# A FRET-Based Indicator for Imaging Mitochondrial Zinc Ion

# **Supporting Information**

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### I. Materials and General Methods

Reagents and solvents were purchased from various commercial sources and used without further purification unless otherwise stated. CH<sub>3</sub>CN (OmniSolv, EMD) were directly used in titration experiments without purification. All reactions were carried out in oven- or flame-dried glassware. Analytical thin-layer chromatography (TLC) was performed using precoated TLC plates with silica gel 60 F254 (EMD) or with aluminum oxide 60 F254 neutral (EMD). Flash column chromatography was performed using 40-63 µm (230-400 mesh ASTM) silica gel (EMD) or alumina (80-200 mesh, pH 9-10, EMD) as the stationary phases. Silica and alumina gel was flame-dried under vacuum to remove absorbed moisture before use. <sup>1</sup>H NMR spectra were recorded at 300 MHz on a Varian Mercury spectrometer. <sup>13</sup>C NMR spectra were recorded at 125 MHz, on a Bruker Avance spectrometer. All chemical shifts were reported in  $\delta$ units relative to tetramethylsilane. High resolution mass spectra were obtained at the Mass Spectrometry Laboratory at FSU: ESI spectra were obtained on a JEOL AccuTOF spectrometer. Spectrophotometric and fluorometric titrations were conducted on a Varian Cary 100 Bio UV-Visible Spectrophotometer and a Varian Cary Eclipse Fluorescence Spectrophotometer, respectively, with a 1-cm standard quartz cell. The fluorescence quantum yield was determined by comparison of the integrated area of corrected emission spectrum with reference of  $N_{N}$ '-di-noctyl-2,6-di-*n*-octylamino-1,4,5,8-naphthalenetetracarboxylic acid diimide ( $\phi_f = 0.53$  in CH<sub>2</sub>Cl<sub>2</sub>) as the reference by the literature method.<sup>1</sup>

## **II.** Syntheses and Characterizations of New Compounds



#### 1. Synthetic scheme for compound 2

**Compound 7.** 2,6-Dibromonaphthalene-1,4,5,8-tetracarboxylic acid bisanhydride  $6^2$  (400 mg, 0.94 mmol) was suspended in glacial acetic acid (20 mL). To the stirred suspension 3-methoxypropylamine (1.0 mL, 9.7 mmol) was added slowly at rt and the mixture was kept at reflux at 120 °C for 10 min. The reaction mixture was cooled to rt and the resulting pale yellow precipitate was collected and washed with glacial acetic acid. The yield of **7** was 198 mg (37%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 8.99 (s, 2H), 4.32 (t, J =7.2 Hz, 4H), 3.52 (t, J = 6.0 Hz, 4H), 3.29 (s, 6H), 2.04 (m, 4H).

**Compound 2.** Compound **7** (50 mg, 0.09 mmol) and 3-methoxypropylamine (2.0 mL) were refluxed under an argon atmosphere for 1 h. Then the reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL) followed by extraction with a dilute HCl solution (0.5 M, saturated with NaCl) three times. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> before the solvent was removed under a reduced pressure and the obtained blue solid was purified by silica chromatography using THF in CH<sub>2</sub>Cl<sub>2</sub> (gradient 0-10%). The yield of **2** was 32 mg (63%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 9.40 (t, 2H), 8.10 (s, 2H), 4.26 (t, J = 7.2 Hz, 4H), 3.51-3.61 (m, 12H), 3.39 (s, 6H), 3.34 (s, 6H), 1.96-2.07 (m, 8H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, 25 °C) 166.1,163.1, 149.2, 125.7, 121.1, 118.3, 101.9, 70.8, 70.2, 59.0, 58.8, 40.6, 38.0, 29.6, 28.3. HRMS (ESI+): calcd. (M+Na<sup>+</sup>) 607.2744, found 607.2754.





**Compound 8.** Compound **7** (50 mg, 0.09 mmol) and 1-aminopentanol (100 mg, 0.97 mmol) were refluxed in THF for 1 h. The solvent was removed under a reduced pressure and the obtained red solid was purified by silica chromatography using THF in  $CH_2Cl_2$  (gradient 0-20%). The yield of **8** was 27 mg (53%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 10.12 (t, 1H), 8.08 (s, 1H), 8.23 (s, 1H), 4.25 (t, 4H), 3.68 (q, 2H), 3.56 (q, 2H), 3.51 (t, 4H), 3.29 (s, 6H), 1.93-2.04 (m, 4H), 1.84 (q, 2H), 1.56-1.68 (m, 4H). MS (ESI-): calcd. 589.4, found 589.4.

