Supporting Information

A highly sensitive and selective turn-on fluorescent chemodosimeter for Cu^{2+} based on BODIPY and its application in bioimaging

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General Information

All chemical reagents were commercially available and of analytical grade. The stock solutions of metal ions were prepared from LiCl, NaCl, KCl, $MgSO_4$, $CaCl_2$, $Ba(NO_3)_2$, $Al(NO_3)_3•9H_2O$, $Pb(NO_3)_2$,$Cr_2(SO_4)_3$, $MnCl_2•4H_2O$, $FeCl_3•6H_2O$, $Co(NO_3)_2•6H_2O$, $Ni(NO_3)_2•6H_2O$, CuCl, $CuCl_2$, $AgNO_3$, $Zn(OAc)_2•2H_2O$, $CdCl_2•H_2O$, $HgCl_2$with doubly distilled water. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were measured on a Bruker Avance III spectrometer. Electrospray mass spectra (ESI-MS) were recorded on a Thermofisher LCQ. Fluorescence spectra were determined on a Perkin Elmer LS-55. UV-vis spectra were measured on a Shimadzu UV-3600. The pH values of sample solutions were monitored by a PHS-3 system. Stock solutions for analysis were prepared (1×10^{-5} mol·L^{-1} for compound **1** in CH_3CN:H_2O=1:1, v/v) immediately before the experiments.

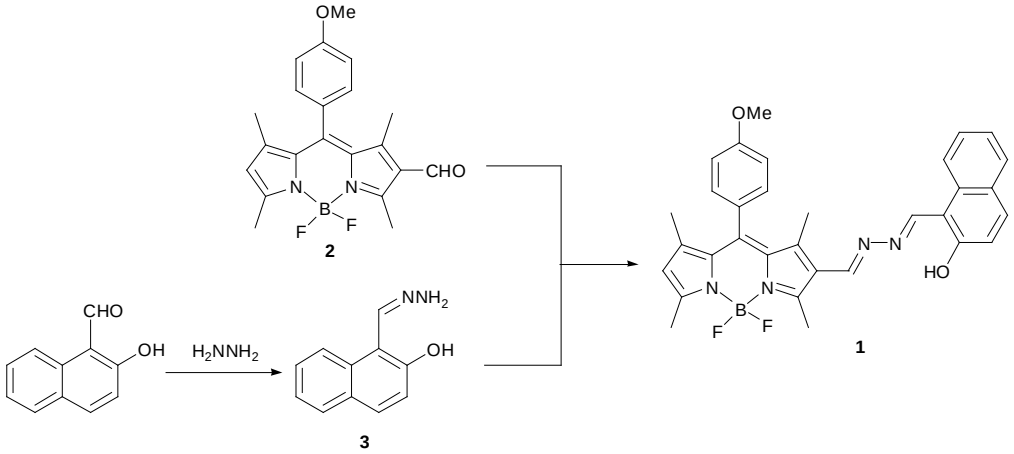
General Procedure for UV-Vis and Fluorescence Studies.

Stock solutions of metal ions were prepared (1×10^{-2} mol/L) in buffer solution pH=6.8. A stock solution of compound **1** (1×10^{-5} mol·L^{-1}) was prepared in CH_3CN:H_2O (1:1, v/v) immediately before the experiments. In experiments, each time a 3 mL solution of compound **1** (10 μM) was filled in a quartz optical cell of 1 cm optical path length, and then the Cu^{2+} stock solution was added. For fluorescence measurements, excitation was provided at 460 nm, and emission was collected from 470 nm to 650 nm. Results are shown as follows.

Cell Culture and Confocal Imaging

HeLa cells was maintained following protocols provided by the American type Tissue Culture Collection. Cells were seeded at a density of 1.0×10^6 cells·mL^{-1}in RPMI-1640 Medium supplemented with 10% fetal bovine serum (FBS), $NaHCO_3$ (2 g/L), and 1% antibiotics (penicillin/streptomycin, 100 U/ml). Cultures were maintained at 37 °C under a humidified atmosphere containing 5% CO_2. Confocal fluorescence imaging studies were performed on aLSM710 confocal laser-scanning microscope (Carl Zeiss Co., Ltd.).The working solution was prepared from a 2mMacetonitrile solution of compound **1**and then it was diluted with PBS to a final concentration of 10 μM. Prior to imaging, the medium was removed and cell imaging was carried out after washing cells with PBS for three times.

Synthesis and Characterization of Fluorescent Probe 1



Synthetic pathway of compound **1**

Formyl-BODIPY 2 was synthesized according to known procedure.[S1]

Synthesis of compound 3

A three-necked flask was charged with 35 ml (0.6 mol) of hydrazine hydrate and heated to 120℃. A solution of 2-hydroxy-1-naphthaldehyde (3.51 g, 20 mmol) in 15 ml of chloroform was added over a period of 4 hours. After cooling to room temperature the aqueous layer was washed with dichloromethane. The combined organic layers were dried over anhydrous magnesium sulfate, filtered, concentrated under reduced pressure and finally washed with a little ethyl acetate. Then compound 3 was obtained as a yellow solid without further purification. ESI-MS: m/z = 187.1 ($M+H^+$, 100%).

