

## Supplementary Information

# A Bright and Specific Turn-on Fluorescence Sensor for Imaging Copper(II) in Living Cells

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### Contents

- 1. Experimental Section**
- 2. Figure S1**  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra of compound **CB1**.
- 3. Figure S2** UV/vis absorbance and fluorescence response of **CB1** (20  $\mu\text{M}$ ) upon the addition of  $\text{Cu}(\text{ClO}_4)_2$ .
- 4. Figure S3** Differential potential voltammetry (DPV) of **CB1** in acetonitrile solution and **CB1** in the presence of  $\text{Cu}^{2+}$ .
- 5. Figure S4** IR spectra of compound **CB1** and  $\text{Cu}^{2+}$ -binding complexation species.
- 6. Figure S5** Job's plot of compound **CB1** and  $\text{Cu}(\text{ClO}_4)_2$ ; The fluorescence of **CB1**- $\text{Cu}^{2+}$  species upon the addition of  $\text{Na}_2\text{S}$  solution.
- 7. Figure S6** ESI-MS spectra of **CB1** in the presence of  $\text{Cu}^{2+}$ .
- 8. Figure S7** Fluorescence responses of **CB1** to  $\text{Cu}^+$  and the subsequent addition of  $\text{Cu}^{2+}$ ; The pH-dependent measurements of **CB1** and **CB1** plus  $\text{Cu}^{2+}$ .
- 9. Figure S8** Optimized geometry of **CB1**- $\text{Cu}^{2+}$ .
- 10. Figure S9** Frontier orbitals of **CB1** and **CB1**- $\text{Cu}^{2+}$  complexation species.

## 1. Experimental Section

### 1.1. Instruments and reagents

The elemental analyses of C, H and N were performed on a Vario EL III elemental analyzer.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were measured on a Varian INOVA 400M spectrometer. API mass spectra were recorded on HP1100LC/MSD spectrometer. ESI mass spectra were carried out on a HPLC-Q-ToF MS spectrometer using methanol as mobile phase. Uv-vis spectra were measured on a HP 8453 spectrometer. The solution fluorescent spectra were measured on Edinburgh FS 920. IR spectra were recorded using KBr pellets on a Vector 22 Bruker spectrophotometer in the  $4000\text{-}400\text{cm}^{-1}$  regions. 4-Diethylaminosalicylaldehyde and all cationic compounds such as perchlorate of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Hg}^{2+}$  and  $\text{Ag}^+$  were purchased from Acros and used as received.  $\text{CH}_3\text{CN}$  for spectra detection was HPLC reagent without fluorescent impurity and  $\text{H}_2\text{O}$  was deionized water. All the other solvents and reagents were of analytic grade.

### 1.2. General procedures of spectra detection

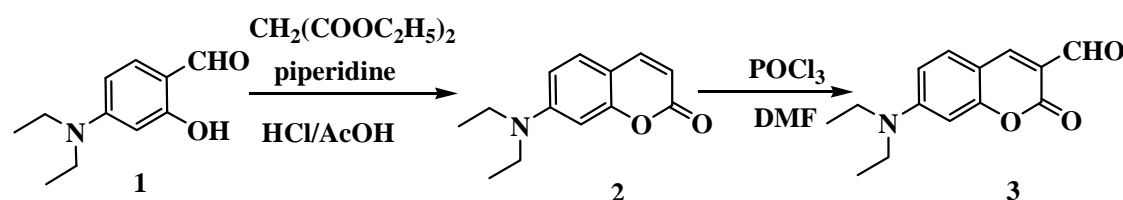
Stock solutions ( $2.0 \times 10^{-5}\text{ M}$ ) of **CB1** were prepared in  $\text{CH}_3\text{CN}:\text{H}_2\text{O}$  (1:1, v/v) solution. The cationic solutions are all in  $\text{H}_2\text{O}$  solutions with a concentration of  $2.0 \times 10^{-2}\text{ M}$  for the spectra analysis. Each time a 2 mL solution of **CB1** was filled in a quartz cell of 1 cm optical path length, and different stock solutions of cations were added into the quartz cell gradually by using a micropipette. The volume of cationic stock solution added was less than 100  $\mu\text{L}$  with the purpose of keeping the total volume of testing solution without obvious change. Excitation wavelength was 468 nm.

### 1.3. Cell Culture

The HeLa cells were grown in H-DMEM (Dulbecco's Modified Eagle's Medium, High Glucose) supplemented with 10 % FBS (Fetal Bovine Serum) in an atmosphere of 5 %  $\text{CO}_2$ , 95% air at 37 °C. Cells ( $5 \times 10^8/\text{L}$ ) were plated on 18 mm glass cover slips and allowed to adhere for 24 hours.

### 1.4. Procedures for synthesis

Synthesis of 7-diethylaminocoumarin (**2**)<sup>S1</sup>.