**Compound 9.** Compound **8** (20 mg, 0.03 mmol) and 3-methoxypropylamine (1.0 mL) were refluxed under an argon atmosphere for 1 h. Then the reaction mixture was diluted with  $CH_2Cl_2$  (50 mL) followed by extraction with a dilute HCl solution (0.5 M, saturated with NaCl) three times. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> before the solvent was removed under a reduced pressure and the obtained blue solid was purified by silica chromatography using THF in  $CH_2Cl_2$  (gradient 0-20%). The yield was 14 mg (70%). <sup>1</sup>H NMR (300 MHz,  $CDCl_3$ , 25 °C) 9.41 (t, 1H), 9.34 (t, 1H), 8.11 (s, 1H), 8.08 (s, 1H), 4.26 (t, J = 7.2 Hz, 4H), 3.71 (q, 2H), 3.47-3.63 (m, 10H), 3.39 (s, 3H), 3.35(s, 6H), 1.98-2.06 (m, 6H), 1.84(q, 2H), 1.53-1.68 (m, 4H). <sup>13</sup>C NMR (125 MHz,  $CDCl_3$ , 25 °C) 166.0, 165.9, 162.9, 162.8, 149.0, 148.9, 125.4, 120.8, 118.0, 101.6, 101.5, 70.6, 70.1, 62.6, 58.8, 58.6, 43.0, 40.4, 37.9, 32.3, 29.5, 29.1, 28.2, 23.4. HRMS (ESI+): calcd. (M+Na<sup>+</sup>) 621.2900, found 621.2919.

**Compound 10.** PPh<sub>3</sub> (42 mg, 0.16 mmol) and imidazole (11 mg, 0.16 mmol) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (4.0 mL) and cooled to 0 °C. Iodine (40 mg, 0.16 mmol) was added and stirred for 30 min. Compound **9** (50 mg, 0.08 mmol) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL) was added dropwise and stirred for 12 h at rt. The solvent was removed under a reduced pressure and the obtained blue solid was purified by silica chromatography using THF in CH<sub>2</sub>Cl<sub>2</sub> (gradient 0-20%). The yield of **10** was 41 mg (70%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 9.44 (t, 1H), 9.36 (t, 1H), 8.16 (s, 1H), 8.10 (s, 1H), 4.27 (t, J = 7.2 Hz, 4H), 3.48-3.64 (m, 10H), 3.39 (s, 3H), 3.35 (s, 6H), 3.22 (t, J = 6.6 Hz, 2H), 1.80-2.08 (m, 10H), 1.59-1.77 (m, 2H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, 25 °C) 166.3, 166.2, 163.2, 149.4, 149.2, 125.9, 121.3, 118.5, 118.3, 102.0, 70.8, 70.2, 59.0, 58.9, 58.8, 43.1, 40.6, 38.1, 33.3, 29.7, 28.6, 28.4, 28.3, 6.6. HRMS (ESI+): calcd. (M+H<sup>+</sup>) 709.2098, found 709.2138.

**Compound 3.** Compound **10** (10 mg, 0.014 mmol) and PPh<sub>3</sub> (8 mg, 0.028 mmol) were dissolved in dry CH<sub>3</sub>CN (5 mL) and heated at 60 °C, for 3 d. The solvent was removed under a reduced pressure and the obtained blue solid was purified by silica chromatography using CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub> (gradient 0-20%) and then washed several times with hexanes. The yield was 8.0 mg (62%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 9.39 (t, 1H), 9.23 (t, 1H), 8.07 (s, 1H), 7.94 (s, 1H), 7.70-7.89 (m, 15H), 4.23 (m, 4H), 3.78-3.87 (m, 2H), 3.41-3.61 (m, 10H), 3.37 (s, 3H), 3.30 (s, 6H), 1.78-2.04 (m, 12H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, 25 °C) 165.9, 162.8, 149.0, 148.8, 135.3, 133.9, 133.8, 130.8, 130.7, 125.5, 125.4, 120.9, 118.5, 118.1, 117.8, 101.6, 70.7, 70.1, 58.9, 58.7, 42.7, 40.5, 37.9, 29.6, 29.0, 28.2, 28.1, 22.6. HRMS (ESI+): calcd. (M-I<sup>-</sup>) 843.3886, found 843.3894.