Synthesis of compound 1

In a three-necked flask, compound **2** (382 mg, 1 mmol) was dissolved in 20 ml of ethanol under reflux. Then, compound **3** (372 mg, 2 mmol) in 20 ml of ethanol was added into the mixture with continuous stirring. Finally the obtained mixture was monitored by TLC. When the compound **2** was no longer continue to disappear, the mixture was filtered while hot. Then, the obtained solid was dissolved in dichloromethane, dried over anhydrous magnesium sulfate, filtered and concentrated. Then compound **1** was obtained by column chromatography using petroleum ether and ethyl acetate as the eluent (190mg, 35%yield). Mp: 259-261℃.^{1}H NMR (500 MHz, $CDCl_3$) ppm, δ: 9.66 (s, 1H, CH=N), 8.65 (s, 1H, CH=N), 8.16 (d, J = 8.5 Hz, 1H, Ar-H), 7.87 (d, J = 8.9 Hz, 1H, Ar-H), 7.79 (d, J = 7.6 Hz, 1H, Ar-H), 7.57 (t, J = 7.8 Hz, 1H, Ar-H), 7.39 (t,J = 7.4 Hz,1H, Ar-H), 7.32 (d, J = 9.0 Hz, 1H, Ar-H), 7.21 (d, J = 8.6 Hz, 2H, Ar-H), 7.07 (d, J = 8.6 Hz, 2H, Ar-H), 6.11 (s, 1H, Pyrrol-H), 3.91 (s, 3H, OCH_3), 2.93 (s, 3H, CH_3), 2.62 (s,3H, CH_3), 1.71 (s, 3H, CH_3), 1.49 (s, 3H, CH_3).^{13}CNMR (126 MHz, $CDCl_3$) ppm, δ: 161.10, 160.69, 159.94, 159.11, 155.87, 145.91,142.95, 141.70, 134.20,133.64,132.91,131.36, 129.45, 129.28, 128.38, 127.88, 126.83, 123.77, 123.07, 120.41, 119.43, 115.01, 55.59, 29.91, 15.12, 14.60, 12.79. ESI-MS: m/z = 551.2 ($M+H^+$).

References:

S1: (a) L. Jiao, C. Yu, J. Li, Z. Wang, M. Wu and E. Hao, *J. Org. Chem*., 2009, **74**, 7525.
(b) J. Ye, W. Ye, C. Xiao, Y. Chen, G. Wang, and W. Zhang, *Chin. J. Org. Chem*., 2012, **32**, 1503.

The Effect of Organic Solvent to the reactivity of compound 1 with Cu^{2+}

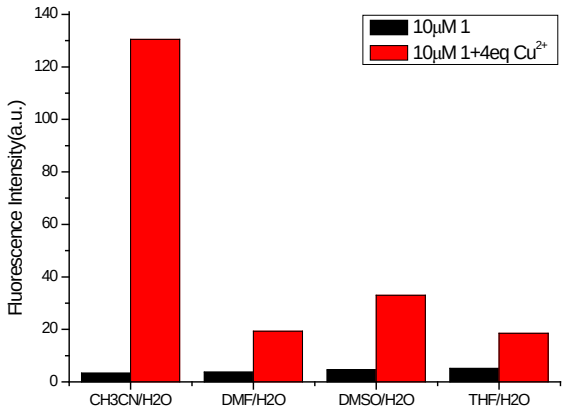


Figure S1. Effect of solvent to the reaction of compound **1**(10 μM) with Cu^{2+} (40 μM, 4 equiv) in H_2O/solvent (1:1, v/v) medium for 10 minutes (λ_{ex}: 460 nm).

