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Supporting Information

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A Bright and Specific Fluorescent Sensor for Mercury in Water, Cells, and Tissue

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Synthetic Materials and Methods. Silica gel P60 (SiliCycle) was used for column chromatography. Analytical thin layer chromatography was performed using SiliCycle 60 F254 silica gel (precoated sheets, 0.25 mm thick). 3,6-Bis[[(1,1-dimethylethyl)dimethylsilyl]oxy]-9H-xanthen-9-one (**6**) was prepared according to literature procedures.[1] Anhydrous DMF was purchased from Acros Organics (Morris Plains, NJ) and was used as received. Cesium carbonate was purchased from Alfa Aesar (Ward Hill, MA) and was used as received. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and were used as received. ^{1}H NMR spectra were collected in $CDCl_3$ or d^6-DMSO (Cambridge Isotope Laboratories, Cambridge, MA) at 25 ºC using either a Bruker AV-300, Bruker AVQ-400, or Bruker AVB-400 spectrometer at the College of Chemistry NMR Facility at the University of California, Berkeley. All chemical shifts are reported in the standard δ notation of parts per million using the peak of residual proton or carbon signals of $CDCl_3$ or d^6-DMSO as an internal reference. Low- and high-resolution mass spectral analyses were carried out at the College of Chemistry Mass Spectrometry Facility at the University of California, Berkeley. Combustion analyses were performed at the Microlab facility at the University of California, Berkeley.

2-[(4-Bromo-3-methylphenyl)-(2-hydroxyethyl)amino]ethanol (2). Under a nitrogen atmosphere, a mixture of *N*,*N*-di(2-hydroxyethyl)-*m*-toluidine (**1**, 20.2 g, 104 mmol) and tetraethylammonium bromide (1.7 g, 8.09 mmol) was dissolved in methanol (2 mL) and dichloromethane (100 mL) and cooled to 0 °C in an ice bath. A solution of bromine (16.5 g, 103 mmol) in dichloromethane (100 mL) was added dropwise to the toluidine/bromide solution while maintaining the reaction temperature at 0 °C. The reaction was allowed to warm slowly to room temperature and stirred overnight under nitrogen. Triethylamine (2.2 mL) was then added, the solution was filtered through silica plug, and the volatiles were removed under reduced pressure. Purification by column chromatography (silica gel, ethyl acetate) delivered **2** as a white solid (27 g, 95% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.27 (1 H, d, *J* = 8.8 Hz), 6.52 (1 H, d, *J* = 3.0 Hz), 6.35 (1 H, dd, *J* = 8.8, 3.0 Hz), 3.75 (4 H, t, *J* = 4.8 Hz), 3.47 (4 H, t, *J* = 4.8 Hz), 2.31 (3 H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 147.30, 138.45, 132.80, 115.11, 112.04, 111.84, 60.81, 55.49, 23.64. HRFAB-MS: Calcd for [M^+]: $C_{11}H_{16}BrNO_2$, 273.0364; Found, 273.0365.

(4-Bromo-3-methylphenyl)-*N*,*N*-bis[2-(*p*-toluenesulfonyl)ethyl]aniline (3). Solutions of diol **2** (26.9 g, 98.2 mmol) in THF (60 mL) and sodium hydroxide (11.2 g, 280 mmol) in water (60 mL) were mixed in a flask and cooled to 0 °C in an ice bath. A solution of *p*-toluenesulfonyl chloride (37.5 g, 196 mmol) in THF (60 mL) was added dropwise to the basic diol solution while maintaining the reaction temperature < 5 °C. The reaction was stirred for an additional 28 h and poured into an ice-water mixture (200 g), and the THF was evaporated under reduced pressure. The reaction was extracted with dichloromethane (5 × 50 mL), the organic layer was dried over Na_2SO_4, and the solvent was removed by

rotary evaporation. Purification by column chromarography (silica gel, 1:2 hexanes/ethyl acetate) afforded **3** as a white crystalline material (24.7 g, 43% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.66 (4 H, d, *J* = 8.3 Hz), 7.24 (4 H, d, *J* = 8.3 Hz), 7.17 (1 H, d, *J* = 8.8 Hz), 6.30 (1 H, d, *J* = 2.6 Hz), 6.12 (1 H, dd, *J* = 8.8, 2.6 Hz), 4.05 (4 H, t, *J* = 5.8 Hz), 3.51 (4 H, t, *J* = 5.8 Hz), 2.40 (6 H, s), 2.24 (3 H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 145.01, 138.67, 132.96, 132.57, 130.04, 127.99, 114.83, 112.82, 111.72, 66.47, 50.56, 23.51, 21.00. HRFAB-MS: Calcd for [M^+]: $C_{25}H_{28}BrNO_6S_2$, 581.0541; Found, 581.0553.