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**Compound 11.** 4-Hydroxybenzaldehyde (1.0 g, 8.19 mmol), 2-t-BOC-aminoethylbromide (1.52 g, 6.87 mmol) and potassium carbonate (1.9 g, 13.75 mmol) were refluxed in acetone (20 mL). After 2 h the solution was cooled to rt and water (15 mL) was added. The aqueous solution was extracted with EtOAc ( $3 \times 30$  mL).The combined organic phases were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, concentrated in vacuo, and purified by flash chromatography on silica using CH<sub>2</sub>Cl<sub>2</sub> as eluent, affording 1.34 g (62%) product **11**. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 9.88 (s, 1H), 7.83 (d, J = 8.4 Hz, 2H), 6.69 (d, J = 9.0 Hz, 2H), 4.98 (s, broad, 1H), 4.10 (t, J = 4.8 Hz, 2H), 3.59 (m, 2H), 1.44 (s, 9H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, 25 °C) 190.9, 163.7, 156.0, 132.0, 130.1, 114.8, 79.6, 67.5, 39.9, 28.4.



**Compound 12.** Sodium hydride, (108 mg, 60% dispersion in mineral oil; 2.71 mmol) was suspended in dry THF (8.0 mL) in a flame-dried flask and it was cooled to 0 °C. Di(ethylene glycol)methyl ether ( 360 mg, 3.0 mmol) was added slowly and stirred under an argon atmosphere for 30 min. The solution was slowly added to 5,5'-bis(bromomethyl)-2,2'-dipyridyl<sup>3</sup> (1.0 g, 5.4 mmol) in THF (50 mL) and stirred for 2 h. Then most of the THF was removed under vacuum. The residue was partitioned between  $CH_2Cl_2$  and brine. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The crude product was purified using silica chromatography, using THF in  $CH_2Cl_2$  (gradient 0-10%). The yield of **12** was 1.1 g (53%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 8.68 (s, 1H), 8.63 (s, 1H), 8.38 (d, J = 8.1 Hz, 2H), 7.82-7.86 (m, 2H), 4.65 (s, 2H), 4.53 (s, 2H), 3.65-3.54 (m, 8H), 3.39 (s, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, 25 °C) 155.8, 154.9, 149.3, 148.6, 137.6, 136.6, 134.1, 133.6, 121.1, 121.0, 72.0, 70.7, 70.6, 69.9, 59.1, 29.7.

**Compound 13.** Compound **12** (700 mg, 1.83 mmol) was dissolved in  $(EtO)_3P$  (2.0 mL). The mixture was heated at 110 °C for 4 h. Excess of  $(EtO)_3P$  was removed under high vacuum in a fume hood. The residue was partitioned between EtOAc and NaHCO<sub>3</sub> (0.1 M). The organic

layer was washed with NaHCO<sub>3</sub> (0.1 M) twice before being dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The solvent was removed under a reduced pressure to afford analytically pure product. Yield: 657 mg (82%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 8.63 (s, 1H), 8.56 (s, 1H), 8.34 (d, J = 7.8 Hz, 2H), 7.75-7.84 (m, 2H), 4.64 (s, 2H), 4.06 (q, 4H), 3.55-3.70 (m, 8H), 3.88 (s, 3H), 3.19 (d, J = 22 Hz, 2H), 1.27 (t, J = 7.2 Hz, 6H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, 25 °C) 155.3, 154.6, 149.9, 148.6, 138.1, 136.4, 133.8, 128.0, 125.5, 120.7, 71.9, 70.6, 69.8, 62.4, 59.0, 31.5, 30.3, 16.4. HRMS (FAB) m/z: calcd (M+Na<sup>+</sup>) 461.1817, found 461.1824.

