

Supporting Information

# Endoplasmic reticulum-selective ratiometric fluorescent probe for imaging copper pool

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## 1. Materials and instrumentation

All fluorescence and UV/Vis absorption spectra were recorded in RF-6000 and UV-2600 spectrophotometer, respectively. NMR was recorded at Varian instrument (400 MHz). All reagents and cationic compounds such as  $[\text{Cu}(\text{MeCN})_4][\text{PF}_6]$  and chloride salts of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Fe}^{3+}$  were purchased from Aldrich and used as received.

## 2. UV/Vis absorption and fluorescence spectroscopic methods

Stock solutions of metal chloride salts were prepared in deionized water.  $\text{Cu}^+$  was delivered in the form of  $[\text{Cu}(\text{MeCN})_4][\text{PF}_6]$  from an acetonitrile stock solution.<sup>1</sup> Stock solutions of synthetic probes were prepared in DMSO. The fluorescence quantum yields ( $\Phi_f$ ) of **1-4** were measured relative to reference materials (4-amino *N*-butyl 1,8-naphthalimide ( $\Phi_f = 0.64$  in ethanol)<sup>2</sup> for **1** and **2**; quinine ( $\Phi_f = 0.54$  in 0.5 M  $\text{H}_2\text{SO}_4$ )<sup>3</sup> for **3** and **4**). All spectra were recorded in HEPES buffer (20 mM, pH = 7.4) containing 1% of DMSO. Excitation was carried out at 410 nm with all excitation slit widths 5 nm.

## 3. Determination of the detection limit

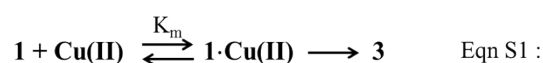
The detection limit was calculated with the following equation:

$$\text{Detection limit} = 3\sigma/k$$

The  $\sigma$  is the standard deviation of blank measurement,  $k$  is the slope between the fluorescence intensity ratios versus analyte concentration. The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of the probe was measured by five times and the standard deviation of blank measurement was achieved. To gain the slope, the ratio of the fluorescence intensities was plotted as a concentration of analyte.

## 4. Kinetic analysis of copper ion-mediated hydrolysis of probe 1

Probe **1** is solved in HEPES buffer (20 mM) at pH 7.4. After an appropriate portion of copper stock solution (5 mM) was added to the solution of **1**, the fluorescence intensity at 545 nm was recorded upon 410 nm excitation using spectrofluorometer (Hitachi, Japan). The fluorescence intensity changes during 600 sec was analyzed and used for kinetic analysis. The kinetic analysis of the measured initial velocity was done based on Eqn. S1 as the following equation.



The initial velocity =  $V_{\text{max}} [\text{Cu ion}] / ([\text{Cu ion}] + K_m)$  where the  $V_{\text{max}}$  value is the presumed maximum velocity when  $[\text{Cu ion}]$  goes to the infinity and the  $K_m$  value is a dissociation constant of the complex between Cu ion and the probe **1**. Other details are described in each figure legend.

## 5. Cell culture and confocal microscopic methods

A human cervical cancer cell line (HeLa) was cultured in Dulbecco's Modified Eagle's Medium (DMEM), with 10% FBS (Gipco), penicillin (100 units/mL), and streptomycin (100  $\mu\text{g}/\text{mL}$ ). One day before imaging, the cells were placed on confocal dishes which were incubated in a humidified atmosphere containing 5% (v/v)  $\text{CO}_2$  at 37 °C. Cell images were obtained using a confocal microscope (Zeiss model LSM 510). Fluorescence images of probes were obtained using excitation wavelengths of 458 nm and 543 nm and band path 475-525 nm and band path 585-615 nm emission filters. Other information is available in the Figure

captions.

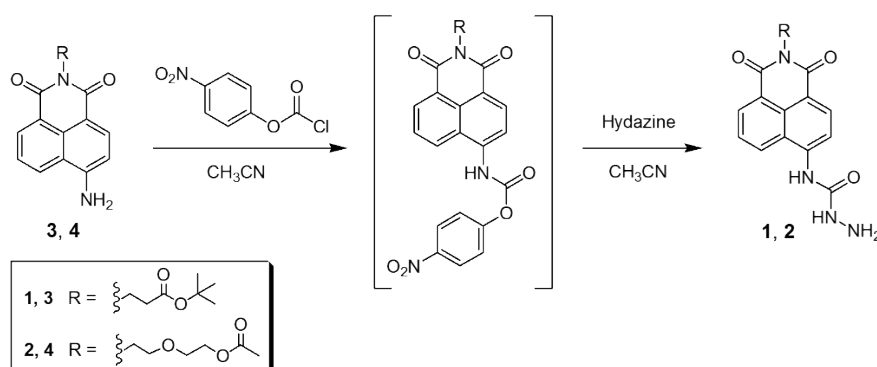
## 6. Co-staining of probe and organelle trackers and confocal microscopic methods

Organelle tracking dyes for co-localization experiments were purchased from Invitrogen. Other information is available in the Figure captions. Live HeLa cells were pretreated with ER-Red (0.5  $\mu\text{M}$ ), Lyso-Red (0.05  $\mu\text{M}$ ), or Mito-Red (0.1  $\mu\text{M}$ ), respectively, for 15 min at 37°C in serum-free high-glucose DMEM media. The cells were subsequently incubated with probe (5  $\mu\text{M}$ ) in serum-free high-glucose DMEM media for 20 min at 37°C, and then washed with PBS. The excitation wavelength and filter set were 458 nm with a 475-525 nm band path (BP) and 543 nm with a > 650 nm long path (LP) for probe and organelle trackers, respectively. Other information is available in the Figure captions.

## 7. MTT assay methods

Cell viability was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells at  $2 \times 10^5/\text{mL}$  were treated with various concentrations of probe **1** or **2** in 96-well plates for 24 h at 37 °C. Then, a solution of MTT in serum free media (5 mg/mL) was added to each well and cells were incubated for 1 h. The water-insoluble formazan was formed during the incubation and DMSO was added to each well. The amount of formazan was then determined by measuring the absorbance at 570 nm using a multi-well plate reader (n = 4).

## 8. Synthesis of compounds 1-4



**Scheme S1.** Synthesis of hydrazide-linked naphthalimides (**1** and **2**) as a ratiometric fluorescent copper probe.

Compounds **3** and **4** were prepared according to the literature procedure.<sup>4-6</sup>

### Synthesis of **1**

Compound **3** (0.1 g, 0.29 mmol) and 4-nitrophenyl chloroformate (0.1 g, 0.49 mmol) were dissolved in  $\text{CH}_3\text{CN}$  (30 mL) at 0 °C under nitrogen atmosphere. After stirring overnight at room temperature, a solution of hydrazine monohydrate (0.028 mL, 0.58 mmol) dissolved in  $\text{CH}_3\text{CN}$  was added to the reaction mixture. After further stirring for 2 h, a yellow solid was

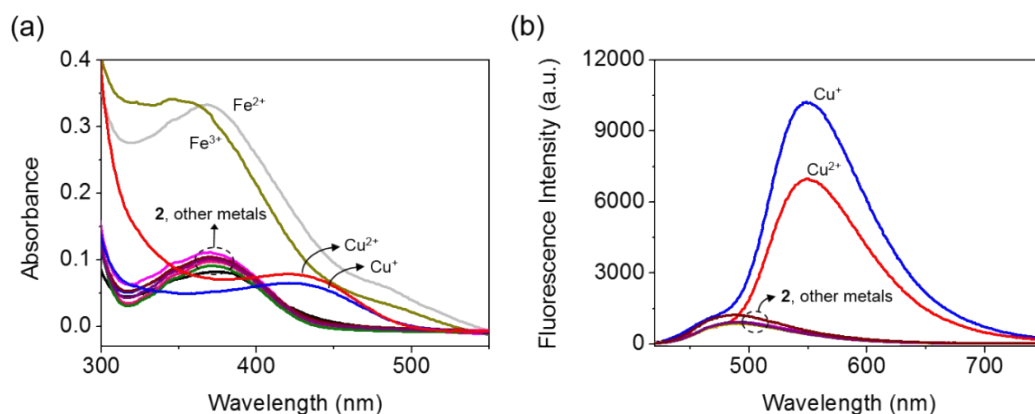
1. D. W. Domaille, L. Zeng and C. J. Chang, *J. Am. Chem. Soc.*, 2010, **132**, 1194–1195.
2. M. S. Alexiou, V. Tychopoulos, S. Ghorbanian, J. H. P. Tyman, R. G. Brown and P. I. Brittain, *J. Chem. Soc. Perkin Trans. 2*, 1990, **5**, 837–842.
3. G. A. Crosby and J. N. Demas, *J. Phys. Chem.*, 1971, **75**, 991–1024.
4. M. H. Lee, J. H. Han, J.-H. Lee, H. G. Choi, C. Kang and J. S. Kim, *J. Am. Chem. Soc.*, 2012, **134**, 17314–17319.
5. T. Guo, L. Cui, J. Shen, R. Wang, W. Zhu, Y. Xu and X. Qian, *Chem. Commun.*, 2013, **49**, 1862–1864.
6. D. Srikun, E. W. Miller, D. W. Domaille and C. J. Chang, *J. Am. Chem. Soc.*, 2008, **130**, 4596–4597.