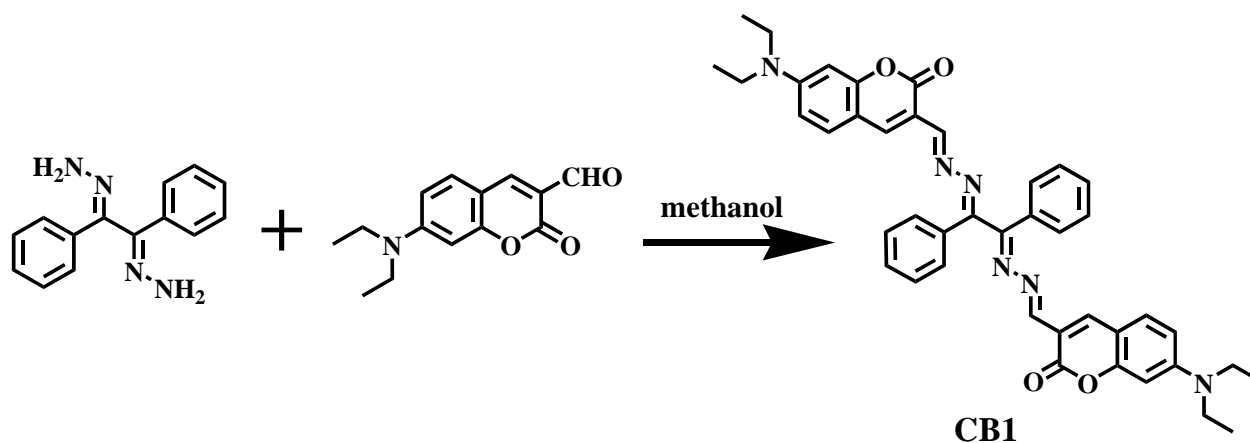
4-Diethylaminosalicylaldehyde (**1**) (1.93 g, 10 mmol), diethylmalonate (3.2 g, 20 mmol) and piperidine (1 mL) were combined in an absolute ethanol solution (30 mL) and stirred for 6 hours under a refluxing condition. After ethanol was removed under reduced pressure, glacial acetic acid (20 mL) and concentrated HCl (20 mL) were added to hydrolyze the reaction with stirring for 6 hours. The solution

was cooled to room temperature and poured into 100 mL ice water. NaOH solution (40%) was added dropwise to modulate pH of the solution to ~5, and a pale precipitate formed immediately. After stirring for 30 minutes, the mixture was filtered, washed with water, dried, then recrystallized with toluene giving compound 2 (1.74 g, 8.0 mmol) in a yield of 80.1%.  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 7.55 (d, 1H,  $J = 9.5$  Hz), 7.23 (d, 1H,  $J = 8.8$  Hz), 6.59 (d, 1H,  $J = 8.8$  Hz), 6.51 (s, 1H), 6.06 (d, 1H,  $J = 9.2$  Hz), 3.42 (q, 4H,  $J = 7.1$  Hz), 1.21 (t, 6H,  $J = 7.1$  Hz).  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 12.4, 45.0, 97.9, 108.9, 109.5, 128.7, 143.6, 150.5, 156.7, 161.0, 162.1.

### Synthesis of 7-diethylaminocoumarin-3-aldehyde (3)<sup>S1</sup>.

Under nitrogen, fresh distilled DMF (2 mL) was added dropwise to  $\text{POCl}_3$  (2 mL) at 20-50°C and stirred for 30 minutes to yield a red solution. Then a portion of 2 (1.50 g, 6.91 mmol, dissolved in 10 mL DMF) was added dropwise to the above solution and yield a scarlet suspension. The mixture was stirred at 60°C for 12 hours and then poured into 100 mL of ice water. NaOH solution (20%) was added to adjust the pH of the mixture to obtain a large amount of precipitate. The crude product was filtered, washed with water, dried and recrystallized with absolute ethanol giving compound 3 (1.20 g, 4.89 mmol) in a yield of 70.8%.  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 10.13 (s, 1H), 8.26 (s, 1H), 7.43 (d, 1H,  $J = 9.0$  Hz), 6.67 (d, 1H,  $J = 9.0$  Hz), 6.50 (s, 1H), 3.49 (q, 4H,  $J = 7.2$  Hz), 1.25 (t, 6H,  $J = 7.2$  Hz).  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 12.4, 45.3, 87.1, 97.2, 108.3, 110.2, 132.5, 145.3, 153.4, 158.9, 161.8, 187.9.

