Highly Sensitive "Turn-on" Fluorescent Sensor for Hg^{2+} in Aqueous Solution based on Structure-Switching DNA

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Electronic Supplementary Information (Experimental section, Figure S1，Dissociation constant calculation)

Experimental section

Sensor Preparation and Mercury Detection:

All oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA) and were purified by HPLC. To prepare the sensor solution, 100 nM Strand A (5'-FAM-TCATGTTTGTTTGTTGGCCCCCTTCTTTCTTA-3') and 400 nM Strand B (5'-ACAAACATGA-BHQ1-3') were added to 100 mM $NaNO_3$ and 10 mM MOPS (3-(N-morpholino)propanesulfonic acid), pH 7.2 buffer solution and kept at room temperature for 1 h to hybridize two strands. Then 500 μL of the sensor solution prepared above was transferred to a cuvette. The cuvette was placed in a fluorimeter (FluoroMax-P; Horiba Jobin Yvon, Edison, NJ) at 25 ^{o}C. The excitation was set at 491 nM and the emission at 518 nm was monitored. After the initial reading, the cuvette was taken out, and a small volume of concentrated Hg^{2+} solution was added. After vortexing, the cuvette was put back into the fluorimeter to continue the kinetics measurement.

Quantum yield of the fluorescent sensor:

The fluorescence intensity of the free FAM and FAM attached to DNA strand A at the same concentration (100 nM) and under the same pH (7.2) was measured and compared. As shown in Figure S1, about 88% of the FAM fluorescence intensity was retained after DNA conjugation to FAM. Since the free FAM in aqueous solution at pH 7.2 has the fluorescence quantum yield of 75% (see

http://www.promega.com/geneticidproc/ussymp8proc/21.html), we estimated the fluorescence quantum yield of FAM after DNA conjugation to be ~ 66%.

Selectivity Assay:

To determine the selectivity of the sensor, 1 μM of various metal ions (M^{2+}) including Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+} was added to the sensor solution individually and the fluorescence increase was monitored using a fluorimeter. In addition, 1 μM Hg^{2+} and 1 μM one of other metal ions (Hg^{2+}-M^{2+} pair) were added together to the sensor solution and the fluorescence response was recorded as well.

Mercury Detection in Pond Water:

A pond water sample was collected from the University of Illinois campus and filtered through 0.22 μm membrane before testment. 180 μL of the pond water was mixed with concentrated buffer and Hg^{2+} solution to make the final volume of 200 μL containing 500 nM Hg^{2+}, 100 mM $NaNO_3$ and 10 mM MOPS at pH 7.2. 300 μL concentrated sensor solution was then mixed with 200 μL of previously prepared pond water and the fluorescence response was monitored by fluorimeter. The final mixture contained 200 nM Hg^{2+} and 100 nM hybrdized DNA.

Figure S1

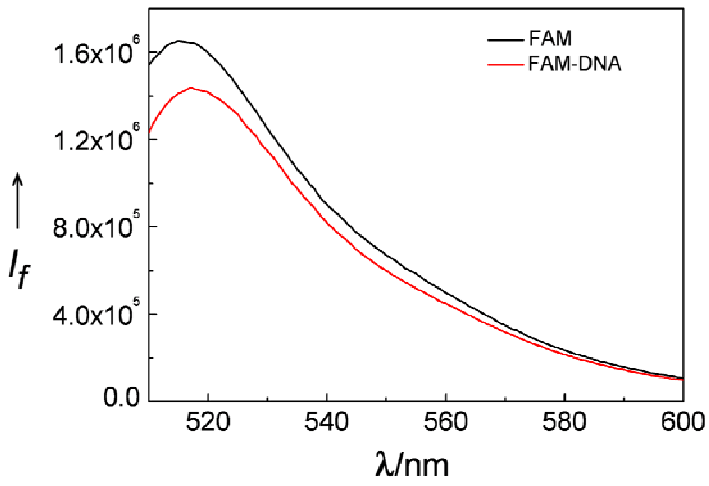

Figure S1: Fluorescence spectra of free FAM (black line) and FAM attached to DNA strand A (red line) at same concentration and buffer conditions (100 nM, pH 7.2 MOPS buffer)

DISSOCIATION CONSTANT CALCULATION

Through fitting calibration curve in Figure 2b using following equation in Origin Software, the dissociation constant (k) was calculated to be 471 nM with a Hill coefficient (n) of 2.4.

$$y = y_0 + (y_m - y_0) * \frac{x^n}{k^n + x^n}$$

k : Dissociation constant

n : Hill coefficient

x : Hg^{2+} concentration

y_0 : Background fluorescence intensity

y_m : Saturated fluorescence intensity