Supporting information for

Development of a Near-infrared Fluorescent Probe for Imaging of Endogenous Cu^{+} in Live Cells

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Materials and instruments: Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan or Agilent 1100 HPLC/MSD spectrometer. NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard. Electronic absorption spectra were obtained on a Labtech UV Power PC spectrometer. Photoluminescent spectra were recorded at room temperature with a HITACHI F4600 fluorescence spectrophotometer with the excitation and emission slit widths at 5.0 and 5.0 nm respectively. Cell imaging was performed with a Nikon Eclipse TE300 inverted microscope. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

Synthesis of probe 3. Compound **1** (0.14 g, 0.20 mmol) and compound **2** [1] (0.20 g, 0.80 mmol) were dissolved in anhydrous DMF (20 mL), and the reaction mixture was heated at 80 ˚C for 4 hours under N_2 atmosphere. After removal of DMF under reduced pressure, the resulting residue was purified by flash column chromatography with ethanol/ CH_2Cl_2 = (0 to 1: 40) as eluent to obtain compound **3** as a blue solid (0.096 g, 0.21 mmol, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.80 (d, *J* = 13.6 Hz, 2H), 7.35 (t, *J* = 7.2 Hz, 4H), 7.16 (t, *J* = 7.6 Hz, 2H), 7.02 (d, *J* = 8.0 Hz, 2H), 5.85 (d, *J* = 13.2 Hz, 2H), 4.06-3.99 (m, 8H), 3.08 (t, *J* = 6.4 Hz, 4H), 2.80-2.72 (m, 8H), 2.59-2.53 (q, 4H), 2.50 (t, *J* = 6.4 Hz, 4H), 1.90-1.84 (m, 2H), 1.69 (s, 12H), 1.42 (t, *J* = 6.4 Hz, 6H), 1.24 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 173.6, 169.0, 142.8, 142.1, 140.6, 128.5, 124.0, 123.8, 122.2, 109.3, 96.2, 53.9, 48.4, 38.8, 32.3, 32.0, 31.8, 29.0, 26.1, 24.8, 21.8, 14.9, 11.9; MS-ESI m/z: 788.3 $[M\text{-}I^-]^+$. HRMS-ESI $[M\text{-}I^-]^+$ calcd for $C_{46}H_{66}N_3S_4^+$, 788.4134; Found 788.4137. Elemental analysis: calcd (%) for $C_{46}H_{66}IN_3S_4 \cdot H_2O$: C 59.14, H 7.34, N 4.50; found C 58.75, H 7.24, N 4.26. The presence of a water molecule is consistent with the hygroscopic nature of the compound.

Cell culture and fluorescence imaging. MG63 cells were seeded in a 12-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) for 24 h. Immediately before the experiments, the MG63 cells were washed with PBS buffer. The MG63 cells were incubated with $CuCl_2$ (0.20 mM) for 7 h or or ascorbic acid (1.0 mM) for 4 h at 37 °C. After washing three times with PBS buffer, the cells were then incubated with the probe **3** (5.0 μM) for 10 min at 37 °C. Subsequently, the cells were washed three times with PBS buffer. The fluorescence images were acquired with a Nikon Eclipse TE300 equipped with a CCD camera. In order to confirm whether the fluorescence turn-on response was indeed induced by Cu^+, membrane-permeable Cu^+ chelator, bis(2-((2-(ethylthio)ethyl)thio)ethyl)amine (BETA) (50 μM) was added to the cells

pretreated with $CuCl_2$ + probe **3** or with ascorbic acid + probe **3**. The fluorescence images were acquired with a Nikon Eclipse TE300 equipped with a CCD camera.

Preparation of the test solution. PBS buffer solution was prepared by dissolving $NaH_2PO_4 \cdot 2H_2O$ (1. 2 g, 7.5 mmol) and $Na_2HPO_4 \cdot 7H_2O$ (4.7 g, 17.5 mmol) in twice-distilled water (1.0 L), and the pH values were adjusted with 1 M NaOH or 1 M HCl. The stock solution of the probe **3** was prepared at 0.15 mM in ethanol. The solutions of various testing species were prepared from $CaCl_2$, $MgCl_2$ $CoCl_2 \cdot 6H_2O$, $CdCl_2 \cdot 1/2H_2O$, $CuCl_2 \cdot 2H_2O$, $FeCl_2$, $Pb(NO_3)_2$, $MnSO_4 \cdot H_2O$, $NiCl_2 \cdot 6H_2O$, $HgCl_2$ and $ZnCl_2$ in the twice-distilled water. $[Cu(MeCN)_4][PF_6]$ test solution (1.5 mM) was prepared in MeCN. The test solution of the probe **3** (2.5 μM) in 3 mL aqueous buffer (pH 7.0, 25 mM PBS buffer with 10% ethanol.) was prepared by placing 0.050 mL of the probe **3** stock solution and 0.25 mL ethanol in 2.7 mL of 25 mM PBS buffer (pH = 7.0). The test solution was shaken well at room temperature before recording the spectra.