### Synthesis of CB1

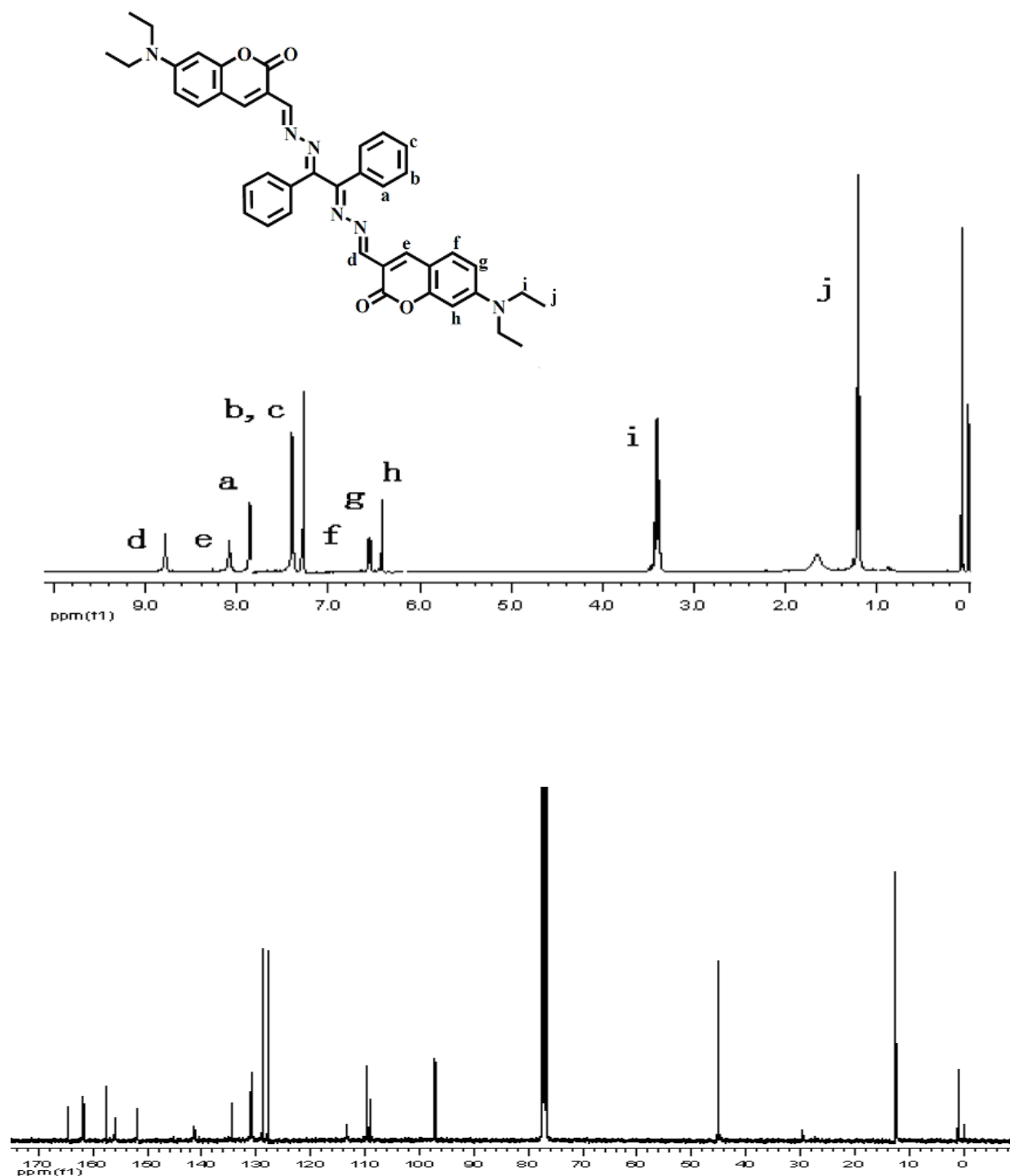


A methanol solution of 7-diethylaminocoumarin-3-aldehyde (3) (2.0 mmol, 0.49 g) and benzildihydrazone (0.24 g, 1 mmol) were mixed and refluxed for 4 hr. Red precipitates formed were filtered, washed with methanol and dried under vacuum. Yield: 73%. Anal Calc. for  $\text{C}_{42}\text{H}_{40}\text{N}_6\text{O}_4$ : H 5.82, C 72.81, N 12.13%. Found: H 5.93, C 72.70, N 12.01%.  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 8.77 (s, 2 H), 8.08 (s, 2 H), 7.86 (d, 4 H,  $J = 8.0$  Hz), 7.39 (m, 6 H), 7.27 (d, 2 H,  $J = 6.8$  Hz), 6.55 (d, 2 H,  $J = 6.8$  Hz), 6.42 (s, 2H), 3.41 (q, 8 H,  $J = 7.0$  Hz), 1.20 (t, 12 H,  $J = 7.0$  Hz).  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 164.5, 161.7, 157.5, 155.9, 151.8, 141.3, 134.4, 131.0, 130.6, 128.7, 127.8, 113.3, 109.5, 108.9, 97.1, 45.0, 12.5.

