Rational Design of “Turn-on” Allosteric DNAzyme Catalytic Beacons for Aqueous Mercury Ions with Ultrahigh Sensitivity and Selectivity

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Experimental Section

Materials: All DNA samples were purchased from Integrated DNA Technologies Inc. (Coralville, IA, USA) and were purified by HPLC by the company. Uranium acetate dihydrate was purchased from Fisher Scientific (Hampton, NH, USA) and other metal salts used in this work include: MgCl₂, CaCl₂, Mn(OAc)₂, Fe(NH₄)₂(SO₄)₂, FeCl₃, CoCl₂, NiCl₂, Cu(NO₃)₂, ZnCl₂, Cd(OAc)₂, Hg(ClO₄)₂, Pb(NO₃)₂, and TbCl₃.

Gel based assay: 1 µM 5'-FAM labeled 39S and 1 µM enzyme were annealed in 10 mM MES buffer pH 5.5 with 300 mM NaNO₃ by heating at 65 °C for 1 min and subsequently cooling slowly to room temperature in 30 min. For the experiment shown in Figure 1c, 8 µL of the
annealed DNAzymes were placed in microcentrifuge tubes and 1 µL of 100 µM Hg(ClO₄)₂ was added. The system was allowed to incubate at room temperature for 10 min and 1 µL of 10 µM UO₂²⁺ was added to initiate the cleavage reaction. The reaction was stopped after 1 min by adding 10 µL of stop buffer containing 8 M urea and 50 mM EDTA. For E₃T, E₄T, E₅T, and E₆T DNAzymes, a 20 min time point was also taken for the samples without Hg²⁺, because the fractions of cleavage for these enzymes were too low at 1 min to allow accurate calculation. The reaction mixture was then loaded to a denaturing 20% polyacrylamide gel and the gel was imaged with a Molecular Dynamics STORM 840 fluorescence imager with excitation wavelength set at 450 nm.

Hg²⁺ detection: The catalytic beacon sensor was prepared by annealing 2 µM of the substrate and enzyme strand shown in Figure 2a in 10 mM MES, pH 5.5 and 300 mM NaNO₃. A large volume of the same buffer was also prepared. For each detection, 25 µL of the annealed sensor was mixed with 475 µL of buffer and the final sensor concentration was 100 nM. The fluorometer (Fluoromax-P, Horiba Jobin Yvon, Edison, NJ) was set at the kinetics mode with 15 sec intervals. The excitation was set at 490 nm and emission at 520 nm. The temperature was set at 24 °C. UO₂²⁺ (0.5 µL of 1 mM) was added to the DNAzyme to make a final concentration of 0.5 µM, and this mixture was used as the sensor for Hg²⁺. The sensor was vortexed and placed into the fluorometer to start the kinetics reading. After the first reading, the cuvette was taken out and a small volume of Hg(ClO₄)₂ or other metals was added. After vortexing, the cuvette was placed back to the fluorometer to continue the reading.