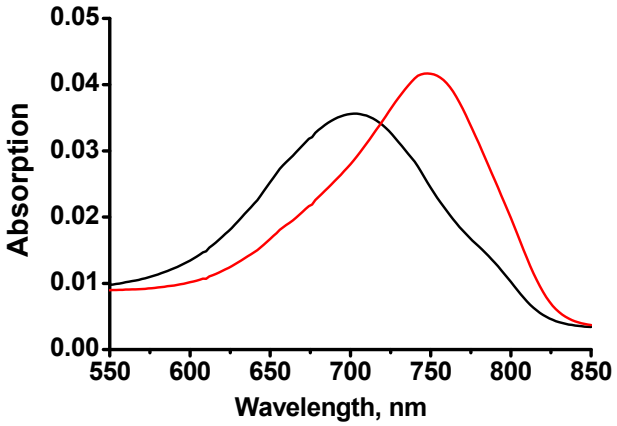

Figure S1. Absorption spectra of the probe **3** (2.5 μM) in the absence (black line) or the presence (red line) of Cu^+ (2.5 μM) in the aqueous buffer (pH 7.0, 25 mM PBS buffer with 10% ethanol.)

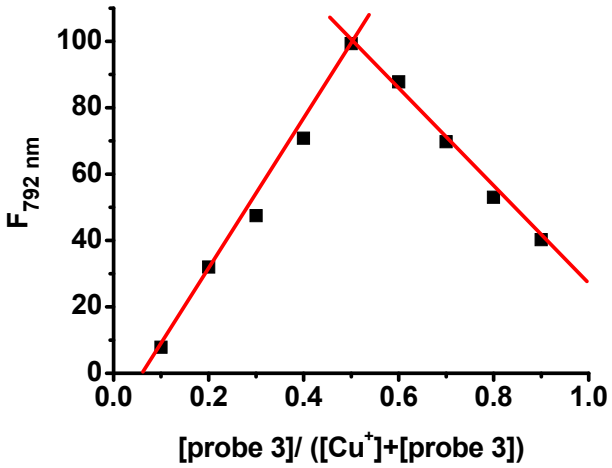

Figure S2. Job's plot of the probe **3** with Cu^{+} ions according to the method of continuous variations. Excitation at 750 nm. The total concentrations of the probe **3** and Cu^{+} were kept constant at 5.0 μM.

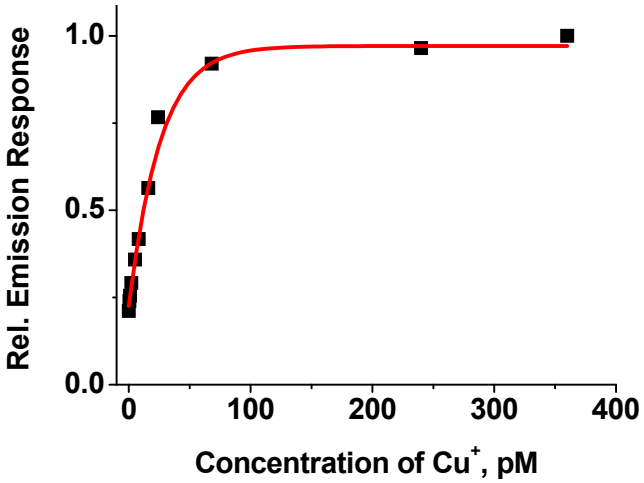

Figure S3. Normalized fluorescence response of the probe **3** (1.0 μM) to free Cu^{+} ions (0, 0.50, 1.0, 2.0, 5.0, 8.0, 16, 24, 68, 240, 360 pM.) for K_d value determination in a thiourea buffered solution (pH 7.0, 25 mM PBS buffer with 10% ethanol, [thiourea] = 100 μM.). Excitation at 750 nm. Emission at 792 nm. The observed K_d value is 6.1×10^{-12} M.

