Electronic Supplementary Information for "A highly selective cyanide sensing in water via fluorescence change and its application to *in vivo* imaging"

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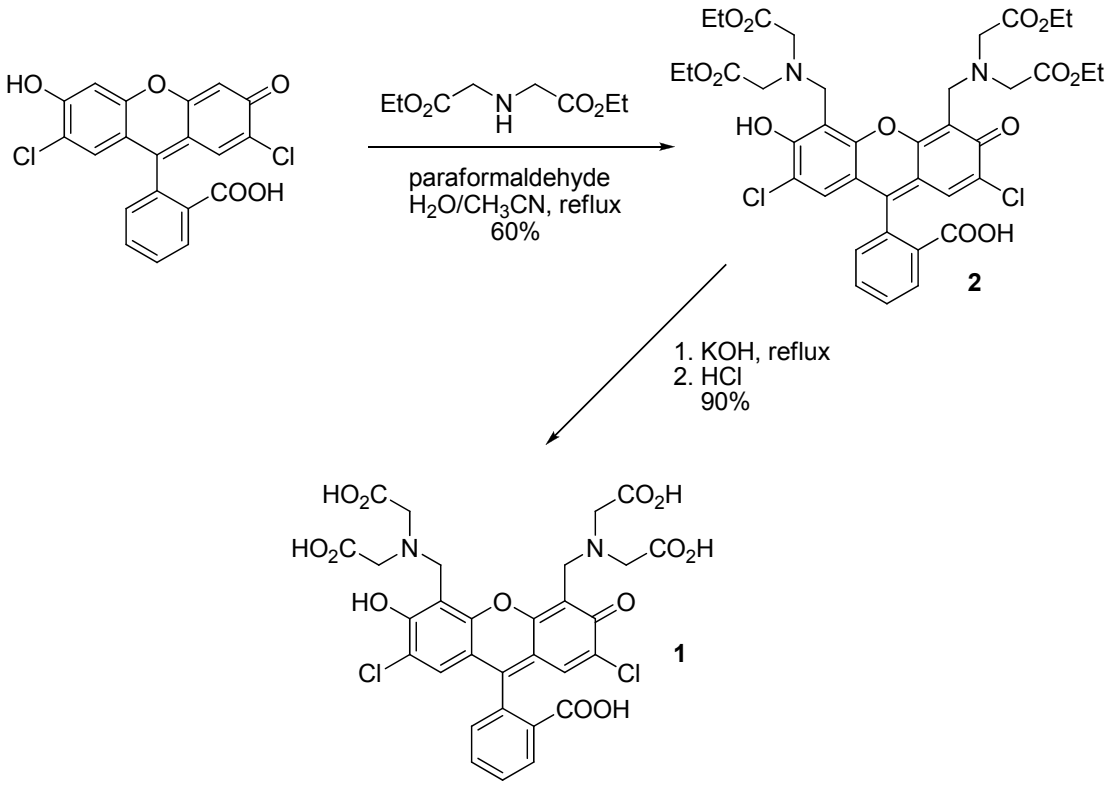
Experimental Section

General methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Flash chromatography was carried out on silica gel 60 (230-400 mesh ASTM; Merck). Thin layer chromatography (TLC) was carried out using Merck 60 F_{254} plates with a thickness of 0.25 mm. Preparative TLC was performed using Merck 60 F_{254} plates with a thickness of 1 mm. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 250 or Varian 500. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). UV absorption spectra were obtained on UVIKON 933 Double Beam UV/VIS Spectrometer. Fluorescence emission spectra were obtained using RF-5301/PC Spectrofluorophotometer (Shimadzu).

Synthesis



Sensor **1**[1] and intermediate **2**[2] were synthesized following the reported procedure.

Compound 2. Diethyl iminodiacetate (1.52 mL, 8.68 mmol) and paraformaldehyde (0.224 g, 7.47 mmol) were combined in 20 mL of CH_3CN and refluxed for 30 min. 2,7,-Dichlorofluorescein (**4**) (1.00 g, 2.49 mmol) in 30 mL of CH_3CN/H_2O (1:1) was added to the solution and the reaction mixture was refluxed for 24 h. The CH_3CN

was removed and the product and the residual water were triturated with 30 mL of boiling ethanol. After cooling to room temperature, 5 mL of ether was added to the solution. The product was precipitated at –4°C, filtered on a frit (1.20 g, 60%); ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 1H, *J* = 6.8 Hz), 7.69 (quintet, 2H, *J* = 7.4 Hz), 7.20 (d, 1H, *J* = 6.2 Hz), 6.69 (s, 2H), 4.30-4.53 (d, 4H, *J* = 14 Hz), 4.21 (q, 8H, *J* = 7.1 Hz), 3.54 (s, 8H), 1.28 (t, 12H, *J* = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ170.6, 169.0, 155.9, 151.6, 148.6, 135.6, 130.6, 128.3, 127.3, 125.8, 124.3, 117.9, 110.9, 109.9, 83.0, 61.6, 54.5, 49.1, 31.2, 14.4.

