 g, 18.33 mmol) and NaOH (3 g, 75.01 mmol) were dissolved in 30 mL of ethanol-water solution, and the resulting reaction mixture was refluxed for 15 min. The cooled reaction solution was then transferred into a beaker, acidized with concentrated hydrochloric acid to pH=2, followed by filtration and evaporation to produce the compound coumarin-3-carboxylic acid (**4**) (4.3 g, yield 90%). 196-197 °C. $^1H$ NMR (500 MHz, DMSO-$d_6$, ppm) $\delta$ 13.27 (s, 1H, COOH), 8.76 (s, 1H, CH), 7.93 (d, $J$ = 7.5 Hz, 1H, Ar-H), 7.75 (t, $J$ = 17.0 Hz, 1H, Ar-H), 7.43 (dd, $J$ = 8.6, 17.0 Hz, 2H, Ar-H); $^{13}C$ NMR (125 MHz, DMSO-$d_6$, ppm) $\delta$: 164.5, 157.2, 155.0, 148.9, 134.8, 130.7, 125.3, 118.9, 118.5, 116.6; *Anal.* Calcd for $C_{10}H_6O_4$: C, 63.16; H, 3.18. Found: C, 63.12; H, 3.16. ESI-MS calcd for m/z = 190.0, found $[M+H]^+$ = 191.1.

***Synthesis of rhodamine B hydrazide (7).*** Rhodamine B (4.8 g, 10.02 mmol), 8 mL hydrazine hydrate (80% in water by weight), and 50 mL methanol were added into a 100 mL flask and heated at reflux till the pink color

disappeared [45]. Then, the cooled reaction solution was poured into distilled water and extracted with ethyl acetate. The combined extracts were dried with sodium sulfate anhydrous, filtered, and evaporated, giving the compound rhodamine B hydrazide (**7**) as a light brown solid (3.2 g, yield of 70%). $^1$H NMR (500 MHz, $CDCl_3$, ppm) $\delta$ 7.93 (d, $J$ = 8.6 Hz, 1H, Ar-H),7.44 (t, $J$ = 6.9 Hz, 2H, Ar-H), 7.10 (d, $J$ = 8.6 Hz, 1H, Ar-H), 6.45 (d, $J$ = 8.6 Hz, 2H, xanthene-H), 6.41 (d, $J$ = 2.3 Hz, 2H, xanthene-H), 6.28 (dd, $J$ = 2.3, 8.6 Hz, 2H, xanthene-H), 3.60 (s, 2H, $NH_2$), 3.33 (dd, $J$ = 6.9, 14.0 Hz, 8H, 4N$\underline{CH_2}CH_3$), 1.15 (t, $J$ = 14.0, 12H, 4N$CH_2\underline{CH_3}$); $^{13}$C NMR (125 MHz, $CDCl_3$, ppm) $\delta$ 166.2, 153.9, 151.6, 148.9, 132.6, 130.1, 128.2, 123.9, 123.1, 108.1, 104.6, 98.0, 66.0, 44.5, 12.7; *Anal.* Calcd for $C_{28}H_{32}N_4O_2$: C, 73.66; H, 7.06; N, 12.27. Found: C, 73.63; H, 7.03; N, 12.29. ESI-MS calcd for m/z = 456.3, found $[M+H]^+$ = 457.4.

***Synthesis of rhodamine B ethylenediamine (8).*** Rhodamine B (4.8 g, 10.02 mmol), 3.01 g ethylenediamine (50.10 mmol), and 50 mL ethanol were added into a 100 mL flask and heated at reflux till the pink color disappeared [46,47]. Then, the cooled reaction solution was poured into distilled water and extracted with ethyl acetate. The combined extracts were dried with sodium sulfate anhydrous, filtered, and evaporated to afford the compound rhodamine B ethylenediamine (**8**) as a light brown solid (3.5 g, yield 72%). $^1$H NMR (500 MHz, DMSO-$d_6$, ppm) $\delta$ 7.77 (d, $J$ = 4.3 Hz, 1H, Ph-H), 7.48 (t, $J$ = 8.0 Hz, 2H, Ph-H), 6.99 (d, $J$ = 4.6 Hz, 1H, Ph-H), 6.37 (s, 2H, xanthene-H), 6.35 (d, $J$ = 3.3 Hz, 2H, xanthene-H), 6.32 (d, $J$ = 8.6 Hz, 2H, xanthene-H), 3.32 (dd, $J$ =6.9, 14.0 Hz, 8H, 4$CH_2$), 2.97 (t, $J$ = 15.0 Hz, 2H, N$CH_2$), 2.19 (t, $J$ = 15.0 Hz, 2H, $CH_2NH_2$), 1.08 (t, $J$ = 14.0 Hz, 12H, 4$CH_3$); $^{13}$C NMR (125 MHz, DMSO-$d_6$, ppm) $\delta$ 167.6, 154.1, 153.1, 148.9, 128.8, 128.7, 124.1, 108.6, 105.6, 97.7, 64.5, 40.4, 12.9; *Anal.* Calcd for $C_{30}H_{36}N_4O_2$: C, 74.35; H, 7.49; N, 11.56. Found: C, 74.32; H, 7.47; N, 11.58. ESI-MS calcd for m/z = 484.3, found $[M+H]^+$ = 485.4.