Absorption and Emission Spectra of compound 1 and in the presence of Cu^{2+}

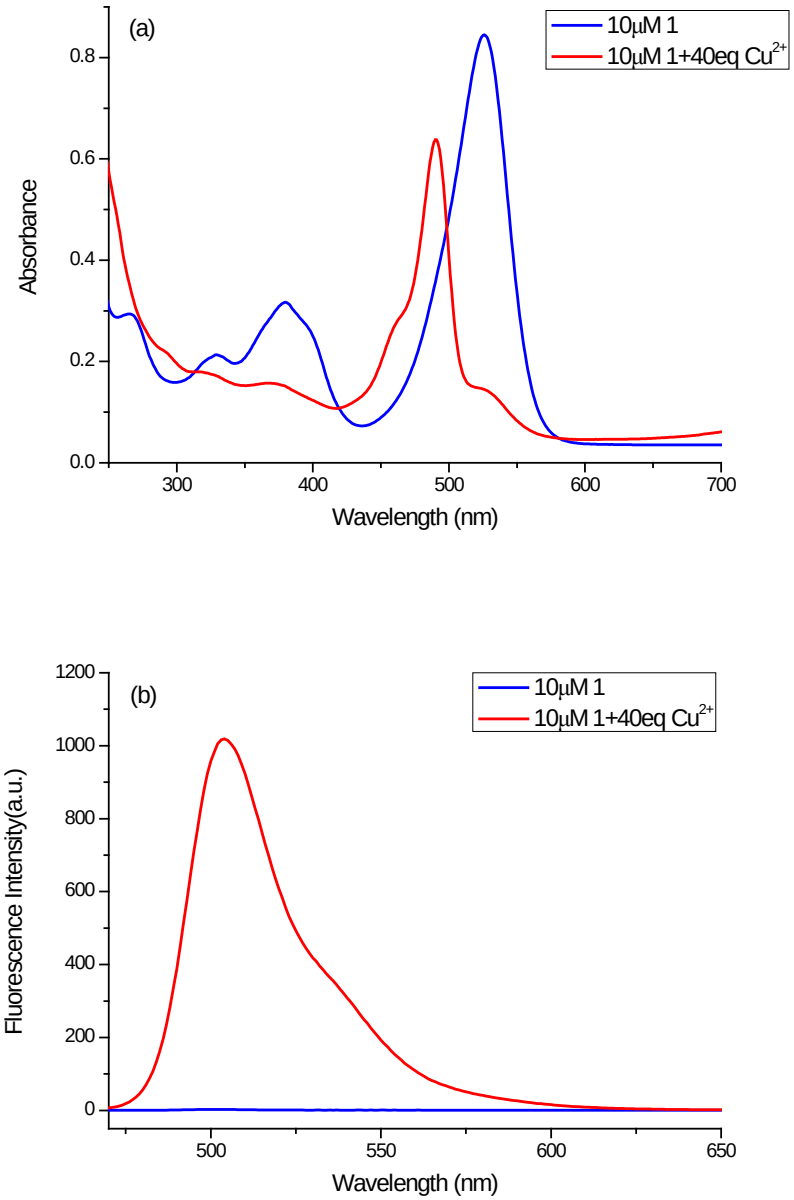


Figure S2. (a) Absorbance and **(b)** fluorescence spectra of compound **1**(10 μM) and Cu^{2+} ions (40 equiv) in H_2O/CH_3CN (1:1, v/v) medium (λ_{ex}: 460 nm).

The Absorption and Fluorescence Responses of 1 to Cu^{2+} and other metal ions

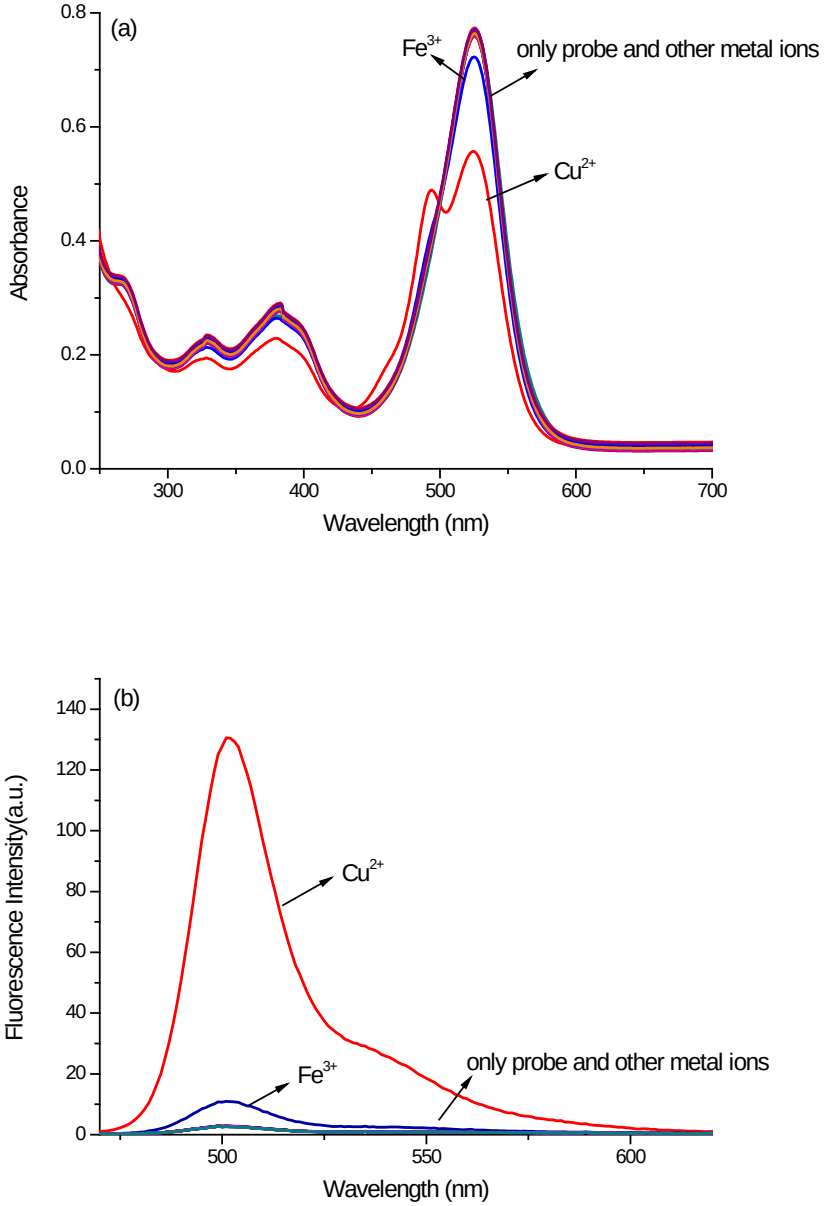


Figure S3. (a) Absorption spectra of compound **1** (10 μM) with different metal ions (40 μM, 4 equiv) in H_2O/CH_3CN (1:1, v/v) medium for 30 minutes and **(b)** fluorescence spectra of compound **1** (10 μM) with different metal ions (40 μM, 4e quiv) in H_2O/CH_3CN (1:1, v/v) medium for 10 minutes (λ_{ex}: 460nm).