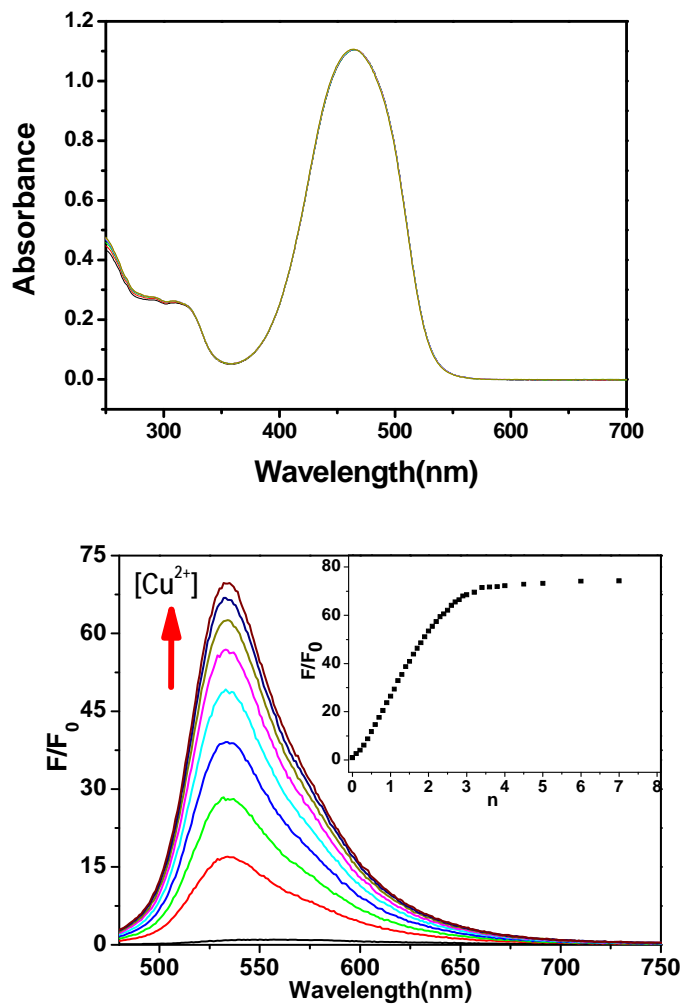
## Preparation of Cu-binding species of CB1 for IR spectra characterization

An acetonitrile solution of **CB1** (70 mg, 0.1 mmol) and  $\text{Cu}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$  (55 mg, 0.15 mmol) were mixed and stirred for about 2 hr, after the solution were removed by rotation evaporation, the residue solid was obtained and dried in vacuum.

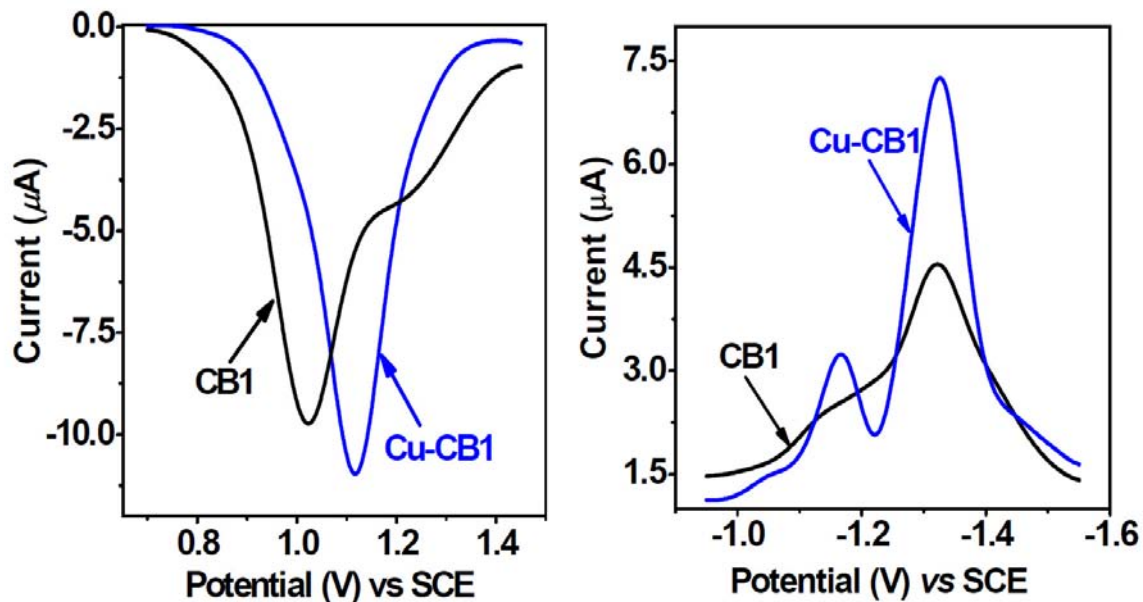
### 2. Figure S1 $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of compound **CB1**.