**Compound 14.** In a flame dried flask compounds **13** (300 mg, 0.68 mmol) and **11** (180 mg, 0.68 mmol) were dissolved in dry THF (20 mL) and cooled to -78 °C. Potassium bis(trimethylsilyl)amide (1.5 mL, 0.5 M in toluene, 0.75 mmol) was added dropwise. Upon completing the addition, the stirring was continued for 3 h while the temperature rose to rt. The reaction mixture was then partitioned between  $CH_2Cl_2$  and water. The aqueous layer was washed with  $CH_2Cl_2$  (50 mL × 3) and the organic portions were combined. The organic portions were dried over Na<sub>2</sub>SO<sub>4</sub> followed by solvent removal under vacuum. The compound was isolated via silica chromatography using 0-25% THF in  $CH_2Cl_2$ . The isolated yield of **14** was 160 mg (43%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 8.73 (s, 1H), 8.64 (s, 1H), 8.37 (d, J = 8.7 Hz, 2H), 7.95 (d, J = 7.8 Hz, 1H), 7.83 (d, J = 7.5 Hz, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 16.8 Hz, 1H), 7.00 (d, J = 16.2 Hz, 1H), 6.91 (d, J = 8.7 Hz, 2H), 5.01 (s, broad, 1H), 4.65 (s, 2H), 4.05 (t, J = 4.8 Hz, 2H), 3.75-3.54 (m, 10H), 3.39 (s, 3H), 1.46 (s, 9H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, 25 °C) 158.8, 155.9, 155.4, 154.4, 148.7, 148.0, 136.5, 133.6, 133.3, 133.2, 130.4, 129.9, 128.1, 122.8, 121.0, 120.7, 114.8, 79.6, 72.0, 70.7, 69.8, 67.3, 59.1, 40.1, 28.4. HRMS (FAB) m/z: calcd (M+Na<sup>+</sup>) 572.2737, found 572.2746.

**Compound 15.** Compound **14** (100 mg, 0.18 mmol) dissolved in  $CH_2Cl_2$  (2.0 mL) was slowly added to trifluoroacetic acid (5.0 mL) at 0 °C. The reaction mixture was stirred at rt for 12 h. Then the reaction mixture was diluted with  $CH_2Cl_2$  (50 mL) followed by extraction with a NaOH solution (0.5 M, saturated with NaCl) three times. The organic layer was dried over  $K_2CO_3$  before the solvent was removed under vacuum to afford analytically pure product **15**. Yield: 75 mg (92%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 8.73 (s, 1H), 8.63 (s, 1H), 8.37 (d, J = 8.1 Hz, 2H), 7.94 (d, J = 8.4 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.48 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 16.2 Hz, 1H), 6.99 (d, J = 16.2 Hz, 1H), 6.92 (d, J = 9.0 Hz, 2H), 4.65 (s, 2H), 4.02 (t, J = 4.8 Hz, 2H), 3.55-3.71 (m, 8H), 3.39 (s, 3H), 3.10 (t, J = 4.8 Hz, 2H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>,

25 °C) 159.2, 155.5, 154.4, 148.7, 147.9, 136.5, 133.6, 133.4, 133.1, 130.5, 129.7, 128.1, 122.6, 121.0, 120.7, 114.8, 72.0, 70.7, 70.3, 69.8, 59.1, 41.6. HRMS (FAB) m/z: calcd (M+Na<sup>+</sup>) 472.2212, found 472.2220.

**Compound 16.** Compound **7** (63 mg, 0.11 mmol) and **15** (50 mg, 0.11 mmol) were refluxed in THF for 1 h. The solvent was removed under a reduced pressure and the obtained red solid was purified by silica chromatography using THF in CH<sub>2</sub>Cl<sub>2</sub> (gradient 0-10%). The yield of **16** was 44 mg (42%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 10.43 (t, 1H), 8.87 (s, 1H), 8.72 (s, 1H), 8.63 (s, 1H), 8.45 (s, 1H), 8.37 (d, J = 7.8 Hz, 2H), 7.94 (d, J = 6.6 Hz, 1H), 7.82 (d, J = 6.9 Hz, 1H), 7.48 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 16.8 Hz, 1H), 6.99 (d, J = 16.8, 1H), 6.95 (d, J = 8.4 Hz, 2H), 4.65 (s, 2H), 4.27-4.38 (m, 6H), 4.04 (q, 2H), 3.70-3.65 (m, 6H), 3.58-3.49 (m, 6H), 3.39 (s, 3H), 3.33 (s, 6H), 2.03 (m, 4H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, 25 °C) 165.9, 162.0, 161.9, 161.5, 158.3, 155.4, 154.5, 152.0, 148.7, 148.0, 138.4, 136.5, 133.7, 133.2, 130.3, 130.2, 128.6, 128.1, 127.4, 123.6, 123.4, 123.0, 121.7, 121.0, 120.7, 120.5, 115.0, 100.7, 72.0, 70.7, 70.6, 69.9, 66.7, 59.1, 58.7, 58.6, 42.6, 39.1, 38.1, 28.1, 28.0. HRMS (ESI) m/z: calcd (M+H<sup>+</sup>) 936.2819, found 936.2793.