***Synthesis of probe 1.*** Compound (**4**) (280 mg, 1.5 mmol) and thionyl chloride (5 mL, 70 mmol) were successively added into a flask, and refluxed for 7 h. After that, the excess thionyl chloride was moved out by distillation, leading to the crude coumarin-3-carboxylic acyl chloride (**5**). To the solution of potassium rhodanide (0.3 g, 3 mmol) dissolving in 8 mL distilled acetonitrile, compound (**5**) was added, and kept stirring for 0.5 h at room temperature. Upon completion, the resulting solution was filtered to give an orange filtrate (coumarin-3-carboxylic isothiocyanate (**6**) filtrate). Without any purification, compound (**7**) (0.34 g, 0.76 mmol) was directly added into the orange filtrate containing compound (**6**), and stirred for 4 h. After removal of the solvent, the residue was purified by column chromatography with petroleum ether/ethyl acetate as the eluent to afford **1** (0.1 g, yield: 20%). $^1$H NMR (500 MHz, $CDCl_3$, ppm) $\delta$ 11.63 (s, 1H, CONH), 11.45 (s, 1H, NHNCO), 8.79 (s, 1H, CH), 8.01 (d, $J$ = 6.9 Hz, 1H, Ph-H), 7.71 (t, $J$ = 15.0 Hz, 1H, Ph-H), 7.64 (d, $J$ = 5.5 Hz, 1H, Ph-H), 7.52-7.47 (m, 2H, Ph-H), 7.39(dd, $J$ = 8.0, 13.0 Hz, 2H, Ph-H), 7.25 (d, $J$ = 6.9 Hz, 1H, Ph-H), 7.15 (d, $J$ = 7.5 Hz, 1H, N$(CH_2CH_3)_2$Ph-H), 6.77(s, 1H, N$(CH_2CH_3)_2$Ph-H), 6.37-6.33(m, 4H, N$(CH_2CH_3)_2$Ph-H), 3.35 (d, $J$ = 6.9 Hz, 8H, 4$CH_2$), 1.17 (t, $J$ = 14.0 Hz, 12H, 4$CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$, ppm) $\delta$ 180.4, 164.1, 160.3, 155.0, 153.9, 151.8, 151.1, 135.7, 133.4, 130.3, 128.8, 128.4, 125.8, 124.1, 123.9, 118.2, 117.1, 116.4, 108.1, 103.8, 97.7, 66.8, 44.5, 29.6, 12.7; *Anal.* Calcd for $C_{39}H_{37}N_5O_5S$: C, 68.10; H, 5.42; N, 10.18. Found: C, 68.12; H, 5.45; N, 10.20. ESI-MS calcd for m/z = 687.3, found $[M+H]^+$ = 688.4.

***Synthesis of probe 2.*** To the coumarin-3-carboxylic isothiocyanate (**6**) filtrate, compound (**8**) (0.37 g, 0.76 mmol) was added, and the resulting solution was kept stirring for 4 h. After removal of the solvent, the residue was purified by column chromatography with petroleum ether/ethyl acetate as the eluent to afford **2** (0.12 g, yield: 23%). $^1$H NMR (500 MHz, $CDCl_3$, ppm) $\delta$ 8.78 (s, 1H, CH), 8.66 (s, 1H, CONH), 7.91 (d, $J$ = 8.6 Hz, 1H, Ph-H), 7.63 (t, $J$ = 17.0 Hz, 2H, Ph-H), 7.43 (dd, $J$ = 3.5, 5.8 Hz, 2H, Ph-H), 7.35 (dd, $J$ = 8.0 Hz, 13.0 Hz, 2H, Ph-H), 7.07 (d, $J$ = 8.0 Hz, 1H, Ph-H), 6.49 (d, $J$ = 9.2 Hz, 2H, N$(CH_2CH_3)_2$Ph-H), 6.36 (d, $J$ = 2.3 Hz, 2H, N$(CH_2CH_3)_2$Ph-H), 6.28 (d, $J$ = 2.3 Hz, 1H, N$(CH_2CH_3)_2$Ph-H), 3.43 (t, $J$ = 12.0 Hz, 2H, $CH_2$-NHCS), 3.29-3.21 (m, 10H, 5$CH_2$), 2.04 (s, 1H, $CH_2$N-H), 1.12 (t, $J$ = 14.0 Hz, 12H, 4$CH_3$); $^{13}$C NMR (125MHz, $CDCl_3$, ppm) $\delta$ 168.9, 161.5, 161.1, 154.5, 153.8, 153.3, 133.7, 132.5, 131.1, 129.8, 128.9, 128.1, 125.1, 123.9, 123.0, 118.8, 116.6, 108.3, 105.6, 97.9, 65.0, 44.4, 39.6, 38.5, 12.7; *Anal.* Calcd for $C_{41}H_{41}N_5O_5S$: C, 68.79; H, 5.77; N, 9.78. Found: C, 68.81; H, 5.79; N, 9.79. ESI-MS calcd for m/z = 715.3, found $[M+H]^+$ = 716.4.

***Procedures for ion detection.*** The stock solutions of probe **1** and **2** ($1.0 \times 10^{-3}$ M) were prepared in ethanol. Metal ions stock solutions ($1.0 \times 10^{-3}$ M) of the nitrate salts of $Li^+$, $Na^+$, $K^+$, $Ag^+$, $Mg^{2+}$, $Ca^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Ba^{2+}$, $Hg^{2+}$, $Pb^{2+}$, $Cr^{3+}$, $Al^{3+}$, and $Fe^{3+}$ ions were prepared in distilled water. The procedures to prepare samples for fluorescence and UV-vis spectra measurement were as follows: 100 μL of 1 mM stock solution of probe was added into a volumetric flask (10 mL), then appropriate amount of analytes was added into the volumetric flask and the mixture was diluted to 10 mL with corresponding solvent. Spectral data were usually recorded at 10 min after the addition of corresponding solvent.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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