Supplementary Material (ESI) for Chemical Communications

Supporting Information

Membrane Blotting for Rapid Detection of Mercury (II) in Water

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1. EXPERIMENTAL

1.1 Materials.

All oligonucleotides were purchased from Integrated DNA Technologies, Inc. (IDT) (Coralville, IA). Hemin and 3,3,5,5-tetramethylbenzidine (TMB) liquid substrate system were purchased from Sigma-Aldrich Inc. (Louis, MO). Triton X-100, 3-(N-morpholino)propanesulfonic acid (MOPS), and all of the metal salts were purchased from Fisher Scientific (Pittsburgh, PA) and used without additional purification. The reaction buffer containing 20 mM MOPS-NaOH, 1 M $NaNO_3$, 40 mM KNO_3, 50 mM NaCl, and 0.1% Triton X-100 with a pH of 7.5 was used for hybridization and colorimetric reaction. The stock hemin solution was prepared in DMSO and stored at -20 °C. Fresh hemin solution was diluted with the reaction buffer before each test. Amersham Hybond™-N+ membrane was purchased from GE Healthcare (Fairfield, CT). Milli-Q water (18 MΩ· cm) was used to prepare all the solutions. The toxic Hg2+ and other heavy metal ions were handled with protective gloves and the waste solutions containing heavy metal ions were disposed properly to avoid polluting the environment.

1.2 Detection in solution

In a typical experiment, 1 μM HgProbe-2 was denatured in the reaction buffer at 85 degree for 2 min and cooled down to RT for 5 min. Then it was mixed with the Hg^{2+} (or other metal ions) solution and the freshly prepared Hemin solution. The final concentrations for HgProbe-2 and hemin were 500 nM and 1 μM, respectively. The mixture was incubated at RT for 2 h. Then, 2 μL of 100 mM H_2O_2 and 2 μL of 100 mM ABTS was quickly added to the above solution to initiate the color reaction. A Varian Cary 50 UV-Vis spectrophotometer was used to record the absorption spectra. For each sample, the absorbance was continuously monitored for 3min at 405 nm with scan rate was set at 1.0 s.

1.3 Detection on membrane

Five microliter of 5 μM HgProbe-2 was carefully applied onto the membrane which was cut into the size of 1 cm × 1 cm. Caution was taken to avoid touching the membrane with the pipette tip. The membrane was left to air dry, and then immersed in 10 mL 0.5× reaction buffer (same reaction buffer as used in solution-based detection) with Hg^{2+}. For environmental water test, equal volume of the water sample was mixed with the reaction buffer. After 2 h of incubation, newly prepared hemin solution was added to the final concentration of 2 μM, and reacted for 1 hour before the membrane was taken out and

applied 50 μl of the commercial TBE substrate solution, which was diluted by half with the reaction buffer, for the color detection. A HP Deskjet F4210 All-in-one Printer was used to record the image of the spots.

1.4 ICP-AES measurement

A commercial Perkin-Elmer Optima 2000 DV inductively coupled plasma atomic emission spectrometer (ICP-AES, Norwalk, CT) was used to quantify the elemental composition of the environmental water samples. The standard solutions were prepared by gradually diluting a mixture of eleven metal ions, i.e., Ca^{2+}, Mg^{2+}, Hg^{2+}, Mn^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+} in 4% (v/v) nitric acid, which was also used to rinse the instrument twice to make sure no memory effect from previous tests. Nitric acid was added to the environmental samples with a final concentration of 4% (v/v) for ICP-AES measurement.

2. SUPPORTING FIGURES

Figure S1. Comparison of the absolute absorbance at 405 nm after 3-min oxidation of ABTS with DNA probes of different sequence designs. The blue bar represents the absorbance without the presence of Hg, and the red bar represents the signal in the present of 500 nM Hg. The reaction conditions were the same as described above in the Experimental section. Measurements were performed in three replicates and the average values were displayed here with the error bars representing the standard deviations. No significant absorbance difference with and without Hg using HgProbe-3 (with no T_4 linkage) and HgProbe-4 (no T-insert within the G-quadruplex structure) were observed.