Compound 1. KOH was added to a stirred solution of **1** (500 mg, 0.62 mmol) in distilled water (25 mL). The reaction mixture was then refluxed for 12 h. After the reaction mixture was cooled to room temperature, 1 M HCl solution was added to make pH 2-3. The resulting precipitate was filtered and washed with small amount of cold distilled water. After drying at 90°C *in vacuo*, compound **1** was obtained in an analytically pure form (402 mg, 90%). m.p. 230 °C, dec.; ^{1}H-NMR (DMSO d_6) 8.03 (d, 1H, *J* = 7.3 Hz), 7.80 (m, 2H), 7.37 (d, 1H, *J* = 7.3 Hz), 6.63 (s, 2H), 4.30 (s, 4H), 3.55 (s, 8H); ^{13}C-NMR (D_2O) 181.4, 173.1, 171.6, 170.1, 155.6, 133.7, 133.4, 132.2, 130.5, 130.2, 130.1, 129.9, 127.4, 111.9, 105.2, 56.7, 50.1.

1. E. J. Jun, J. A. Kim, K. M. K. Swamy, S. Park and J. Yoon, *Tetrahedron Lett.,* 2006, **47**, 1051.
2. Y. J. Jang, E. J. Jun, Y. J. Lee, Y. S. Kim, J. S. Kim and J. Yoon, *J. Org. Chem.,* 2005, **70**, 9603.

Fluorescent study

Stock solutions (1 mM) of sodium salts of CN^-, SCN^-, $H_2PO_4^-$, HSO_4^-, NO_3^-, $CH_3CO_2^-$, F^-, Cl^-, Br^- and I^- were prepared in double distilled water. Stock solutions of host (0.01 mM) were also prepared in double distilled water. Test solutions were prepared by placing 4-40 μL of the probe stock solution into a test tube, adding an appropriate aliquot of each anion stock, and diluting the solution to 4 mL with HEPES (0.02 M, pH 7.4). Final concentration of the probe was either 6 μM or 6 μM. Perchlorate, chloride or acetate salts of copper (II) solution (1 mM) were prepared in double distilled water.
For all measurements, excitation was at 505 nm. Both excitation and emission slit widths were either 1.5 nm or 3 nm.

Fabrication and design of the microfluidic device and fluorescence imaging of sensor 1, copper ion and cyanide

The microfluidic device was fabricated using soft lithography with PDMS (polydimethyl siloxane).[3] The microfluidic device were composed of three major

functional parts: two loading channels, chaotic mixer, and an outlet for fluorescence measurement.[4] In each loading channel, an inlet port was connected to a syringe pump (PHD2000 Infusion, Harvard Apparatus, Holliston, MA, USA) which controlled the flow rate of loaded sample. The microchannel in the chaotic mixer had herring-bone shaped obstacles on its wall, which caused chaotic advection and enhanced mixing of laminar flows.[4] The outlet channel is a flat-wall one for the easiness in the fluorescence measurement. The channels were 250 μm wide,100 μm deep and the overall size of the microfluidic device was 9.60 mm long and 2.48 mm wide. The loading and outlet channels were ended by holes (600 μm diameter).
Fluorescence images of sensor **1** were captured under a fluorescent stereomicroscope (SMZ 1500, Nikon Co.) with a Peltier-cooled CCD camera (SPOT INSIGH™, Diagnostic instruments, Sterling Heights, MI, USA) and further analyzed using a Java-based image processing program, IMAGE-J (NIH, Bethesda, USA).

3. Y. Xia and G. M. Whitesides, *Annu. Rev. Mater. Sci.*, 1998. **28**, 153.
4. (a) S. K. Kwon, S. Kou, H. N. Kim, X. Chen, H. Hwang, S.-W. Nam, S. H. Kim, K. M. K. Swamy, S. Park and J. Yoon, *Tetrahedron Lett.*, 2008, **49**, 4102; (b) J.-A. Kim, H. Hwang, E. J. Jun, S.-W. Nam, K.-M. Lee, S. H. Kim, J. Yoon, S. Kang and S. Park, *Bull. Korean Chem. Soc.*, 2008, **29**, 225.