Absorption and Fluorescence Titration Spectra of compound 1

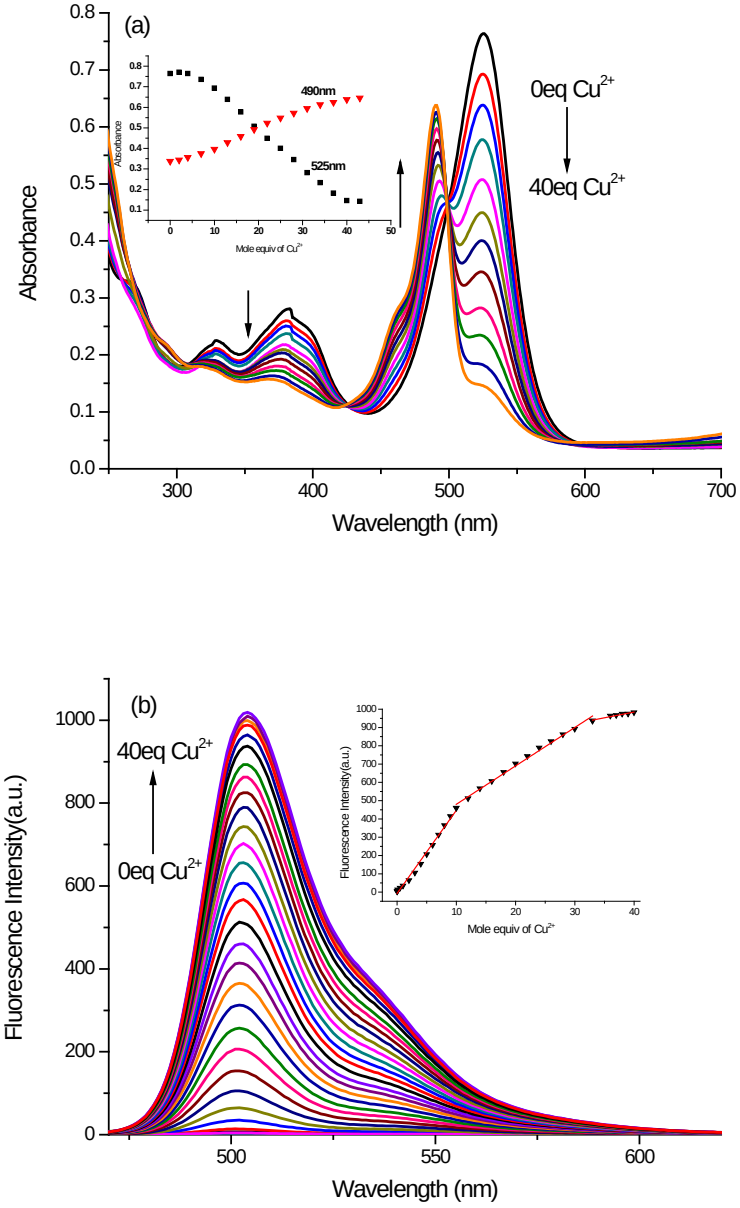


Figure S4. (a) Absorbance spectra of compound **1** (10 μM) towards Cu^{2+} ions (0 to 40 equiv) in H_2O/CH_3CN (1:1, v/v) medium (inset: plot of absorbance depending on the equiv of Cu^{2+} at 525 nm and 490 nm) and **(b)** fluorescence spectra of compound **1** (10 μM) towards Cu^{2+} ions (0，0.01, 0.02，0.04，0.1，1，2，3，4，5，6，7，8，9，10，12，14，16，18，20，22，24，26，28，30，33，36，37，38，39，40 equiv) in H_2O/CH_3CN (1:1, v/v) medium (λ_{ex}: 460 nm).

The Effect of pH to the reactivity of compound 1 with Cu^{2+}

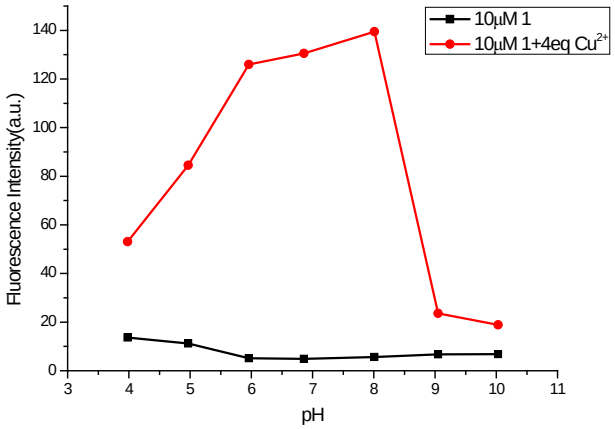


Figure S5. Effect of pH to the reaction of compound **1** (10 μM) with Cu^{2+} (40 μM, 4 equiv) in H_2O/CH_3CN (1:1, v/v) medium for 10 minutes (λ_{ex}: 460 nm).

The Effect of different cations to the reactivity of compound 1 with Cu^{2+}

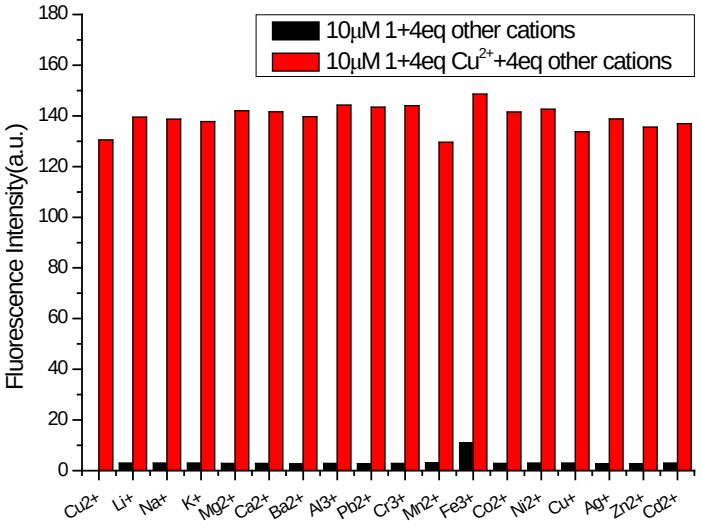


Figure S6. Fluorescence responses of **1** (10 μM) to various cations in H_2O/CH_3CN (1:1, v/v) medium. The black bars represent the emission intensities of **1** in the presence of 40 μM of alkali (IA) and alkaline earth (IIA) metals and for other cations of interest. The red bars represent the change of the emission that occurs upon the subsequent addition of 40 μM of Cu^{2+} to the above solution. The intensities were recorded at 507 nm, excitation at 460 nm.