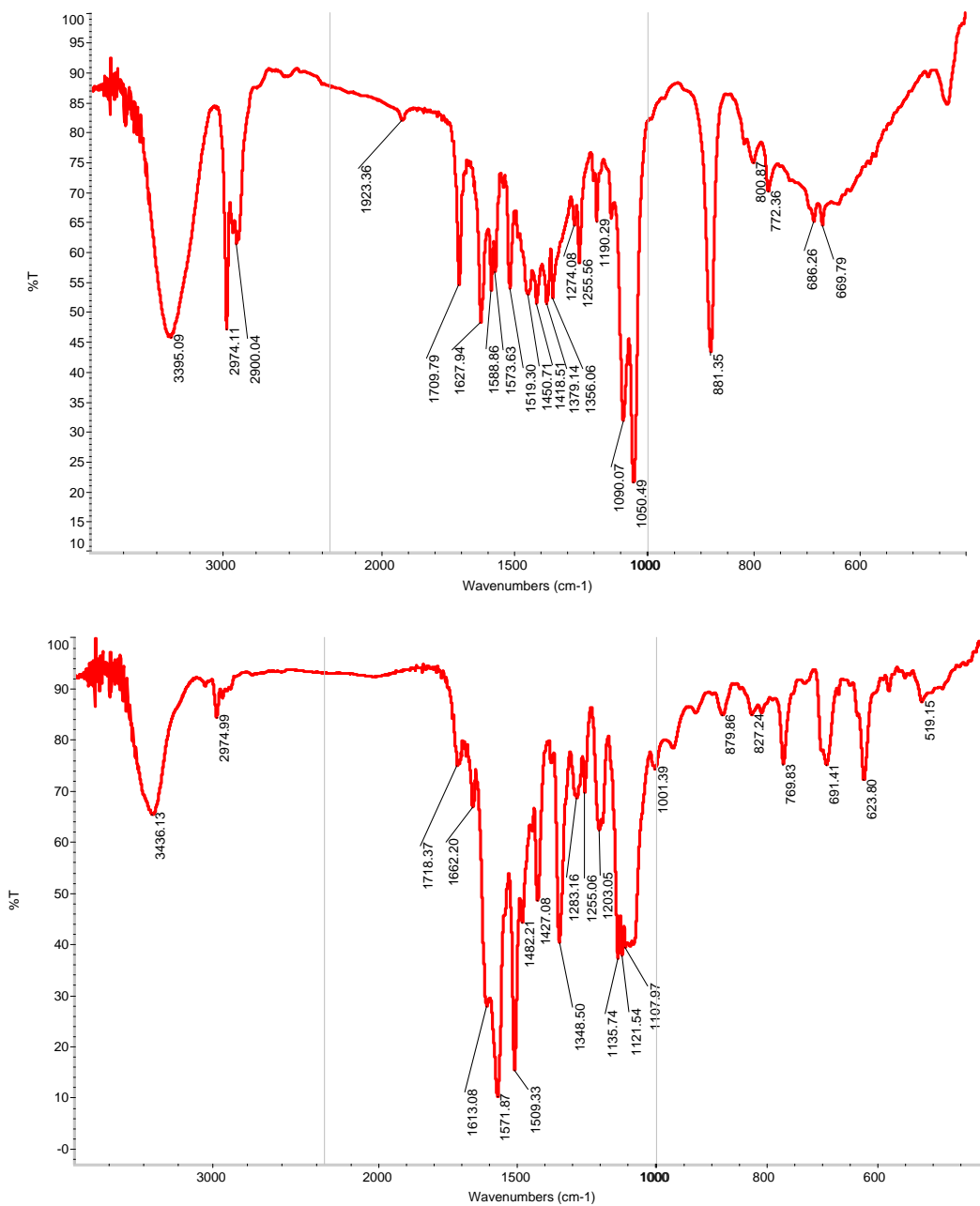
**3. Figure S2** Top: UV/vis absorbance response of **CB1** (20  $\mu\text{M}$ ) when added 0, 2, 4, 6, 8, 10, 15, 20 equiv.  $\text{Cu}(\text{ClO}_4)_2$  in  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (1:1, v/v). Bottom: Fluorescence response of compound **CB1** (20  $\mu\text{M}$ ) in  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (1:1, v/v) upon the addition of increasing concentrations of  $\text{Cu}^{2+}$ . Inset: Titration curve of fluorescence of compound **CB1** vs the equiv. of  $\text{Cu}^{2+}$ .



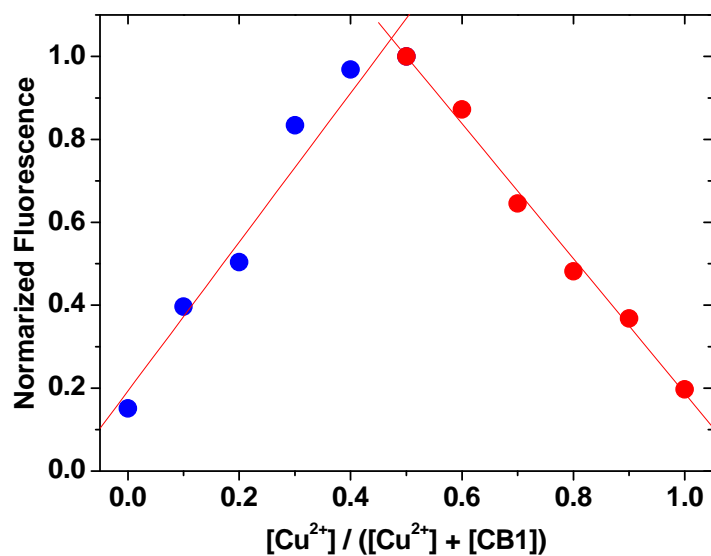
**4. Figure S3** Differential potential voltammetry (DPV) of **CB1** (0.5 mM) in acetonitrile solution and **CB1** (0.5 mM) in the presence of  $\text{Cu}^{2+}$  (0.5 mM) (named as **Cu-CB1**). 0.1 M  $n\text{-Bu}_4\text{NPF}_6$  as electrolyte. Scan rate is  $25 \text{ mV}\cdot\text{s}^{-1}$  and  $-25 \text{ mV}\cdot\text{s}^{-1}$  for the oxidization and reduction process, respectively.