formed in the reaction mixture. The organic solvent was evaporated and resulting crude was dissolved in MeOH (2 mL). By using excess diethyl ether, compound **1** (0.06 g, 53%) was obtained as a yellow solid. FAB-MS  $m/z$   $[M+H]^+$  calc. 399, obs. 399.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  1.32 (s, 9H); 2.57 (t,  $J = 7.3$  Hz, 2H); 4.25 (t,  $J = 7.2$  Hz, 2H); 7.87 (t,  $J = 6.6$  Hz, 1H); 8.42 (d,  $J = 8.2$  Hz, 1H); 8.49~8.52 (m, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-}d_6$ )  $\delta$  27.9, 33.8, 35.9, 80.4, 125.2, 131.1, 132.8, 163.1, 163.7, 170.5.

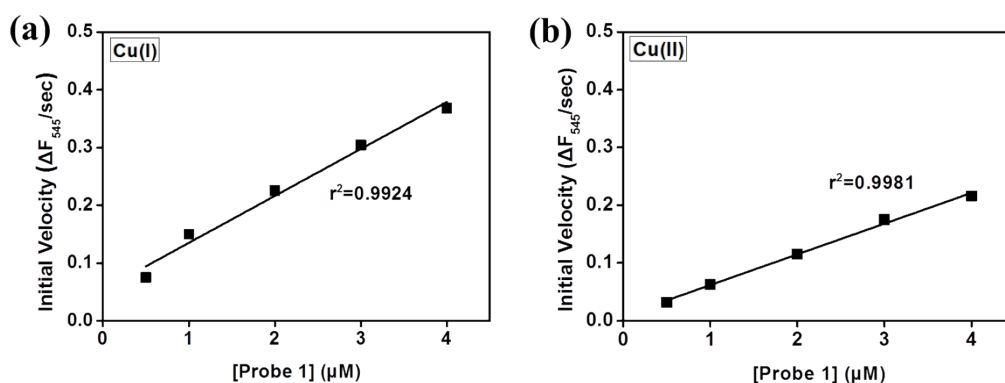
### Synthesis of **2**

Compound **2** was synthesized using a modification of the procedure used to obtain compound **1**. Using **4** (0.1 g, 0.29 mmol) and 4-nitrophenyl chloroformate (0.09 g, 0.49 mmol), compound **2** was obtained in the form of a yellow solid in 61% yield. ESI-MS  $m/z$   $[M-H+Na]^+$  calc. 422.120, obs. 422.950.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6/\text{CDCl}_3$ )  $\delta$  1.95 (s, 3H); 3.72 (t,  $J = 4.7$  Hz, 2H); 3.79 (t,  $J = 6.1$  Hz, 2H); 4.14 (t,  $J = 4.7$  Hz, 2H); 4.36 (t,  $J = 6.1$  Hz, 2H); 7.76 (d,  $J = 7.5$  Hz, 1H); 7.85 (s, 1H); 8.47 (d,  $J = 8.4$  Hz, 1H); 8.55~8.59 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-}d_6/\text{CDCl}_3$ )  $\delta$  20.4, 38.3, 63.0, 67.2, 67.9, 114.2, 122.0, 128.6, 130.5, 132.4, 163.1, 163.7, 170.1.

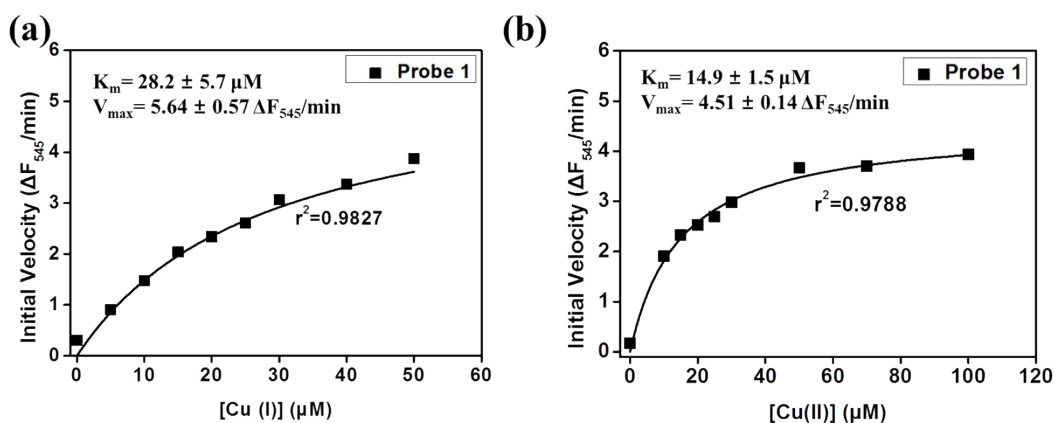
## 9. Additional Data



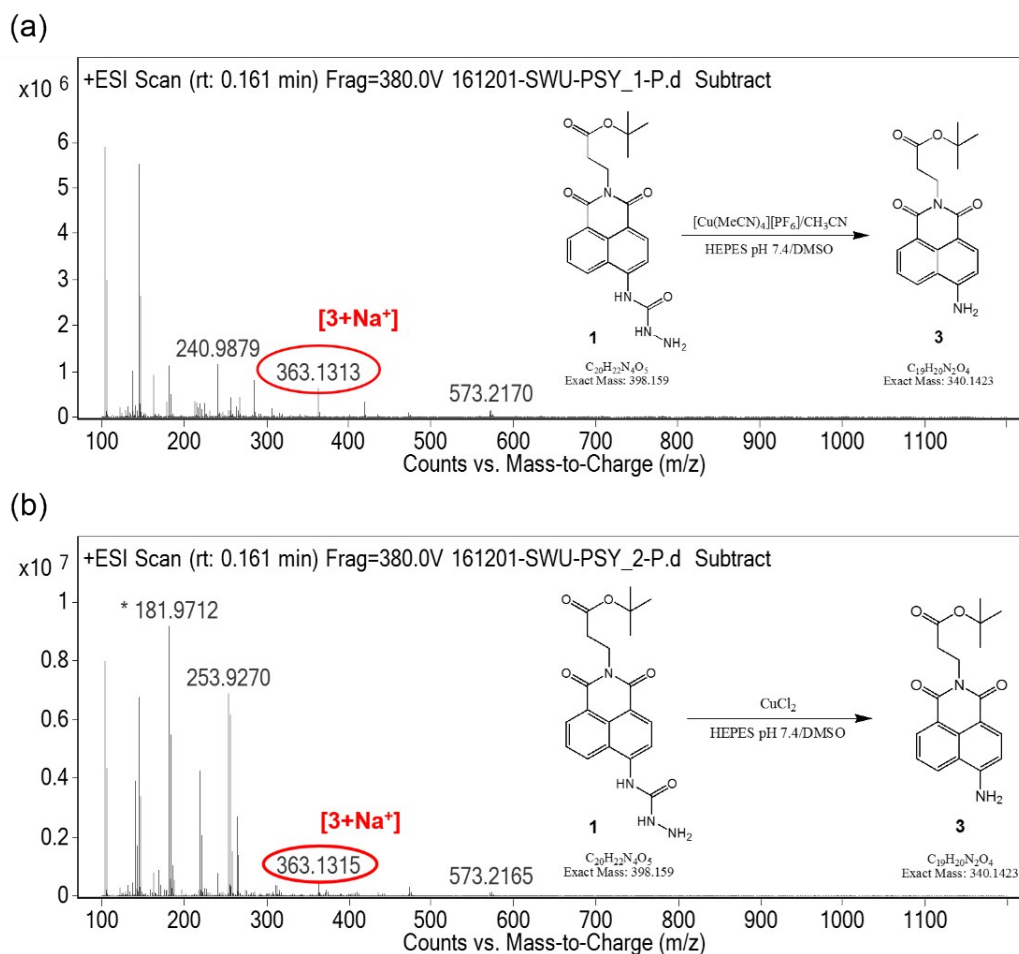
**Figure S1.** (a) UV/Vis absorption and (b) fluorescence spectra of **2** (5.0  $\mu\text{M}$ ) in the presence of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cu}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Fe}^{3+}$  ions (20 equiv, respectively) in HEPES buffer (20 mM, pH = 7.4) containing 1% DMSO at room temperature, and incubation time: 1 h. Excitation was effected at 400 nm.