HgProbe-1: 5'- AGGGACGGGA TTC TTT CTT AAAA TTG TTT GTT TG**T**GGAGGGT-3'
HgProbe-2: 5'- AGGGACGGGA TT CTT AAAA TTG TT TG**T**GGAGGGT-3'
HgProbe-3: 5'- AGGGACGGGA TTT TTT TG**T**GGAGGGT-3'
HgProbe-4: 5'- AGGGACGGGA TTC TTT CTT AAAA TTG TTT GTT TG-GGAGGGT-3'

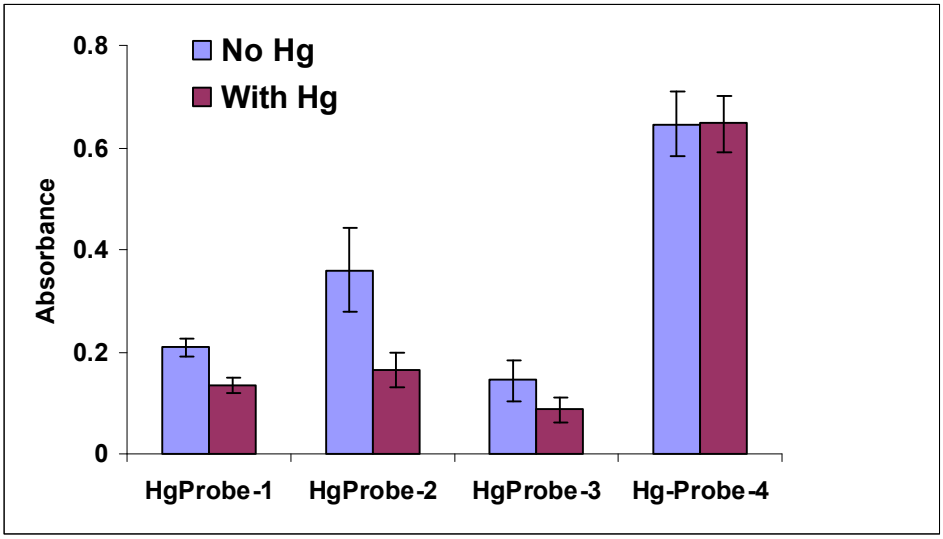

Figure S2. Selectivity study of the solution-based assay. M represents different metals chosen in the study. The metal ions of 1 or 100 μM (except Hg, the higher concentration for Hg was 2 μM) were incubated with HgProbe-2 using the reaction conditions indicated in the above Experimental section. The adjusted absorbance equals to the absorbance with the metal ion minus that without the metal ion. Measurements were performed in three replicates and the average values were displayed here with the error bars representing the standard deviations.

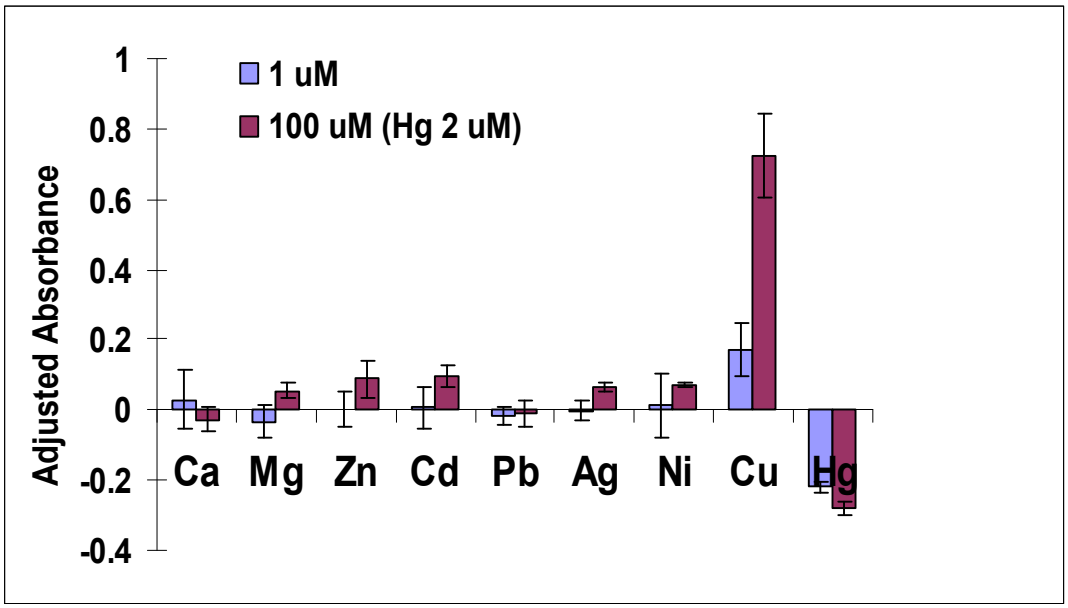

Table S1. Ion concentrations in environmental water samples measured by ICP-AES.

Concentration (μM)	Mg and Ca	Zn	Cu	Pb	Hg
Creek	Saturated	1.81	1.52	1.39	Not detectible
Bay	Saturated	8.18	0	1.37	Not detectible