**Compound 4.** Compound **16** (25 mg, 0.026 mmol) and 3-methoxypropylamine (2.0 mL) were refluxed under an argon atmosphere for 1 h. Then the reaction mixture was diluted with  $CH_2Cl_2$  (50 mL) followed by extraction with a dilute HCl solution (0.5 M, saturated with NaCl) three times. The organic layer was dried over  $K_2CO_3$  before the solvent was removed under a reduced pressure and the obtained blue solid was purified via silica chromatography using THF in  $CH_2Cl_2$  (gradient 0-30%).Yield of **4** was 15 mg (62%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 9.66 (t, 1H), 9.45 (t, 1H), 8.72 (s, 1H), 8.63 (s, 1H), 8.36 (d, J = 9.0 Hz, 2H) 8.20 (s, 1H), 8.12 (s, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.48 (d, J = 9 Hz, 2H), 7.16 (d, J = 16.8 Hz, 1H), 6.95 (d, J = 16.8 Hz, 1H), 6.97 (d, J = 8.4 Hz, 2H), 4.64 (s, 2H), 4.28-4.33 (m, 6H), 3.94 (q, 2H), 3.49-3.71 (m, 16H), 3.39 (s, 6H), 3.35 (s, 6H), 2.02 (m, 6H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, 25 °C) 166.2, 166.0, 163.0, 158.6, 155.4, 149.4, 148.9, 148.7, 148.0, 136.5, 133.6, 133.3, 133.1, 130.4, 130.1, 128.1, 125.8, 125.6, 122.8, 121.4, 121.1, 121.0, 120.7, 118.6, 118.0, 115.0, 102.6, 101.7, 72.0, 70.7 70.6, 70.0, 69.8, 66.7, 59.1, 58.9, 58.6, 42.5, 40.4, 37.9, 29.5, 28.2. HRMS (ESI) m/z: calcd (M+H<sup>+</sup>) calcd 945.4398, found 945.4387.



4. Synthetic scheme for compound 5

**Compound 17.** Compound **16** (20 mg, 0.02 mmol) and 1-aminopentanol (20 mg, 0.2 mmol) were dissolved in DMF (2.0 mL) and heated at 110 °C under an argon atmosphere for 1 h. The solvent was removed under a reduced pressure. The blue residue obtained was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) followed by extraction with a dilute HCl solution (0.5 M, saturated with NaCl) three times. The organic layer was dried over anhydrous  $Na_2SO_4$  before the solvent was removed under a reduced pressure and the obtained blue solid was purified by silica chromatography using THF in CH<sub>2</sub>Cl<sub>2</sub> (gradient 0-50%). The yield of **17** was 11 mg (53%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 9.66 (t, 1H), 9.38 (t, 1H), 8.72 (s, 1H), 8.63 (s, 1H), 8.37 (d, J = 8.4 Hz, 2H), 8.21 (s, 1H), 8.09 (s, 1H), 7.93 (d, J = 10.8 Hz, 1H), 7.81 (d, J = 10.8 Hz, 1H), 7.47 (d, J = 9.0 Hz, 2H), 7.17 (d, J = 16.2 Hz, 1H), 7.00 (d, J = 16.2 Hz, 1H), 6.96 (d, J = 9.0 Hz, 2H),4.64 (s, 2H), 4.25-4.34 (m, 6H), 3.94 (q, 2H), 3.57-3.71 (m, 8H), 3.48-3.57 (m, 8H), 3.39 (s, 3H), 3.34 (s, 6H), 2.02 (m, 4H), 1.86 (q, 2H), 1.61-1.70 (m, 4H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>, 25 °C) 166.3, 163.2, 158.8, 155.6, 154.6, 149.4, 149.1, 148.8, 148.1, 136.7, 133.8, 133.5, 133.3, 130.5, 130.3, 128.2, 126.0, 125.8, 123.0, 121.5, 121.2, 121.1, 120.9, 118.6, 118.2, 115.2, 102.7, 101.7, 72.2, 70.9, 70.8, 70.0, 66.9, 62.8, 59.3, 58.8, 43.2, 42.6, 38.1, 32.5, 29.3, 28.4, 23.6. HRMS (ESI) m/z: calcd (M+H<sup>+</sup>) 959.4554, found 959.4554.