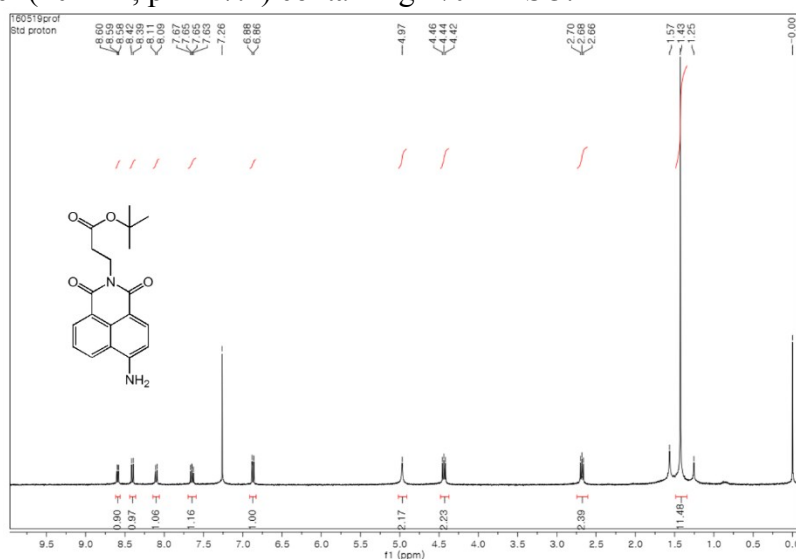
**Figure S2.** Dependency of fluorogenic rate on [probe 1]. The initial velocity was defined as the fluorescence change in the solution during 600 sec after the copper ions ( $50 \mu\text{M}$  for  $\text{Cu}^+$  and  $100 \mu\text{M}$  for  $\text{Cu}^{2+}$ ) were added. The black boxes (■) represent the velocity data using **1** upon measuring emission at 545 nm with excitation at 410 nm, and the solid lines represent the data fitting lines based on a linear regression.



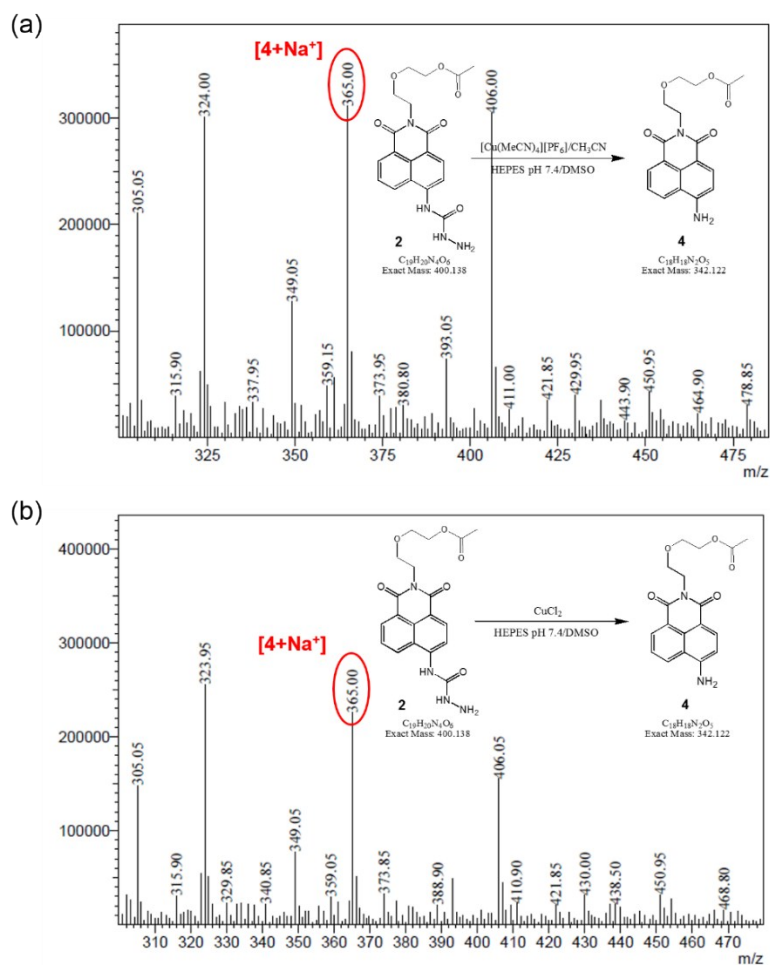
**Figure S3.** Initial velocity measurement and kinetic analysis based on 1:1 complex formation prior to slow hydrolysis. The initial velocity was defined as the fluorescence change in the solution during 600 sec after the copper ions were added. The black boxes (■) represent the velocity data using  $5.0 \mu\text{M}$  of **1** upon measuring emission at 545 nm with excitation at 410 nm. The solid lines represent the data fitting lines based on a non-linear regression to the hyperbolic equation in the section 3 in supplementary information.



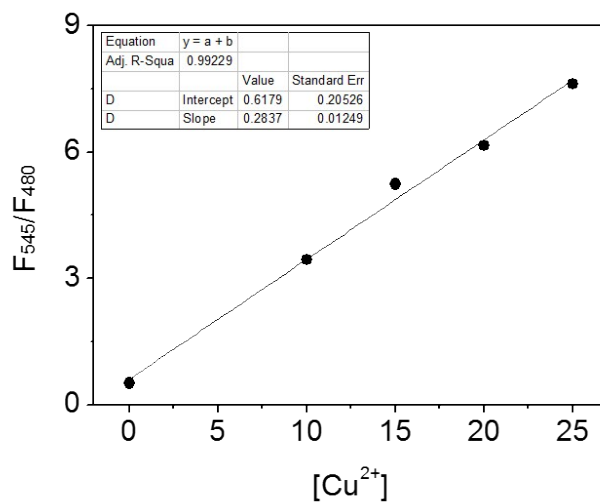
**Figure S4.** ESI-MS spectra of **1** in the presence of an excess amount of (a)  $\text{Cu}^+$  or (b)  $\text{Cu}^{2+}$  ions in HEPES buffer (20 mM, pH = 7.4) containing 1% DMSO.



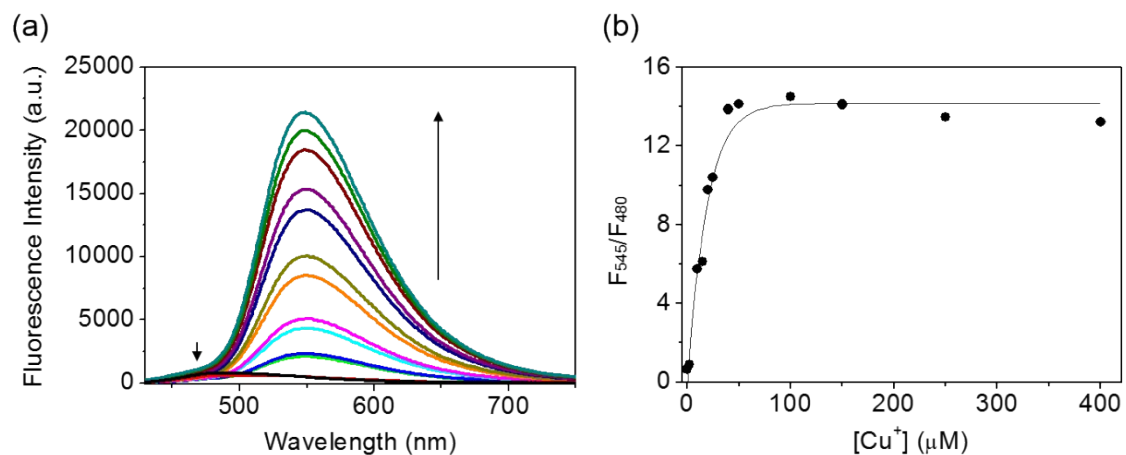
**Figure S5.**  $^1\text{H}$ -NMR spectrum of amino naphthalimide (**3**) in  $\text{CDCl}_3$ . The amino naphthalimide (**3**) was produced from a reaction of **1** with  $\text{CuCl}_2$  in HEPES buffer (pH = 7.4).