13-(4-Bromo-3-methylphenyl)-1,4,7,10-tetrathia-13-azacyclopentadecane (5). To a mixture of cesium carbonate (4.24 g, 13.0 mmol) suspended in dry DMF (250 mL) at 60 °C under a nitrogen atmosphere was added dropwise a solution of ditosyl **3** (5.80 g, 9.96 mmol) and 3,6-dithiaoctane-1,8-dithiol **4** (2.14 g, 9.98 mmol) in dry DMF (500 mL). After the addition was completed, the reaction was stirred at 60 °C under nitrogen for an additional 2 days and the solvent was removed under reduced pressure. The remaining residue was stirred with water (200 mL) and extracted with dichloromethane (5 × 100 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed by rotary evaporation. Purification by flash column chromatography (silica gel, 2:1 dichloromethane/hexanes) gave macrocycle **5** as a white powder (2.30 g, 48% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32 (1 H, d, *J* = 8.8 Hz), 6.50 (1 H, d, *J* = 3.0 Hz), 6.35 (1 H, dd, *J* = 8.8, 3.0 Hz), 3.56-3.50 (4 H, m), 2.81-2.74 (16 H, mm), 2.35 (3 H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 146.08, 138.92, 133.23, 114.27, 111.86, 111.30, 52.33, 33.18, 33.01, 32.67, 29.36, 23.74. HRFAB-MS: Calcd for [M^+] $C_{17}H_{26}BrNS_4$, 451.0131; Found, 451.0119.

6-Hydroxy-9-[4-(1,4,7,10-tetrathia-13-azacyclopentadec-13-yl)-3-methylphenyl]-xanthen-3-one (7, Mercury Green 1, MG1). Under a nitrogen atmosphere, *n*-BuLi (1.6 M in hexanes, 0.25 mL) was added dropwise to solution of receptor **5** (155 mg, 0.343 mmol) in anhydrous THF (5 mL) cooled to –78 °C. The resulting solution was stirred at –78 °C under nitrogen for an additional 1 h. Then, a solution 3,6-bis[[(1,1-dimethylethyl)dimethylsilyl]oxy]-9H-xanthen-9-one **6** (157 mg, 0.343 mmol) in anhydrous THF (5 mL) was added dropwise to the lithiate solution at –78 °C over a period of 20 min. The resulting orange solution was allowed to warm to room temperature and quenched with water (20 mL). The reaction was extracted with dichloromethane (5 × 50 mL) and the solvent was removed by rotary evaporation. Acetic acid (10 mL) was added to the residue and the reaction was stirred at 60 °C for 30 min. The solvent was removed under reduced pressure and the crude product was dry loaded onto silica gel. Purification by flash column chromatography (silica gel, 9:1:1 dichloromethane/methanol/ethyl acetate) furnished MG1 as an orange-brown solid (53 mg, 27% yield). ^{1}H NMR (d_6-DMSO, 300 MHz): δ 7.00-6.97 (3 H, m), 6.79-6.59 (6 H, m), 3.57 (4 H, m), 2.79 (16 H, m), 1.94 (3 H, s). HRFAB-MS: Calcd for [MH^+] $C_{29}H_{32}NO_3S_4$, 584.1421; Found, 584.1416. Anal. Calcd for $C_{30}H_{33}NO_3S_4$•CH_3OH: C, 60.45; H, 6.06; N, 2.27. Found: C, 60.32; H, 5.80; N, 1.98.

Spectroscopic Materials and Methods. Millipore water was used to prepare all aqueous solutions. All spectroscopic measurements were performed in 20 mM HEPES buffer, pH 7. Absorption spectra were recorded on a Varian Cary 50 spectrophotometer (Walnut Creek, CA) and fluorescence spectra were recorded using a Photon Technology International Quanta Master 4 L-format scanning spectrofluorometer (Lawrenceville, NJ)

equipped with an LPS-220B 75-W xenon lamp and power supply, A-1010B lamp housing with integrated igniter, switchable 814 photon-counting/analog photomultiplier detection unit, and MD5020 motor driver. Samples for absorption and emission measurements were contained in 1-cm × 1-cm quartz cuvettes (1.4 or 3.5-mL volume, Starna, Atascadero, CA). Fluorescence quantum yields were determined by reference to fluorescein in 0.1 N NaOH (Φ = 0.95).[2] The binding affinity of Hg^{2+} to MG1 was measured in 20 mM HEPES, pH 7. Excitation was provided at 495 nm, and collected emission was integrated from 500 to 700 nm. The apparent dissociation constant (K_d) was determined using the following equation: $F = (F_{max}[Hg^{2+}] + F_{min}K_d)/(K_d + [Hg^{2+}])$, where F is the observed fluorescence, F_{max} is the fluorescence for the Hg^{2+}:MG complex, and F_{min} is the fluorescence for the free MG1 dye.