**Compound 18.** PPh<sub>3</sub> (6.0 mg, 0.02 mmol) and imidazole (2.0 mg, 0.02 mmol) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (4.0 mL) and cooled to 0 °C. Iodine (5.0 mg, 0.02 mmol) was added and stirred for 10 min. Compound **17** (10 mg, 0.01 mmol) dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> was added dropwise and stirred for 12 h at rt overnight. The solvent was removed under a reduced pressure and the obtained blue solid was purified by silica chromatography using THF in CH<sub>2</sub>Cl<sub>2</sub> (gradient 0-30%). The yield of **18** was 6.0 mg (54%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) 9.68 (t, 1H), 9.39 (t, 1H), 8.73 (s, 1H), 8.67 (s, 1H), 8.37 (d, J = 8.4 Hz, 2H), 8.22 (s, 1H), 8.09 (s, 1H), 8.01 (d, J = 10.8 Hz, 1H), 7.81 (d, J = 10.8 Hz, 1H), 7.48 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 16.2 Hz, 1H), 6.96 (m, 3H), 4.65 (s, 2H), 4.25-4.34 (m, 6H), 3.93 (m, 2H), 3.61-3.73 (m, 6H), 3.46-3.59 (m, 8H), 3.38 (s, 3H), 3.35 (s, 6H), 3.23 (t, J = 6.6 Hz, 2H), 1.86-2.00 (m, 6H), 1.72-1.58 (m, 4H). HRMS (ESI) m/z: calcd (M+H<sup>+</sup>) calcd 1069.3572, found 1069.3562.

**Compound 5.** Compound **18** (6.0 mg, 0.006 mmol) and PPh<sub>3</sub> (3.0 mg, 0.012 mmol) were dissolved in dry CH<sub>3</sub>CN (4.0 mL) and heated at 60 °C for 3 d. The solvent was removed under a reduced pressure and the obtained blue solid was purified via silica chromatography using CH<sub>3</sub>OH in CH<sub>2</sub>Cl<sub>2</sub> (gradient 0-30%). The yield of product was 4.0 mg (59%).<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, 25 °C) 9.67 (t, 1H), 9.37 (t, 1H), 8.72 (s, 1H), 8.63 (s, 1H), 8.37 (d, J = 8.4 Hz, 2H), 8.35 (s, 1H), 8.22 (s, 1H), 7.94 (d, J = 10.8 Hz, 1H), 7.93-7.60 (m, 16H), 7.47 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 16.2 Hz, 1H), 7.02 (d, J = 16.2 Hz, 1H), 6.97 (d, J = 9.0 Hz, 2H), 4.64 (s, 2H), 4.24-4.33 (m, 8H), 3.95 (m, 2H), 3.62-3.73 (m, 6H), 3.58 (m, 8H), 3.38 (s, 3H), 3.34 (s, 6H), 1.86-2.00 (m, 6H), 1.58-1.72 (m, 4H). HRMS (ESI) m/z: calcd (M-I) 1203.5360, found 1203.5312.

Compound	λ <sub>abs</sub> [nm]	$\epsilon/10^3  [M^{-1}  cm^{-1}]$	$\lambda_{em}$ [nm]	φ <sub>f</sub>
1	345	34	428	$0.28^{a}$
$[Zn(1)]^{2+,b}$	374	28	528	$0.46^{a}$
2	607	22	638	0.51 <sup>c</sup>
3	607	23	639	$0.48^{c}$
4	346 (D) 600 (A)	76 22	633	0.41 <sup>c</sup>
$[Zn(4)]^{2+, d}$	360 (D) 600 (A)	55 21	633	0.31 <sup>e</sup>
5	347 (D) 602 (A)	81 23	633	0.28 <sup>c</sup>
$[Zn(5)]^{2+, d}$	363 (D) 602 (A)	67 21	633	0.29 <sup>e</sup>

III. Table S1. Spectroscopic data of 1-5 and ZnCl<sub>2</sub> complexes of 1, 4 and 5 in CH<sub>3</sub>CN.

[a]  $\lambda_{ex} = 365$  nm, [b] data taken from previous work.<sup>4</sup> [c]  $\lambda_{ex} = 565$  nm, [d] Zn(ClO<sub>4</sub>)<sub>2</sub> was added in 10 equiv. relative to **4** or **5**. [e]  $\lambda_{ex} = 400$  nm.