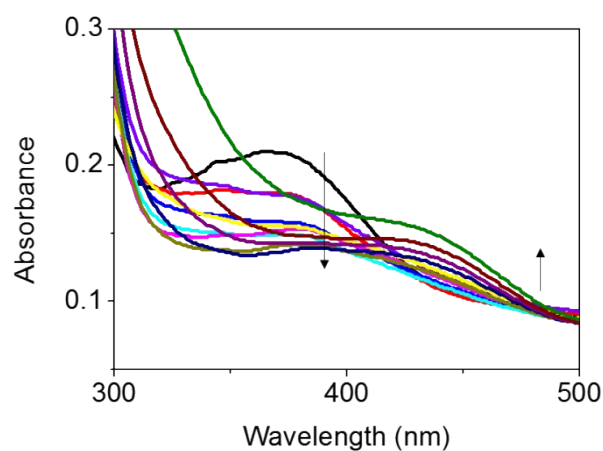
**Figure S6.** ESI-MS spectra of **2** in the presence of an excess amount of (a)  $\text{Cu}^+$  or (b)  $\text{Cu}^{2+}$  ions in HEPES buffer (20 mM, pH = 7.4) containing 1% DMSO.



**Figure S7.** Plot showing the linear relationship ( $R^2 = 0.99$ ) between the  $F_{480}/F_{545}$  and  $[\text{Cu}^{2+}]$  (0, 10, 15, 20, 25  $\mu\text{M}$ ).  $F_{480}$  and  $F_{545}$  indicate the fluorescence intensity of **1** at 480 nm and at 545 nm, respectively. All data were acquired in HEPES buffer (20 mM, pH = 7.4) containing 1% DMSO, with excitation effected at 410 nm.

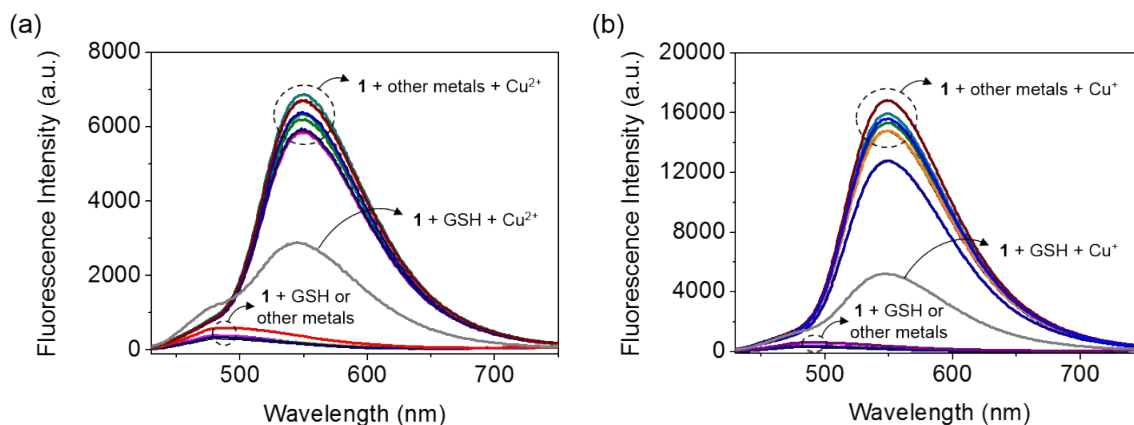


**Figure S8.** (a) Fluorescence changes of **1** ( $5.0 \mu\text{M}$ ) upon the addition of various concentrations of  $\text{Cu}^+$  ions ( $0 \sim 400 \mu\text{M}$ ). (b) Plot of  $F_{545}/F_{480}$  versus  $[\text{Cu}^+]$ . All spectra were acquired in HEPES buffer ( $20 \text{ mM}$ ,  $\text{pH} = 7.4$ ) containing  $1\%$  DMSO. Excitation was effected at  $410 \text{ nm}$ .

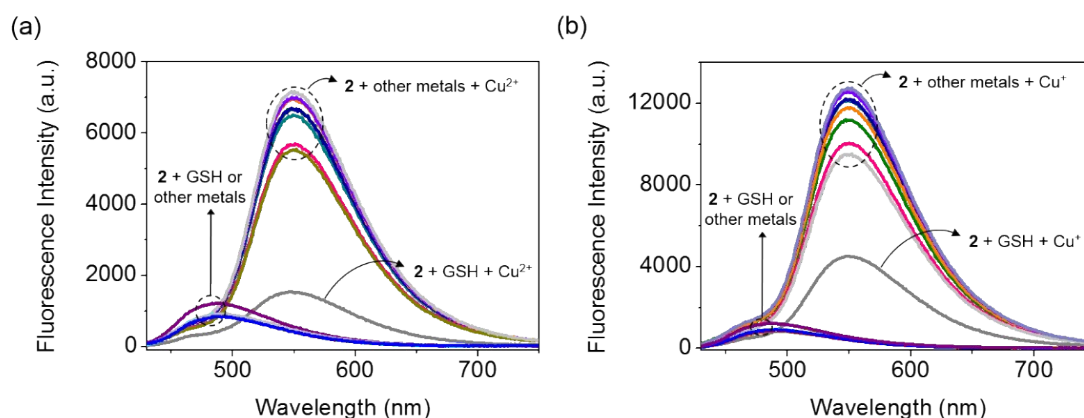


**Figure S9.** UV/Vis absorption changes of **1** ( $5.0 \mu\text{M}$ ) upon the addition of various concentrations of  $\text{Cu}^{2+}$  ions ( $0 \sim 400 \mu\text{M}$ ). All spectra were acquired in HEPES buffer ( $20 \text{ mM}$ ,  $\text{pH} = 7.4$ ) containing  $1\%$  DMSO.