Fluorescence imaging of *C. elegans* exposed to sensor 1, copper ion and cyanide

The *C. elegans* wild-type strain N2 was obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, USA). Dozens of nematodes were maintained at 25℃ on NGM (nematode growth medium) agar plates (0.3% NaCl, 0.25% peptone, 2.5% agar, 5 mg/ml cholesterol, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM KH_2PO_4) previously seeded with *Escherichia coli* OP50 as described elsewhere.[5] The nematodes were synchronized by the alkali-bleaching method.[5] To evaluate the effect of copper and cyanide on the fluorescence of sensor **1** in *C. elegans*, the NGM agar plates including sensor-1, $Cu(ClO_4)_2$ and NaCN were prepared. In details, 250 μl of 12.48 μM sensor-1 in NGM buffer were dropped onto NGM agar plates previously and dried at 25℃ before transferring nematodes on the plates. After exposed to sensor for 3 h., the nematodes were washed three times with NGM buffer (0.3% NaCl, 5 mg cholesterol/ml, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM KH_2PO_4). Then, the half of worms were collected and prepared for microscopic imaging and the other half were transferred onto NGM plate containing 6.24 μM $Cu(ClO_4)_2$ and incubated for 3 h. at 25℃. After washing with NGM buffer, the half of the worms were prepared for observation and the other half were transferred to NGM plate containing of 12.48 μM NaCN and incubated for 3 h. at 25℃. After incubation, the worms were washed three times with NGM buffer before mounted onto a glass slide with Faramount™ aqueous mounting medium (Dako, Glostrup, Denmark). The fluorescent images of the

mounted nematodes were obtained by using a confocal laser scanning microscope (LSM510, Carl Zeiss) at 200 × magnifications.

5. M. J. Beanan and S. Strome, *Development*, 1992, **116**, 755.

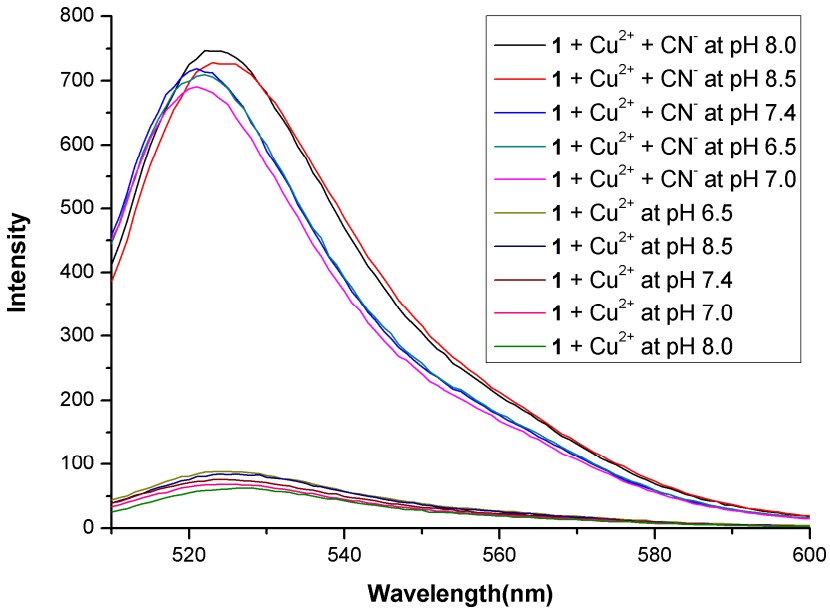


Figure-S1. Fluorescence changes of **1** (6 µM) in the presence of Cu^{2+} (1 eq.) and cyanide (100 eq.) at differential pH (pH 6.5; 0.02 M MOPS, pH 7.0; 0.02 M HEPES, pH 7.4; 0.02M HEPES, pH 8.0; 0.02M HEPES, pH 8.5; 0.02M phosphate buffer) (excitation at 505 nm).