## **IV. Additional figures**



**Fig. S1** Normalized emission spectra of **1** (blue),  $[Zn(1)]^{2+}$  (green), and absorption spectrum of **2** (red). The shaded area represent the spectral overlap between the emission of  $[Zn(1)]^{2+}$  and absorption of **2**.



**Fig. S2** Fluorescence intensity increase (F/F<sub>0</sub> at 630 nm) of **4** (3.0  $\mu$ M), with increasing [ZnCl<sub>2</sub>]. The solid line is the fitting curve of F/F<sub>0</sub> *vs.* [Zn<sup>2+</sup>] based on a 1:1 binding isotherm equation.<sup>5</sup> Dissociation constant K<sub>d</sub> = 9.1  $\mu$ M.



Fig. S3 Fluorescence spectral change of 4 (2.5  $\mu$ M) with different concentrations of ZnCl<sub>2</sub> (0-16  $\mu$ M) in CH<sub>3</sub>CN ( $\lambda_{ex} = 600$  nm). Inset shows variation of fluorescence intensity at 630 nm as a function of [Zn<sup>2+</sup>].



Fig. S4 Ratio of emission intensity at 630 nm on excitation of 4 (3.0  $\mu$ M) at 400 and 600 nm *vs*. [ZnCl<sub>2</sub>] in CH<sub>3</sub>CN.



Fig. S5 Effect of addition of ZnCl<sub>2</sub> (0-10  $\mu$ M) on emission spectrum of an equimolar mixture of 1 (2.5  $\mu$ M) and 2 (2.5  $\mu$ M) in CH<sub>3</sub>CN.  $\lambda_{ex} = 400$  nm.



Fig. S6 Excitation spectrum of 4 (2.5  $\mu$ M,  $\lambda_{em}$  = 640 nm) in presence of ZnCl<sub>2</sub> (16  $\mu$ M) in CH<sub>3</sub>CN.



Fig. S7 Fluorescence spectroscopic responses of 4 (3.0  $\mu$ M,  $\lambda_{ex}$  400 nm) to various metal ions in 1:9 water/CH<sub>3</sub>CN mixture at pH 7.2 (30 mM of MOPS buffer). Black bars represent intensity in the presence of various metal ions (perchlorate salt). Metal ion concentrations of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> were 1.0 mM and for other ions 30  $\mu$ M. Red bars represent the fluorescence following subsequent addition of Zn(ClO<sub>4</sub>)<sub>2</sub> (30  $\mu$ M).



**Fig. S8** Absorption spectra for the titration of **4** (2.5  $\mu$ M) with ZnCl<sub>2</sub> (0-22  $\mu$ M) in water-CH<sub>3</sub>CN (1:9) mixture (MOPES buffer; 10 mM, pH = 7.2). Insert shows variation of absorbance at 400 nm as a function of [Zn<sup>2+</sup>].



**Fig. S9** (A) Emission spectra for the titration of **4** (2.5  $\mu$ M) with ZnCl<sub>2</sub> (0-22  $\mu$ M) in water-CH<sub>3</sub>CN (1:9) mixture (MOPES buffer, 10 mM, pH = 7.2,  $\lambda_{ex}$  = 400 nm). Inset shows variation of absorbance at 640 nm as a function of [Zn<sup>2+</sup>].



Fig. S10 Effect of pH on emission spectra of 1 (red line at pH = 10.42 and blue line at pH = 2.68). Inset shows variation of fluorescence intensity at 460 nm *vs*. pH ( $\lambda_{ex}$  = 380 nm).



Fig. S11 Absorption spectra for the titration of indicator 5 (2.4  $\mu$ M) with Zn(ClO<sub>4</sub>)<sub>2</sub> (0-7.4  $\mu$ M) in CH<sub>3</sub>CN.



Fig. S12 (A) Fluorescence spectral change of 5 (2.4  $\mu$ M) with different concentrations of Zn(ClO<sub>4</sub>)<sub>2</sub> (0-14  $\mu$ M) in CH<sub>3</sub>CN ( $\lambda_{ex} = 400$  nm).