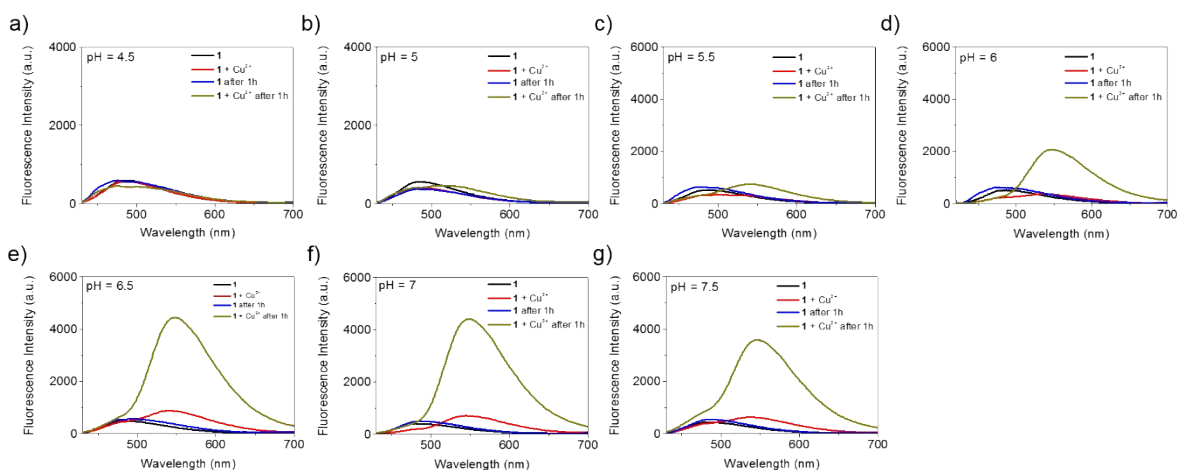




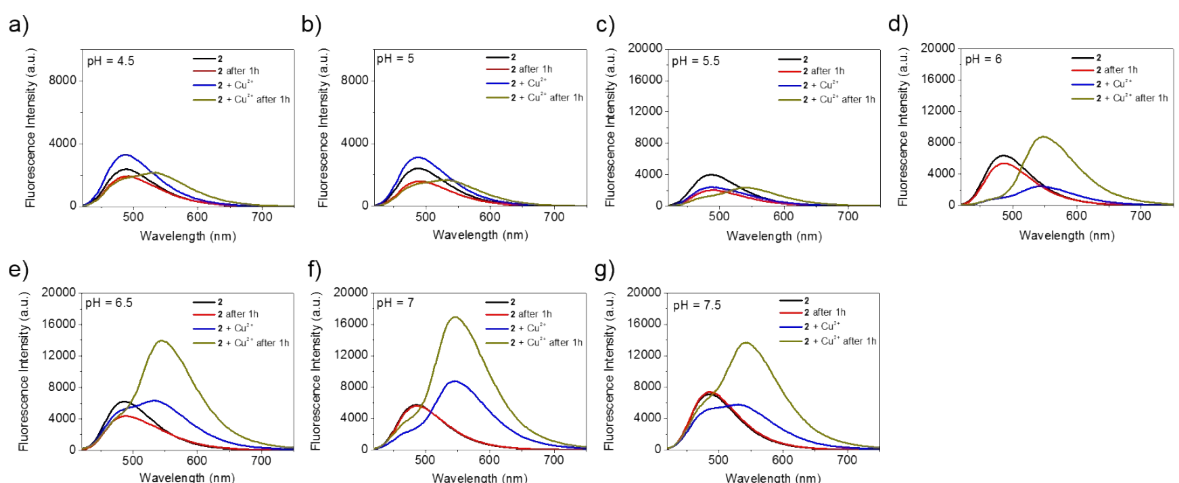
**Figure S10.** Fluorescence spectra of **1** (5.0  $\mu\text{M}$ ) with (a)  $\text{Cu}^{2+}$  and (b)  $\text{Cu}^+$  (100  $\mu\text{M}$ , respectively) in the presence of other metals ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  ions; 100  $\mu\text{M}$ , respectively) and GSH (100  $\mu\text{M}$ ). All spectra were acquired after 2 h in HEPES buffer (20 mM, pH = 7.4) containing 1% DMSO at room temperature. Excitation was effected at 410 nm.



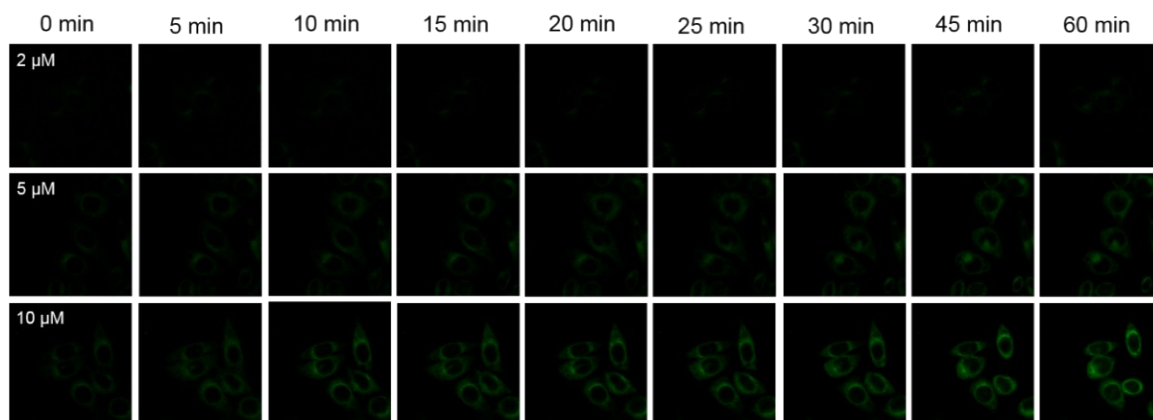
**Figure S11.** Fluorescence spectra of **2** (5.0  $\mu\text{M}$ ) with (a)  $\text{Cu}^{2+}$  or (b)  $\text{Cu}^+$  (100  $\mu\text{M}$ , respectively) in the presence of other metals ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  ions; 100  $\mu\text{M}$ , respectively) and GSH (100  $\mu\text{M}$ ). All spectra were acquired after 2 h reaction time in HEPES buffer (20 mM, pH = 7.4) containing 1% DMSO at room temperature. Excitation was effected at 400 nm.



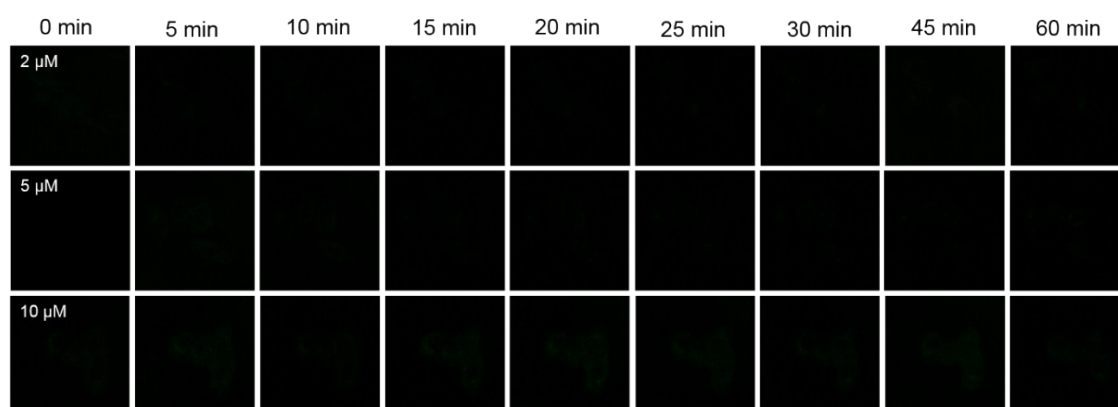
**Figure S12.** The influence of pH on the fluorescence spectra of **1**. All data were obtained in the absence and presence of  $\text{Cu}^{2+}$  ions (10 equiv) in different pH buffer solutions. Some cases were acquired 1 h after addition of the  $\text{Cu}^{2+}$  at room temperature, with an excitation at 410 nm.



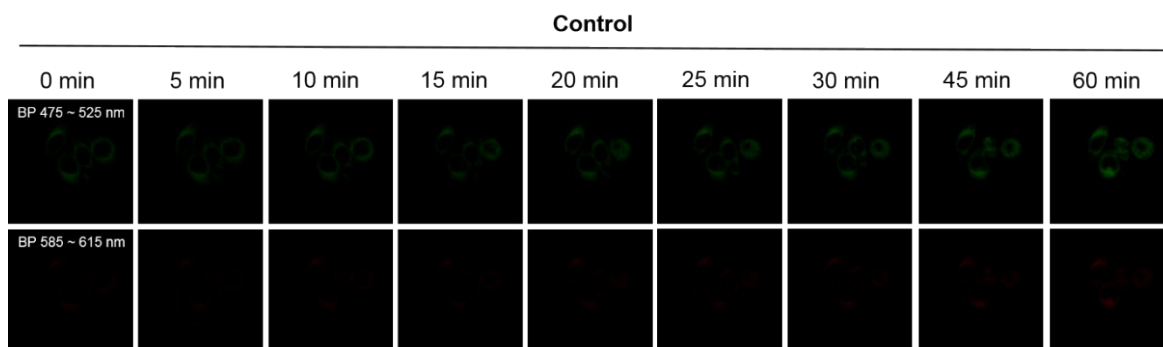
**Figure S13.** The influence of pH on the fluorescence spectra of **2**. All data were obtained in the absence and presence of  $\text{Cu}^{2+}$  ions (10 equiv) in different pH buffer solutions. Some cases were acquired 1 h after addition of the  $\text{Cu}^{2+}$  at room temperature, with an excitation at 410 nm.