Determination of the Apparent Dissociation Constant: The apparent dissociation constant (K_d) for the probe **3**/Cu^+ complex was determined based on a procedure reported by Chang's group. [1] The fluorescence intensity was measured as a function of Cu^+ concentration in a buffered thiourea solution (pH 7.0, 25 mM PBS, [thiourea] = 100 μM). The apparent dissociation constant (K_d) was calculated by using the equation: $F - F_{min} = \Delta F = [Cu^+](F_{max} - F_{min})/(K_d + [Cu^+])$, where F is the observed fluorescence, F_{max} is the fluorescence for the probe **3**/Cu^+ complex, and F_{min} is the fluorescence for the free probe **3**. When plot the reciprocal of △F against the reciprocal of concentration of Cu^+, as shown in Figure S4, a linear relation equation was obtained: Y= A + BX. K_d was calculated from B/A. Free [Cu^+] was calculated by using the stability constants for thiourea: $\beta_{12} = 2.0 \times 10^{12}$, $\beta_{13} = 2.0 \times 10^{14}$, $\beta_{14} = 3.4 \times 10^{15}$. [2]

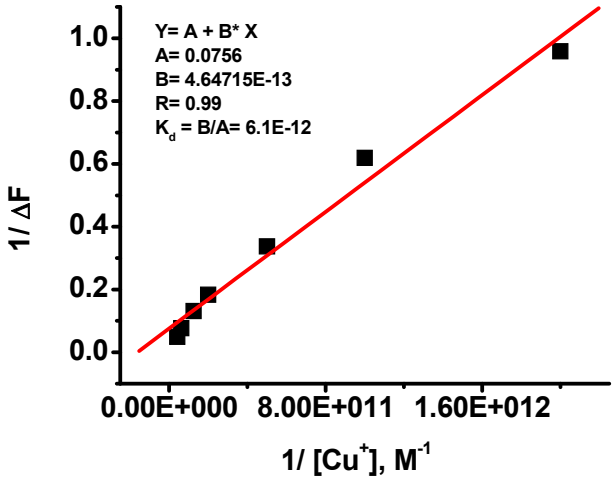

Figure S4. Benesi–Hildebrand plot for Cu^+-bound probe **3** (2.5 μM). Note: the Y axis denotes the reciprocal of △F, and the X axis denotes the reciprocal of the concentration of Cu^+.

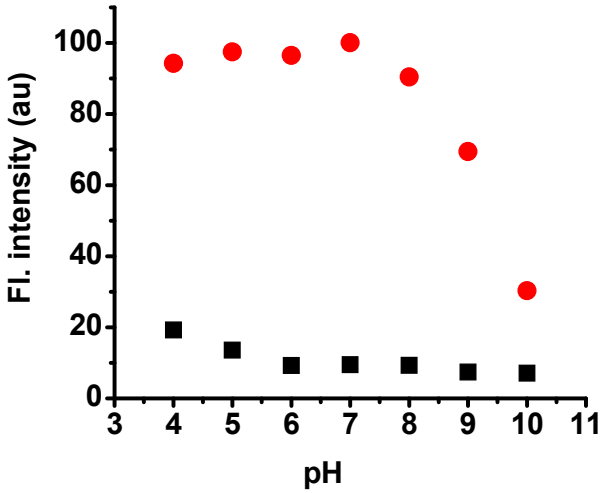

Figure S5. The pH influence on the fluorescence intensity of the probe **3** (2.5 μM) in the absence (■) or presence (●) of Cu^{+} (2.5 μM) in buffered solution (25 mM PBS buffer with 10% ethanol.). Excitation at 750 nm. Emission at 792 nm.

References:

1. L. Zeng, E. W. Miller, A. Pralle, E. Y. Isacoff, C. J. Chang, *J. Am. Chem. Soc.* **2006,** *128,* 10-11.
2. A. E. Martell, R. M. Smith, *Critical Stability Constants,* Plenum Press: New York, 1989.