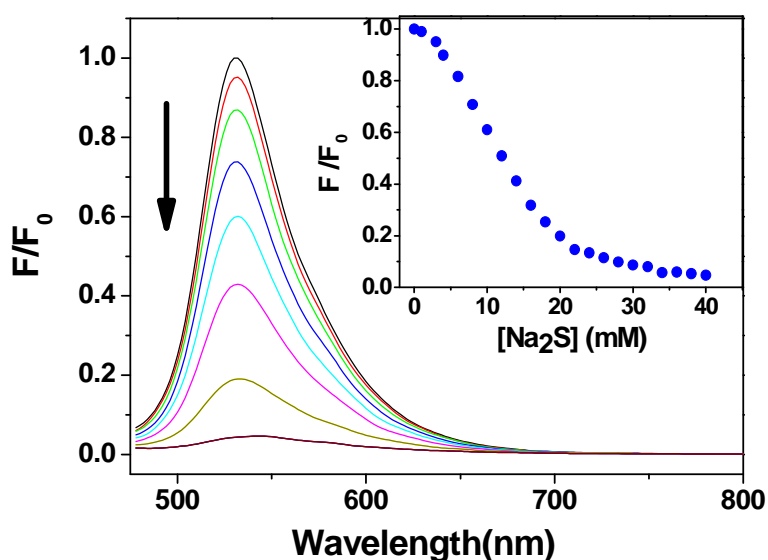
**5. Figure S4** IR spectra of compound **CB1** (top picture) and Cu<sup>2+</sup>-binding complexation species (bottom one). The stretching band at the stretching band at 1718 cm<sup>-1</sup> corresponding to the carbonyl group of coumarin was shifted to 1707 cm<sup>-1</sup> of the Cu<sup>2+</sup>-binding complexation species.



**6. Figure S5a** Binding analysis using the method of Job's plot. Fluorescence intensity at 534 nm in  $\text{CH}_3\text{CN}:\text{H}_2\text{O}$  (1:1, v/v) of compound **CB1** varied upon addition of  $\text{Cu}(\text{ClO}_4)_2$  with an excitation at 468 nm. The total concentration was 40  $\mu\text{M}$ .