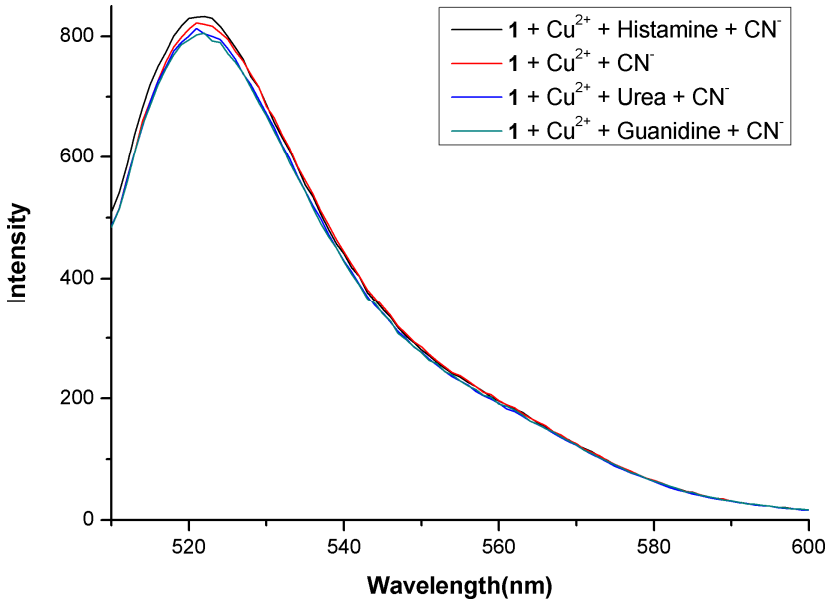


Figure-S2. Fluorescence changes of **1** (6 µM) with Cu^{2+} (1 eq.) and cyanide (100 eq.) in the presence of histamine, urea and guanidine (100 eq.) at pH 7.4 (0.02 M HEPES) (excitation at 505 nm).

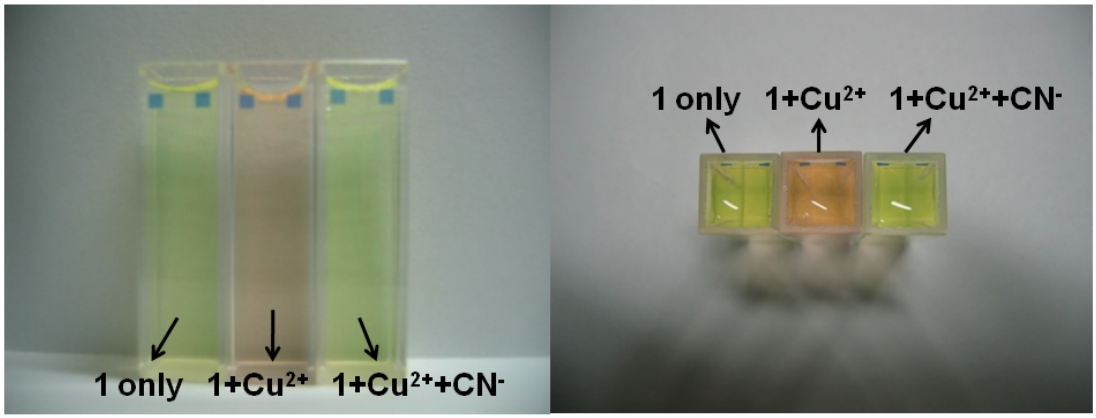


Figure-S3. Colorimetric changes of probe **1** (10 μM) with Cu^{2+} (1 eq.) and cyanide (100 eq.) at pH 7.4 (0.02 M HEPES).

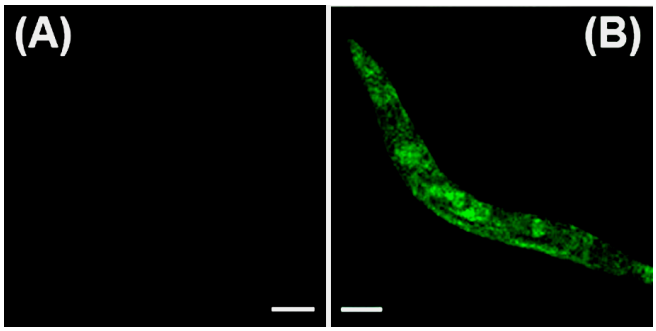


Figure-S4. The fluorescence images of *C. elegans*. (A) Control fluorescent image of *C. elegans* without incubation with probe **1**. (B) The fluorescent image of *C. elegans* previously exposed to copper sensor. Three-days-old young adult of *C. elegans* was incubated with 124.78 μM probe **1**. The scale bars represent 50 μm.