The Effect of different anions to the reactivity of compound 1 with Cu^{2+}

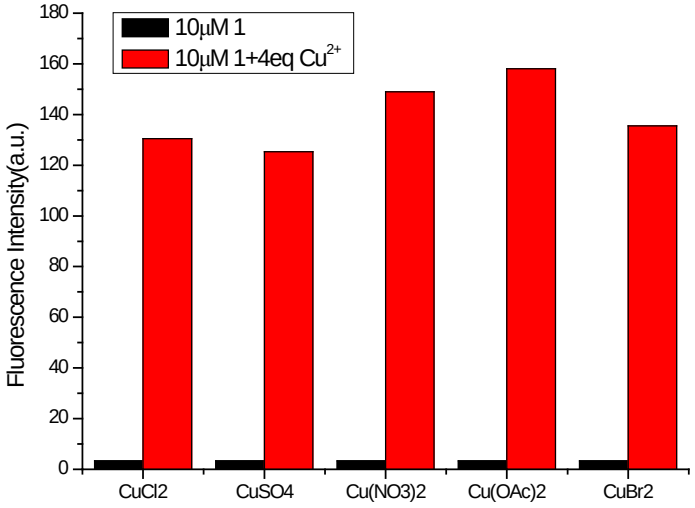


Figure S7. Effect of different anions to the reaction of compound **1** (10 μM) with Cu^{2+} (40 μM, 4 equiv) in H_2O/CH_3CN (1:1, v/v) medium for 10 minutes (λ_{ex}: 460nm).

Determination of Detection Limit

The detection limit was calculated based on the fluorescence titration. Before addingCu^{2+}, the emission intensity of compound **1** was measured by 10 times and the standard deviation of blank measurements was determined. Then a good linear relationship between the fluorescence intensity and Cu^{2+} concentration could be obtained in the 0-1μM（R^2=0.9764）. The detection limit is then calculated with the equation: detection limit = 3σbi/m, where σbi is the standard deviation of blank measurements; m is the slope between intensity versus sample concentration. The detection limit was measured to be **0.1 μM**.

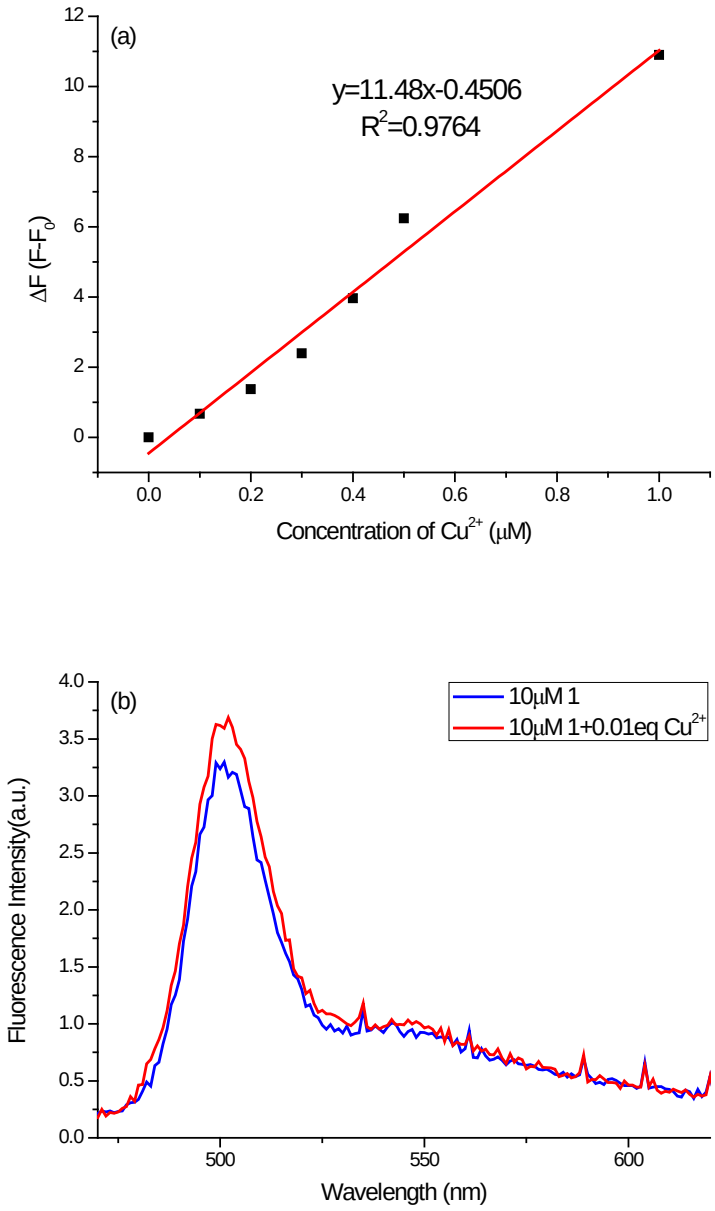


Figure S8. (a) Fluorescence changes of compound **1** (10 μM) upon addition of Cu^{2+} (0.1 to 1.0μM, 0.01 to 0.1 equiv) and **(b)** Fluorescence spectra of compound **1** (10 μM) in the presence of Cu^{2+} (0.1 μM, 0.01 eq) in H_2O/CH_3CN (1:1, v/v) medium (λ_{ex}: 460nm).