**Fig. S13** (A) Absorption spectra of **5** (2.5  $\mu$ M) with ZnCl<sub>2</sub> (0-32  $\mu$ M) in water-CH<sub>3</sub>CN (1:9) mixture (MOPES buffer; 10 mM, pH = 7.2).



Fig. S14 Emission spectra of 5 (2.5  $\mu$ M) with ZnCl<sub>2</sub> (0-32  $\mu$ M) in water-CH<sub>3</sub>CN (1:9) mixture (MOPES buffer; 10 mM, pH = 7.2,  $\lambda_{ex}$  = 400 nm).

### V. Fluorescence Microscopy

The localization properties of 2-5 were determined via costaining experiments using mCerulean3 TOMM-20, a mitochondrial-specific fusion of the cyan fluorescent protein (CFP) mCerulean3. mCerulean3 is a bright monomeric FP with an emission maximum of 475 nm. HeLa S3 cells were seeded onto Bioptechs Delta-T dishes 48 h prior to experimentation, then transfected about 24 h prior to loading the indicator using an Effectene Transfection Reagent Kit (Qiagen) and 1  $\mu$ g of DNA. A 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 (Invitrogen) supplemented with 12.5% fetal bovine serum was used as the culture medium. On the day of experimentation cells were rinsed twice with media and incubated with indicator **5** (1.8  $\mu$ M) for 30 min. Prior to imaging cells were again rinsed twice with media, then provided with 1 mL of fresh media.

All cells were imaged using an Olympus Fluoview FV1000 confocal laser scanning microscope with an Olympus PLAPO 60x oil-immersion objective (NA = 1.4). During experimentation cells were maintained at 37 °C and 5.0% CO<sub>2</sub> using a Bioptechs Delta T4 Culture Dish Controller. Individual cells were imaged sequentially in two channels, using a 543 nm Helium-Neon laser line to excite the indicator and a 405 nm diode laser line to excite the FP. Cells were selected by the quality of FP expression, as the indicators tended to load homogenously into the cells. All data was collected using FluoView Software (Olympus). Laser power and detection settings were optimized for each image to fill LUTs. A pinhole size of 200  $\mu$ m was used for each image. Merged-channel images were produced using Elements software (Nikon).

For performing zinc(II) supplementation experiments, HeLa S3 cells were seeded onto Bioptechs Delta-T dishes approximately 24 h prior to the addition of ZnCl<sub>2</sub>. The culture medium is the same as used for the colocalization experiments (1:1 DMEM to Ham's F12 with Fetal Bovine Serum). Cells were rinsed twice with media and incubated with fluorescent indicator **5** (1.8  $\mu$ M) for 30 min. The dish was again rinsed twice with media and incubated an additional 10 min in 1 mL of media, with solutions containing either 50  $\mu$ M ZnCl<sub>2</sub> and 5  $\mu$ M sodium pyrithione or 0  $\mu$ M ZnCl<sub>2</sub> and 5  $\mu$ M sodium pyrithione. For each experiment, laser power and detection settings were optimized to fill LUTs for the first cell imaged in the presence of 50  $\mu$ M ZnCl<sub>2</sub>, with the same settings being used for each subsequent image and for cells imaged without supplemental ZnCl<sub>2</sub>.



Fig. S15 Confocal fluorescence images of live HeLa (S3) cells transfected with mCer3 TOMM20 that were incubated with 3 (9.0  $\mu$ M, A-C) and 2 (52  $\mu$ M, D-F) for 30 min. Left: Green channel,  $\lambda_{ex}$  405 nm,  $\lambda_{em}$  425-475 nm; Middle: red channel,  $\lambda_{ex}$  543 nm,  $\lambda_{em}$  580-680 nm; Right: merged image. Scale bar: 10  $\mu$ m.



Fig. S16 DIC and fluorescence overlay images of HeLa (S3) cells incubated with compound 5 (1.8  $\mu$ M). (A) No zinc(II) added, and (B) in the presence of 50  $\mu$ M ZnCl<sub>2</sub>. A 405 nm diode laser line was used for excitation.  $\lambda_{em}$  580-680 nm. Scale bar: 10  $\mu$ m. The gray scale fluorescence images are shown in Fig. 5.

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