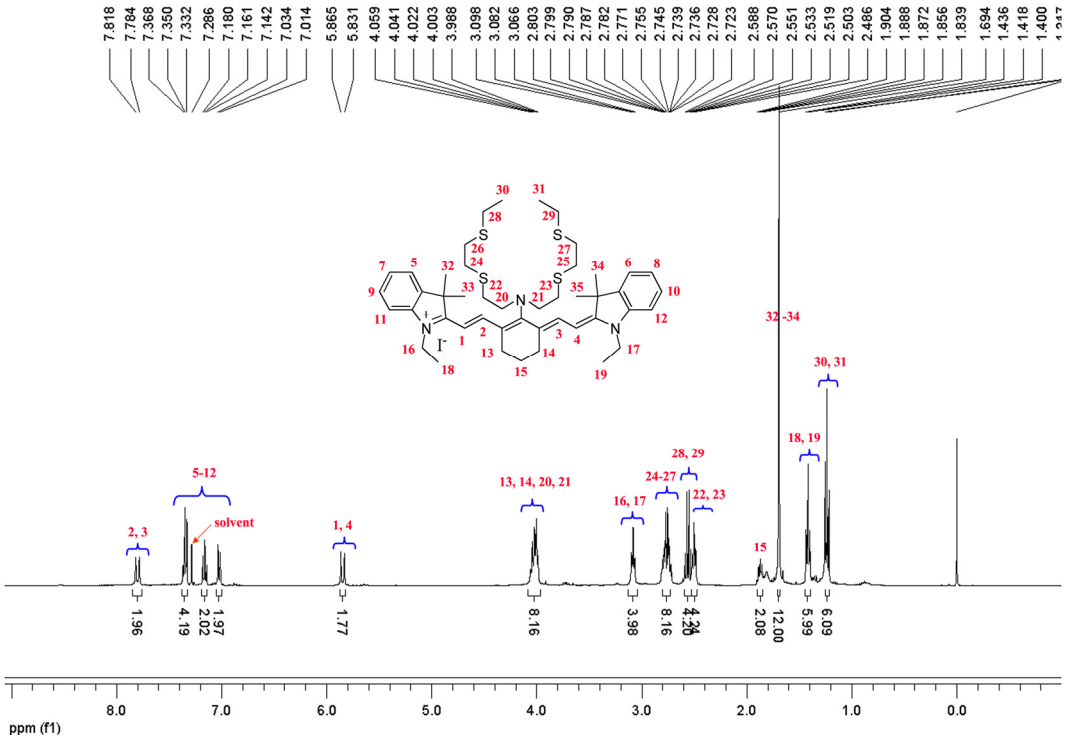

Figure S6. ^{1}H NMR spectrum of the probe **3**.

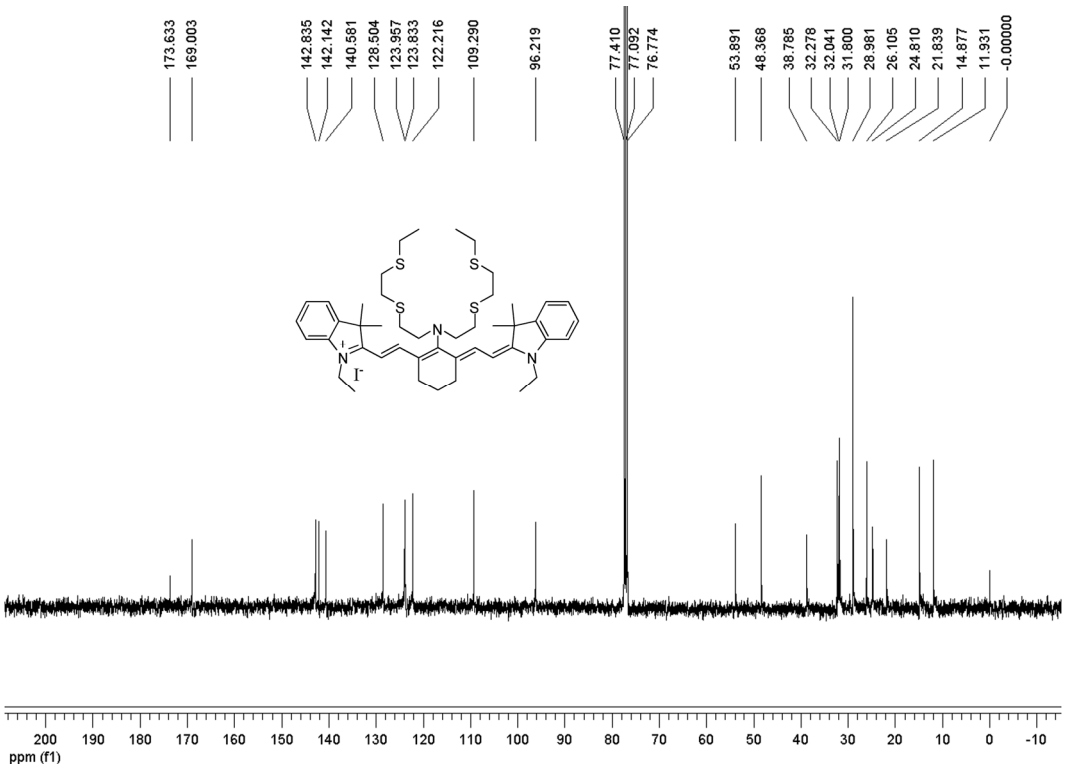

Figure S7. ^{13}C NMR spectrum of the probe **3**.