^{1}H NMR、^{13}C NMR and MS spectrum

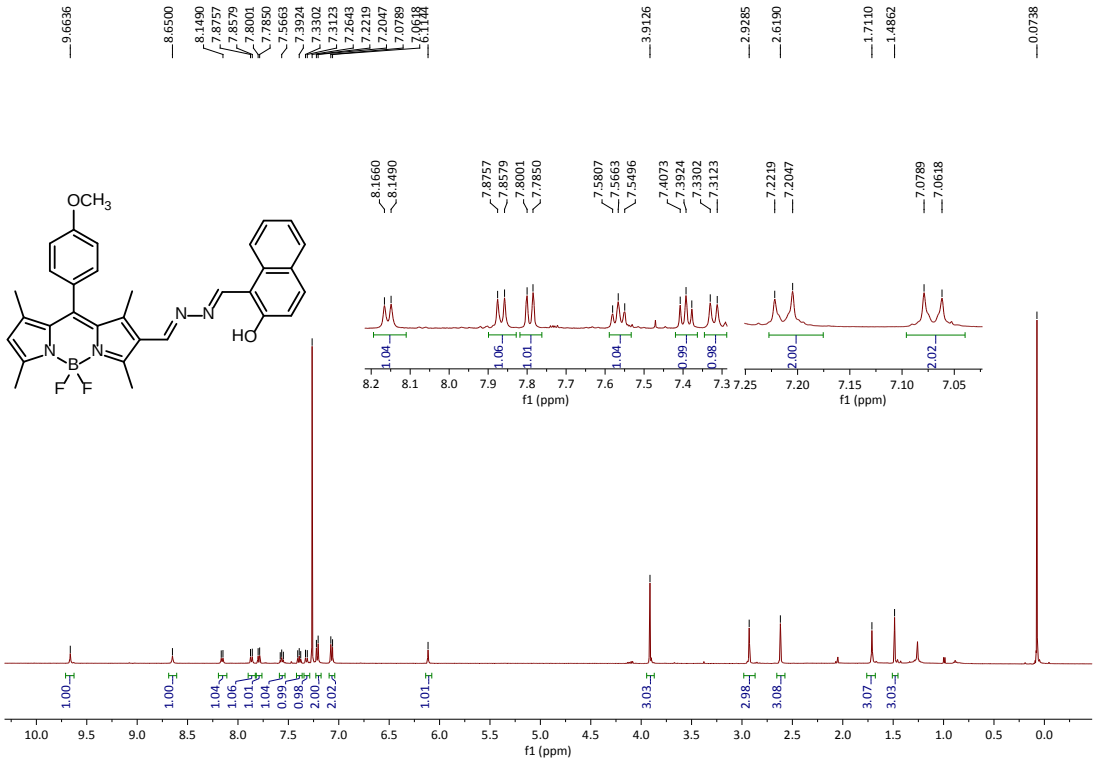


Figure S9. ^{1}H NMR spectrum of compound **1** in $CDCl_3$

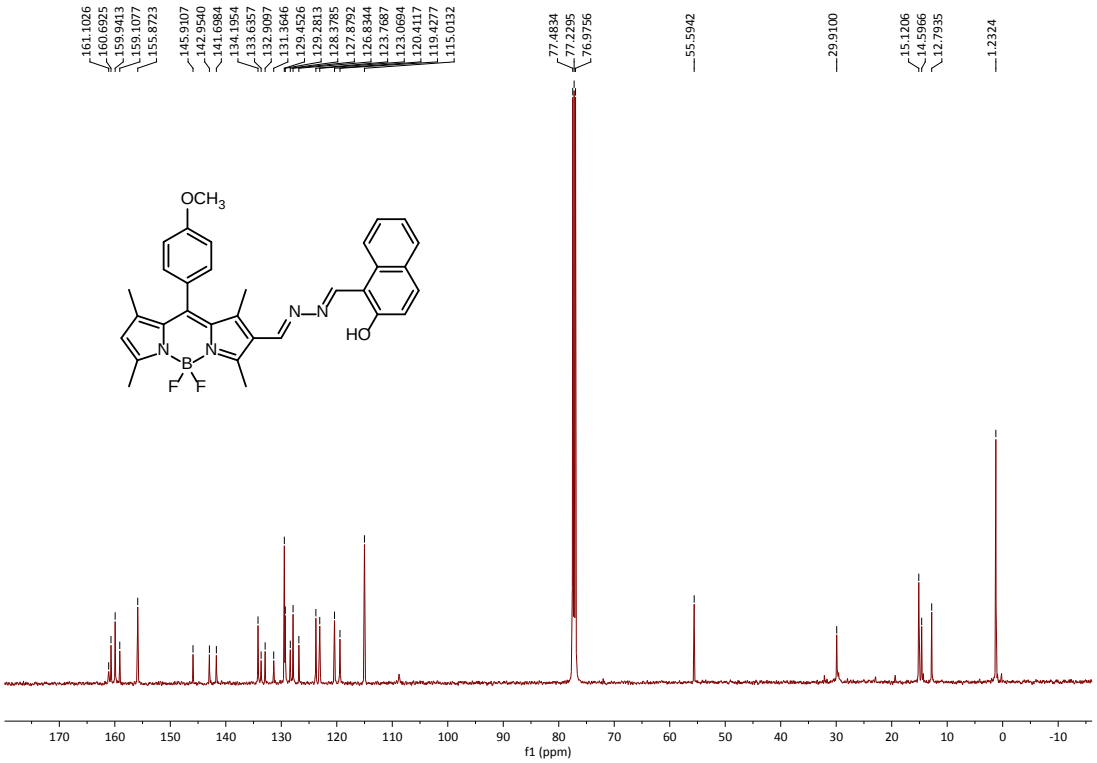


Figure S10. ^{13}C NMR spectrum of compound **1** in $CDCl_3$

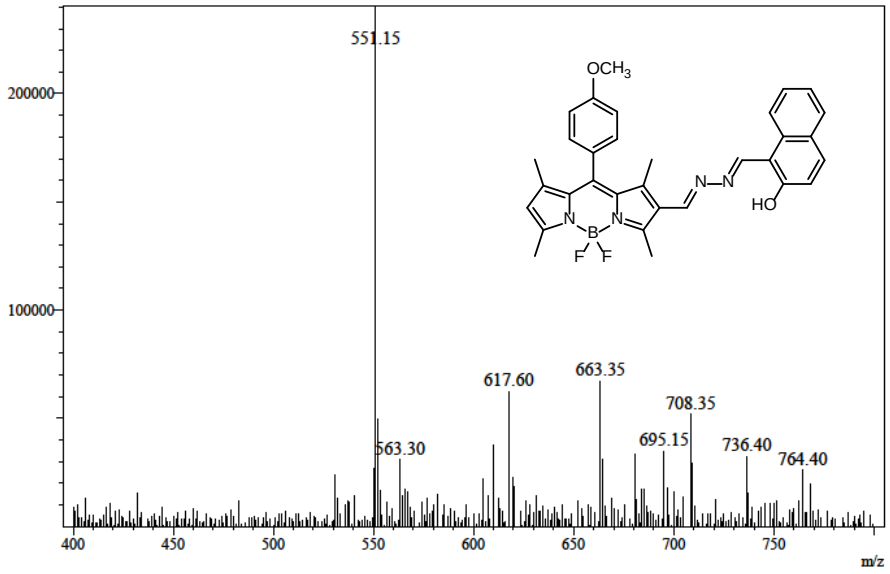
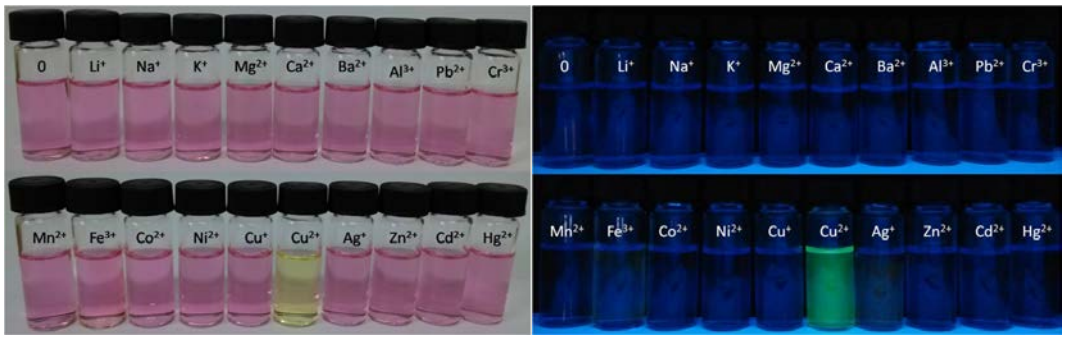


Figure S11. MS spectrum of compound **1**

The observed visual color change to the reaction



(a) Visible light (b) UV light (365 nm lamp)

Figure S12. Observed visual color and fluorescence change of compound **1**(10 μM) upon addition of 4 equiv of different metal ions in CH_3CN/H_2O (1:1, v/v) medium for 3 hours.