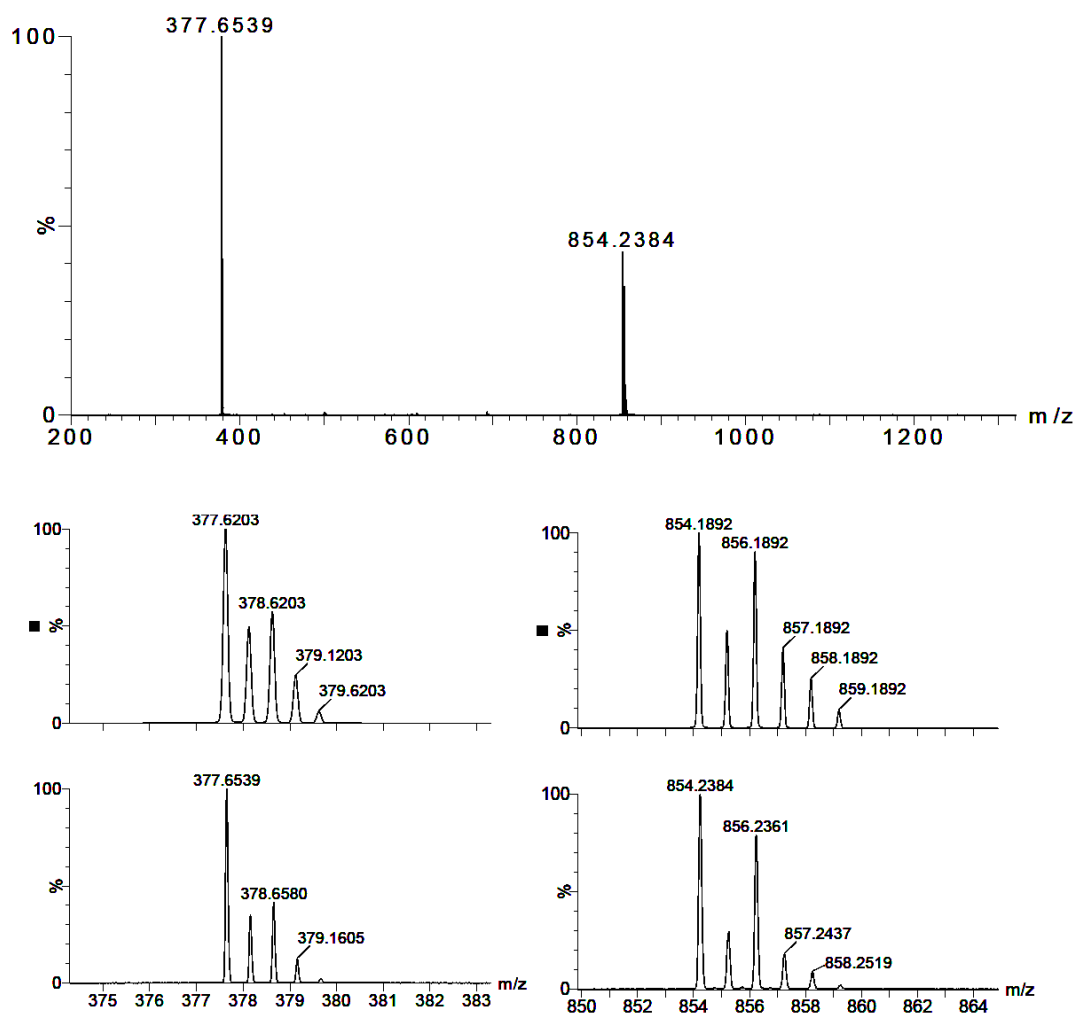


**Figure S5b** Fluorescence of **CB1**– $\text{Cu}^{2+}$  (20  $\mu\text{M}$ , 4 equiv.  $\text{Cu}^{2+}$ ) systems, showing the diminishing of intensity upon the addition of  $\text{Na}_2\text{S}$  solution. The insert: fluorescence of **CB1**– $\text{Cu}^{2+}$  (20  $\mu\text{M}$ ) at 534 nm as a function of  $\text{Na}_2\text{S}$  concentration in  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (1:1, v/v) solution.

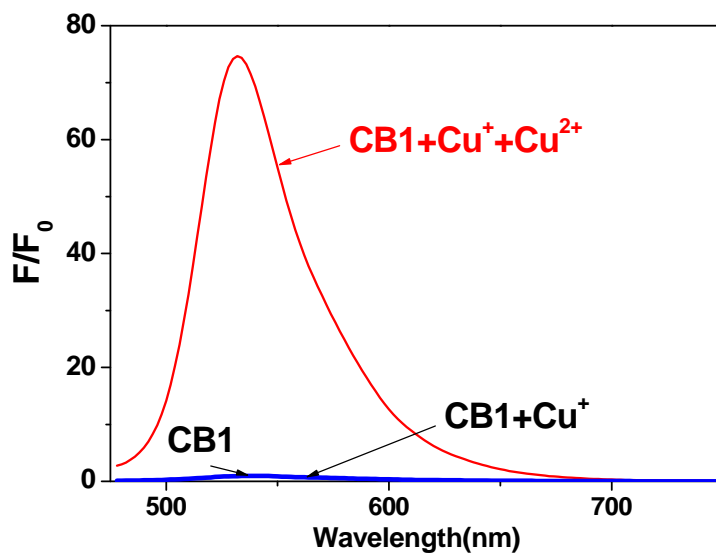




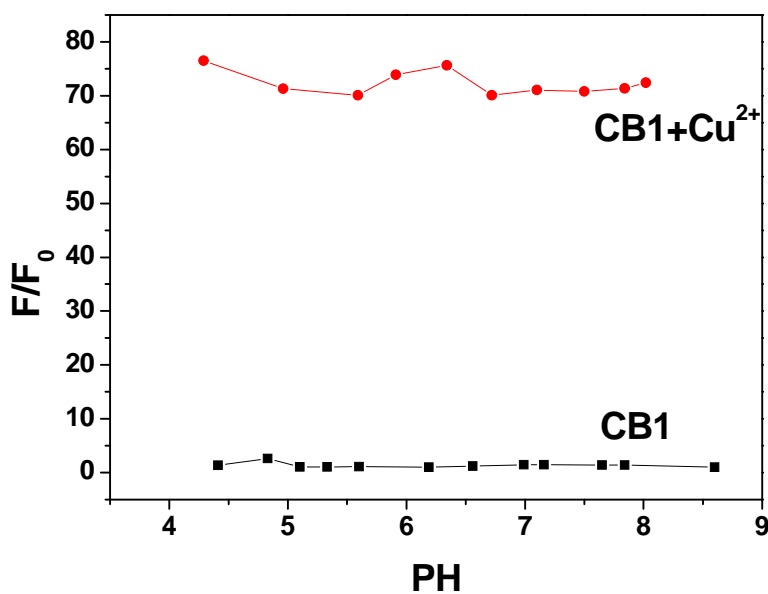
7. **Figure S6** ESI-MS spectra of **CB1** in the presence of  $\text{Cu}^{2+}$  exhibited two peaks at  $m/z = 377.65$  and  $854.24$ , assignable to  $[\text{Cu-CB1}]^{2+}$  and  $[(\text{Cu-CB1})(\text{ClO}_4)]^+$  complexation species, respectively. The high resolved spectra exhibit the measured (bottom pictures) and simulated (top ones) isotopic patterns at  $m/z$  377.6 and  $m/z$  854.2, respectively.