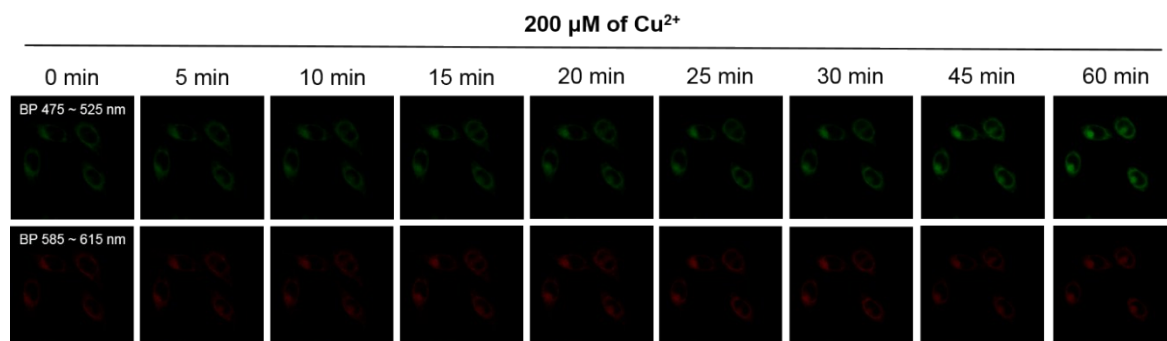
**Figure S14.** Confocal microscopy images of HeLa cells showing the fluorescence response as a function of time upon incubation of different concentrations of probe **1**. The cells were incubated with **1** (2  $\mu$ M, 5  $\mu$ M, and 10  $\mu$ M, respectively) at 37 °C in serum free high glucose DMEM media. The excitation wavelength and filter set are 458 nm with a 505 ~ 550 nm band path.



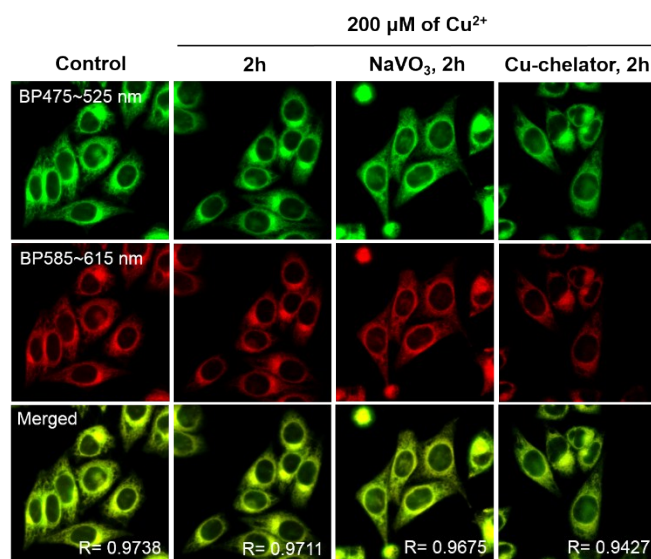
**Figure S15.** Confocal microscopy images of HeLa cells showing the fluorescence response as a function of time upon incubation of different concentrations of probe **2**. The cells were incubated with **2** (2  $\mu$ M, 5  $\mu$ M, and 10  $\mu$ M, respectively) at 37 °C in serum free high glucose DMEM media. The excitation wavelength and filter set are 458 nm with a 505 ~ 550 nm band path.



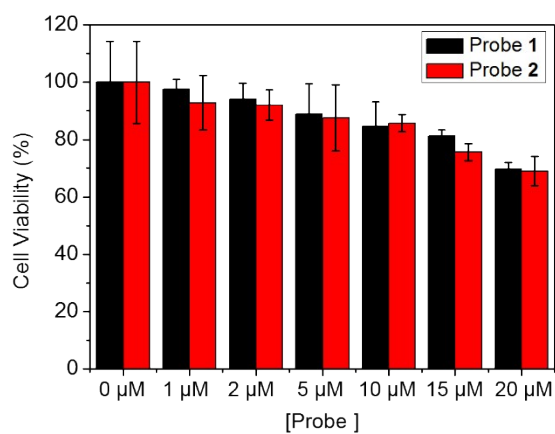
**Figure S16.** Confocal microscopy images of HeLa cells showing the fluorescence response as a function of time upon incubation of 5  $\mu\text{M}$  of probe **1** in serum free high glucose DMEM media at 37  $^{\circ}\text{C}$ . The images were obtained using excitation at 458 nm with a 475 ~ 525 nm band path (BP) filter and 543 nm with a 585 ~ 615 nm band path (BP) filter.



**Figure S17.** Confocal microscopy images of copper-overloaded HeLa cells showing the fluorescence response as a function of time upon incubation of probe **1**. HeLa cells were pretreated with 200  $\mu\text{M}$  of  $\text{CuCl}_2$  for 120 min at 37  $^{\circ}\text{C}$ , which were then washed with PBS. The cells were subsequently incubated with 5  $\mu\text{M}$  of **1** in serum free high glucose DMEM media at 37  $^{\circ}\text{C}$ . The images were obtained using excitation at 458 nm with a 475 ~ 525 nm band path (BP) filter and 543 nm with a 585 ~ 615 nm band path (BP) filter.

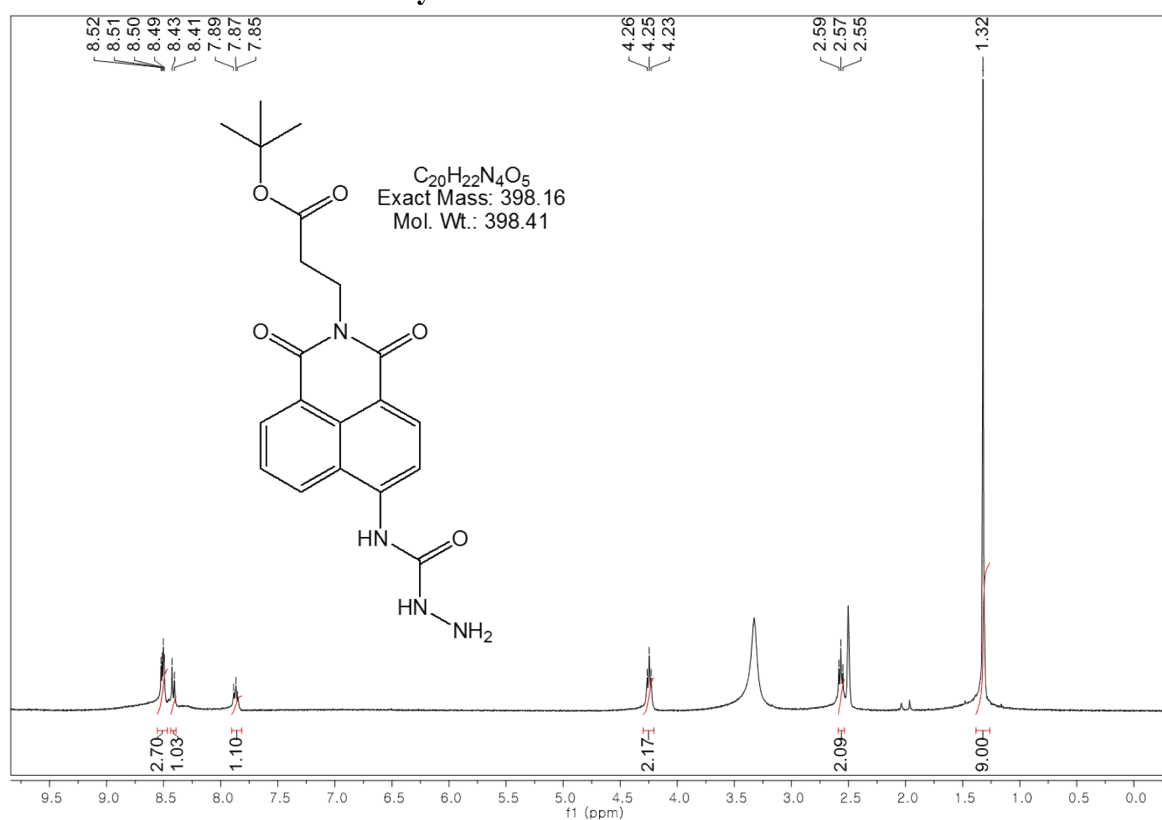


**Figure S18.** Confocal microscopy images of **3** in HeLa cells.  $\text{Cu}^{2+}$ -overloaded HeLa cells were prepared by treatment with  $\text{CuCl}_2$  (200  $\mu\text{M}$ ) for 2 h in the absence and presence of pretreatment of  $\text{NaVO}_3$  (100  $\mu\text{M}$ ) for 24 h at 37  $^{\circ}\text{C}$ . The  $\text{Cu}^{2+}$ -overloaded cells were also treated with Cu-chelator (3,6-dithia-1,8-octanediol, 1 mM) for 30 min at 37  $^{\circ}\text{C}$ . The cells were incubated with 1  $\mu\text{M}$  of **3** for 20 min at 37  $^{\circ}\text{C}$  in serum free high glucose DMEM media. The fluorescent confocal images of **3** were then collected using an excitation wavelength and filter set of 458 nm with a 475–525 nm band path (BP) and 543 nm with a 585–615 nm band path (BP). *R* values in the merged images indicated Pearson's correlation coefficients.