Fish Assays. OmniTrace Ultra grade nitric acid (EM Science) was used for digestion experiments. All glassware was rinsed with dilute nitric acid and millipore water before use. Microwave digestions were carried out using a CEM Discover Labmate microwave synthesizer. Samples of fish tissue (100-200 mg) were dissected from frozen whole specimens after scale removal and digested in nitric acid (200-500 μL) at 180 °C with 300-watt microwave irradiation for 5-10 min. The resulting solutions were neutralized with 10 N NaOH and HEPES buffer. Final solutions for MG1 analysis were brought to 20 mM HEPES, pH 7, using 1 μM MG1 for assays.

Preparation and Staining of Cell Cultures. HEK 293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% Fetal Bovine Serum (FBS, Invitrogen) and glutamine (2 mM). One day before imaging, cells were passed and plated on 18-mm glass coverslips coated with poly-L-lysine (50 μg/mL, Sigma, St. Louis, MO). Immediately before the experiments, cells were washed with DMEM, incubated with the probe in DMEM, washed with PBS, and imaged. Experiments to assess mercury uptake were performed in the same media supplemented with 4-5 ppm $HgCl_2$.

Fluorescence Imaging Experiments. Imaging experiments were performed with a Zeiss LSM510 META/NLO Axioplan 2 laser scanning microscope and a 40x water-immersion objective lens. Excitation of MG1-loaded cells at 488 nm was carried out with an argon ion laser, and emission was collected in a window from 494 nm to 558 nm using a META detection system. MG1-AM (1-5 μM) was incubated with live cell samples for 15-75 min at 37 °C. Additions of TPEN were performed directly on the microscope stage.

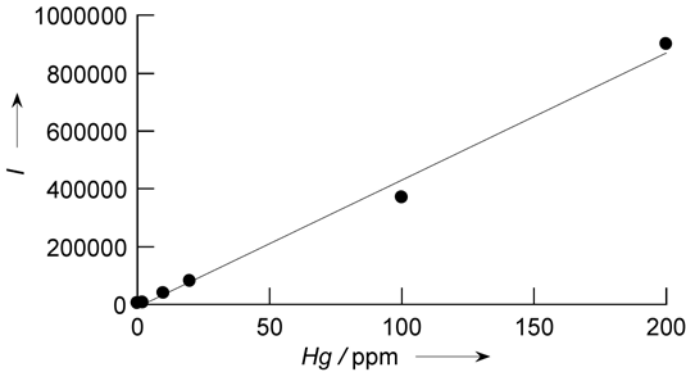

Figure S1. Linear emission response of 1 μM MG1 to ppb concentrations of aqueous Hg^{2+}. Excitation was provided at 495 nm and the emission intensity was measured at 513 nm. Spectra were acquired in 20 mM HEPES, pH 7.

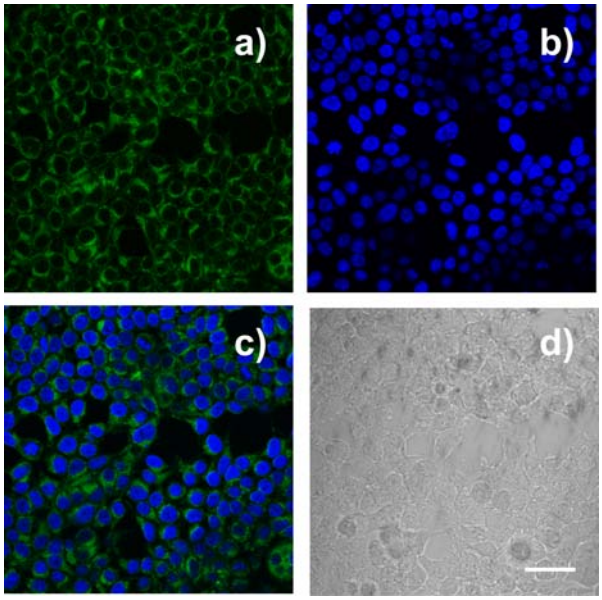

Figure S2. Confocal fluorescence imaging of mercury levels and cell viability in live HEK 293T cells using MG1 and Hoescht 33342. Cells were loaded with 1 μM MG1-AM for 30 min at 37 °C, followed by 4 ppm mercury ($HgCl_2$) and Hoescht 33342 for an additional 30 min at 37 °C. a) Green fluorescent channel showing mercury-induced MG1 fluorescence. b) Blue fluorescent channel showing cell viability by Hoescht 33342 staining. c) Overlay of panels a) and b). d) Brightfield image of cells in panels a)-c). Scale bar = 40 μm.

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[2] Brannon, J. H.; Magde, D. *J. Phys. Chem.* **1978**, *82*, 705-709.