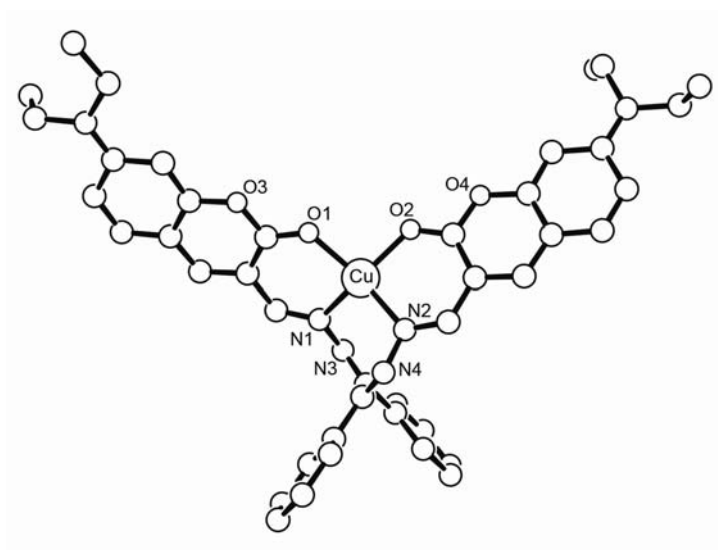
**8. Figure S7a** Fluorescence responses (534 nm) of **CB1** (20  $\mu\text{M}$ ) to  $\text{Cu}^+$  (80  $\mu\text{M}$  as perchlorates) and the subsequent addition of 80  $\mu\text{M}$  of  $\text{Cu}^{2+}$  to the  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (1:1, v/v) aqueous solution with excitation wavelength at 468 nm.



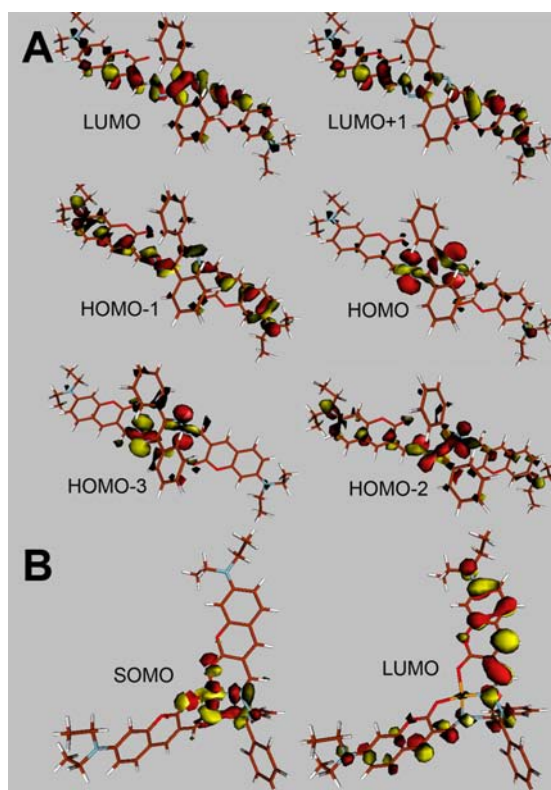
**Figure S7b** The pH-dependent fluorescence response of **CB1** (20  $\mu\text{M}$ ) and **CB1** plus  $\text{Cu}^{2+}$  (80  $\mu\text{M}$ ) in  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$  (1:1, v/v).



**9. Figure S8** Optimized geometry of **CB1**-Cu<sup>2+</sup>. Selected bond lengths (Å) and bond angles(°): Cu-N1 2.095, Cu-O1 2.082, N1-Cu-N2 95.9, O1-Cu-O2 113.2, N1-Cu-O1 88.1.



10. **Figure S9** Frontier orbitals of **CB1** (A) and **CB1-Cu<sup>2+</sup>** complexation species (B) based on their optimized geometries.



**Computational Details** All calculations were carried out using Density Functional Theory as implemented in the Jaguar 6.0 suite<sup>S3</sup> of ab initio quantum chemistry programs. Geometry optimizations were performed with the B3LYP<sup>S4</sup> functional and the 6-31G\*\* basis set with no symmetry restrictions. Transition metals was represented using the Los Alamos LACVP basis.<sup>S5</sup> Time-dependent DFT calculations using the BLYP<sup>[S4a,S4c]</sup> functional were used to compute electronic excitation energies and compared to experiments. These calculations are carried out using the Amsterdam Density Functional (ADF 2008) package,<sup>S6</sup> utilizing the triple-basis set (ZORA/TZP) and the frozen-core approximation. The models used in this study consist of ~90 atoms, which represent the non-truncated substrates that were also used in the experimental work. These calculations challenge the current state of computational capabilities, whereas the numerical efficiency of the Jaguar program allows us to accomplish this task in a bearable time frame.

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