**Figure S19.** Cell viability of probes **1** and **2** in HeLa cells. The cells were treated with various concentrations of probes for 24 h at 37°C, and MTT assay was then performed.

### 10. <sup>1</sup>H / <sup>13</sup>C NMR and MS Analysis



**Figure S20.** <sup>1</sup>H-NMR spectrum of **1** in DMSO-*d*<sub>6</sub>.

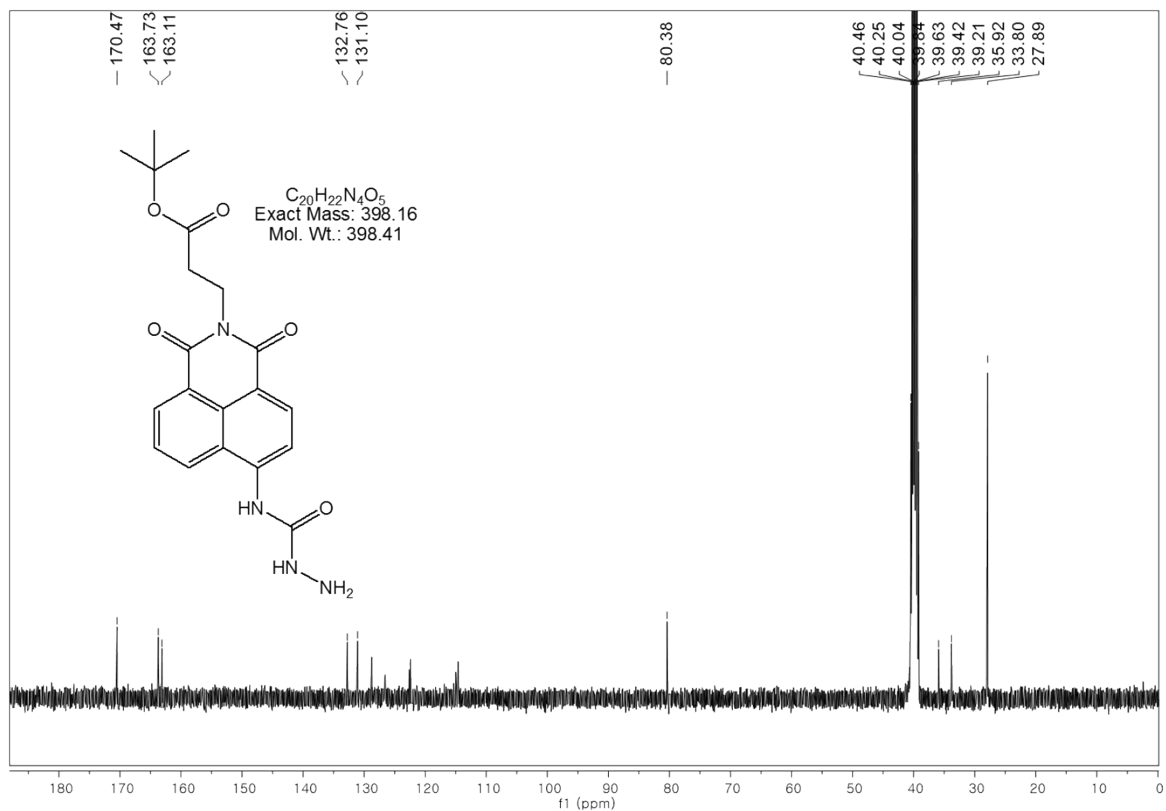


Figure S21.  $^{13}C$ -NMR spectrum of 1 in  $DMSO-d_6$ .