The change of the ^{1}H NMR spectrum to the reaction

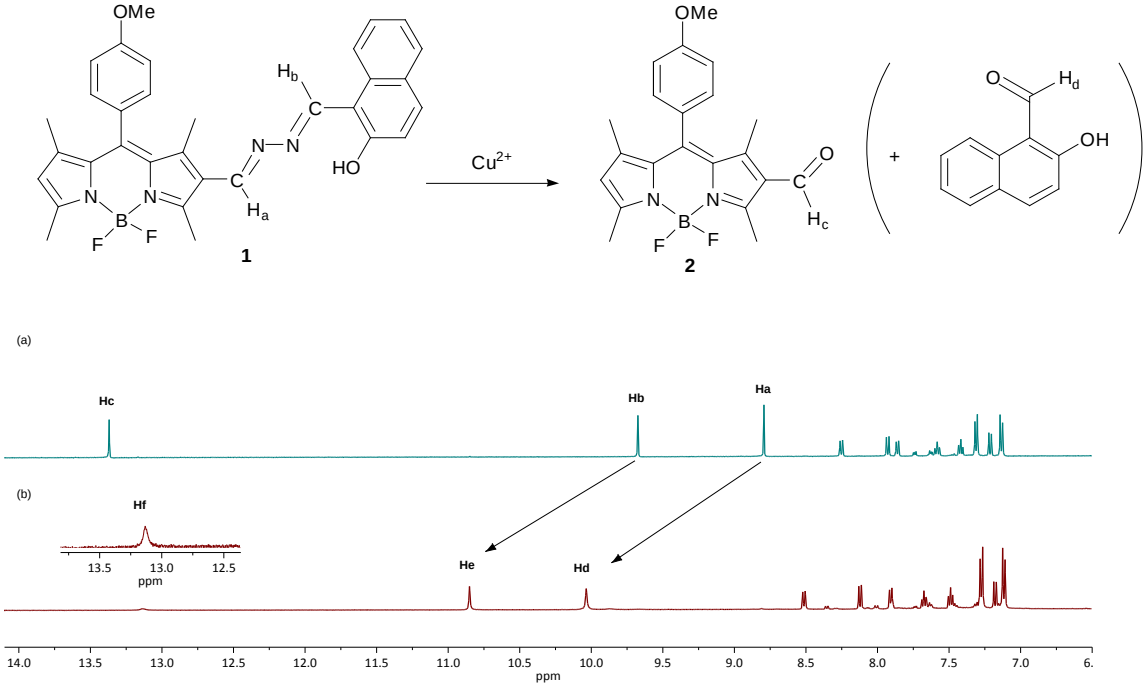


Figure S13. Proposed sensing mechanism and the change of the ^{1}HNMR spectrum to the reaction: **(a)** ^{1}H NMR spectrum of compound **1** (1.0 mM) in CD_3CN. **(b)** ^{1}H NMR spectrum of compound **1** (1.0 mM) upon addition of 1 equiv of Cu^{2+} in CD_3CN (detection after 3 hours).

Fluorescent imaging of Cu^{2+} in HeLa cells

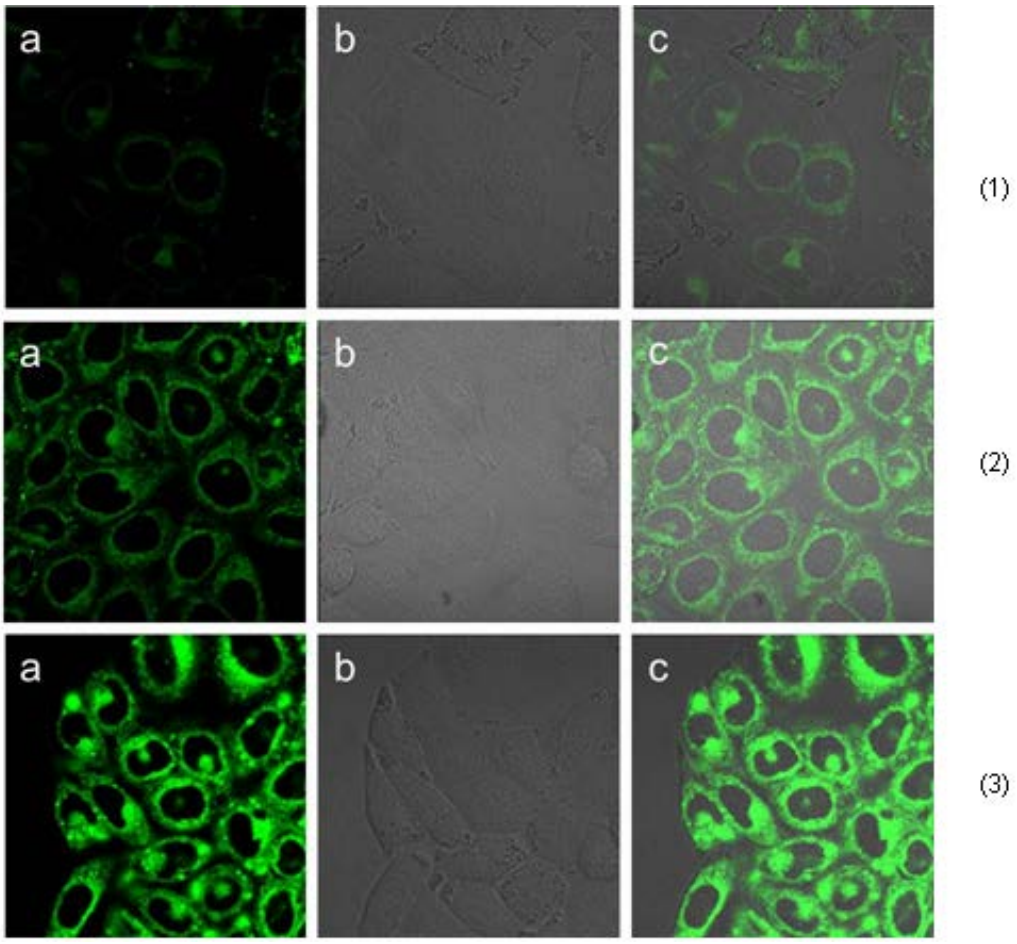


Figure S14. Confocal fluorescence images of living Hela cells: (1a) cells loaded with 10 μM probe **1** for 30 min. (1b) bright field image of 1a. (3c) overlaid images of panels 1a and 1b. (2a) probe-loaded cells treated with 20 μM Cu^{2+} for 1 h. (2b) bright field image of 2a. (2c) overlaid images of panels 2a and 2b. (3a) probe-loaded cells treated with 20 μM Cu^{2+} for 3 h. (3b) bright field image of 3a. (3c) overlaid images of panels 3a and 3b (λ_{ex}: 488 nm, band path for detection: 490-650 nm).

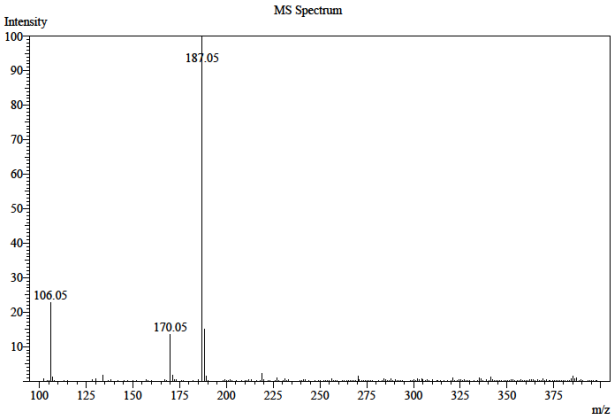


Figure S15. MS spectrum of compound **3**