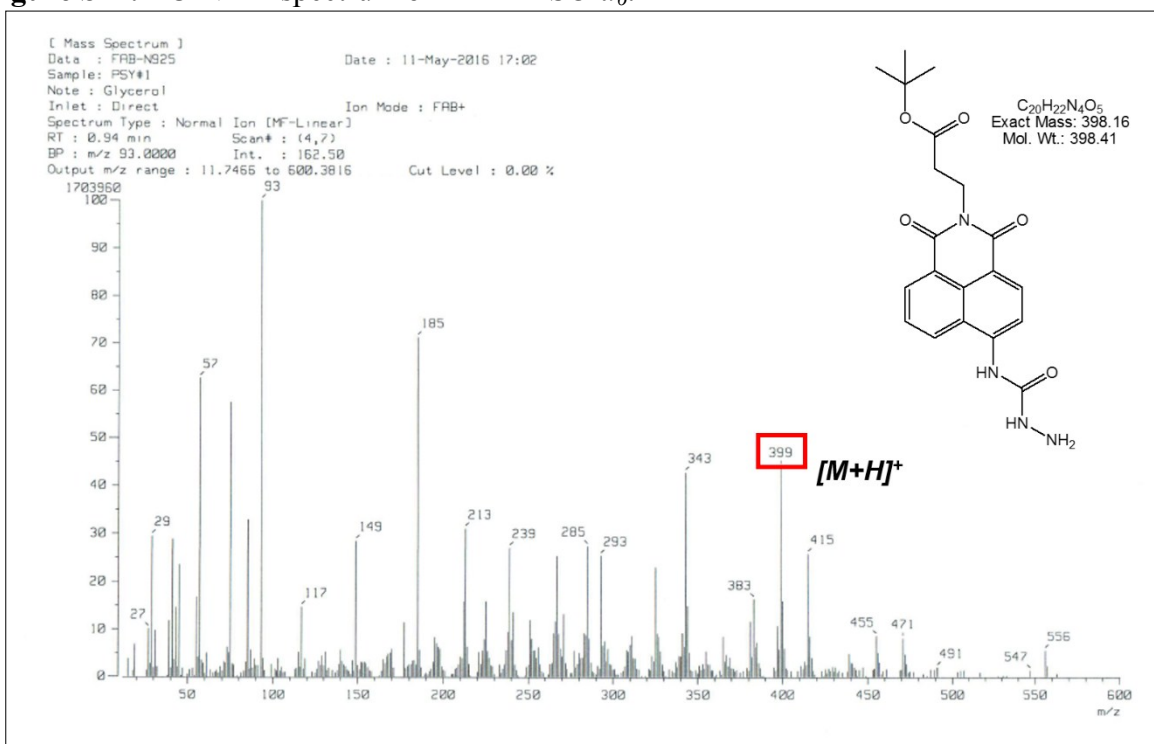
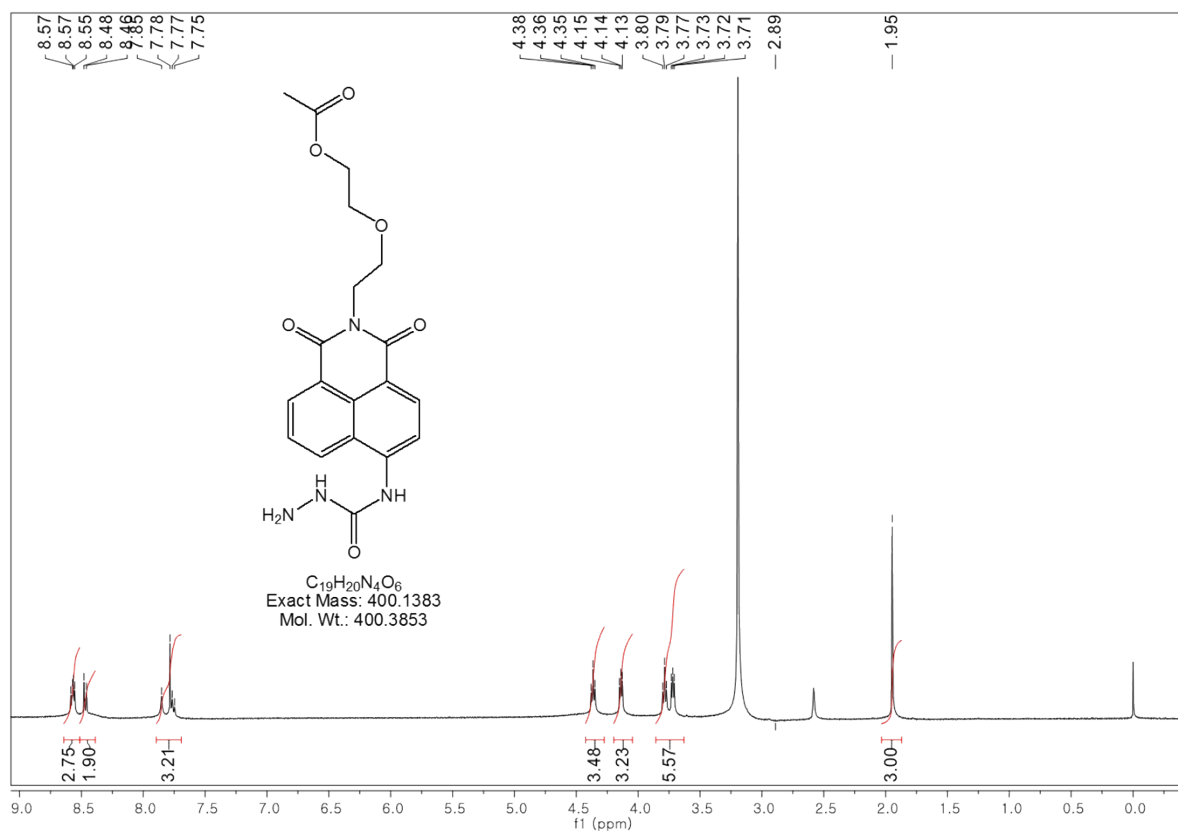
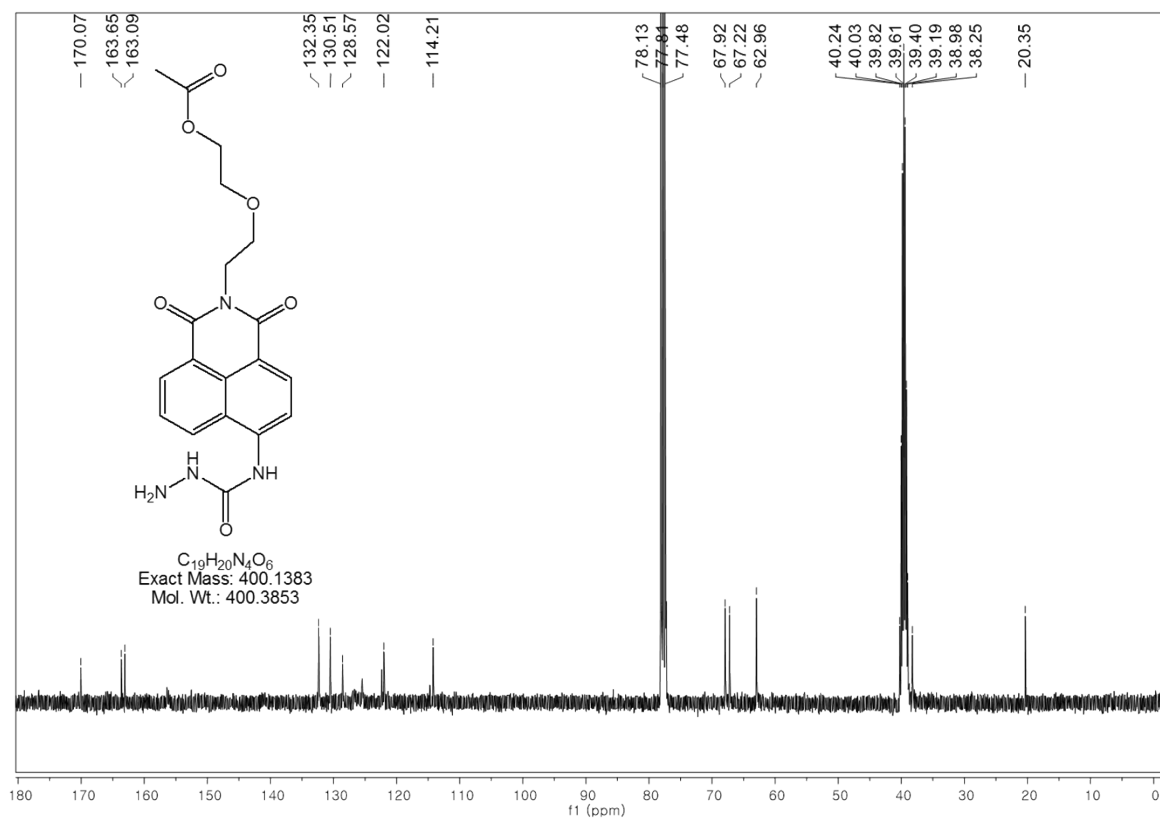


Figure S22. FAB-MS spectrum of 1.



**Figure S23.**  $^1H$ -NMR spectrum of **2** in  $DMSO-d_6$  containing small amount of  $CDCl_3$ .



**Figure S24.**  $^{13}C$ -NMR spectrum of **2** in  $DMSO-d_6$  containing small amount of  $CDCl_3$ .

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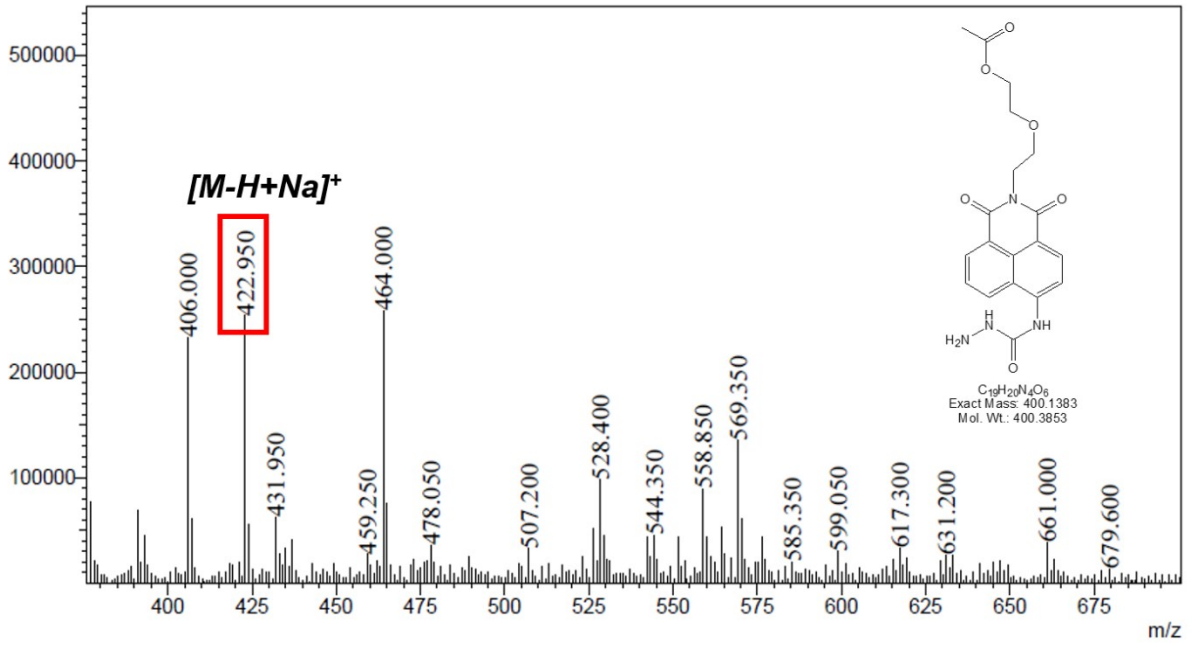


Figure S25. ESI-MS spectrum of 2.