Oligonucleotide-Based Fluorescence Probe for Sensitive and Selective Detection of Mercury(II) in Aqueous Solution

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ABSTRACT

In this paper we unveil a new homogeneous assay—using TOTO-3 and the polythymine oligonucleotide T33—for the highly selective and sensitive detection of Hg\(^{2+}\) in aqueous solution. The fluorescence of TOTO-3 is weak in the absence or presence of randomly coiled T33. After T33 interacts specifically with Hg\(^{2+}\) ions through T–Hg\(^{2+}\)–T bonding, however, its conformation changes to form a folded structure that preferably binds to TOTO-3. As a result, the fluorescence of a mixture of T33 and TOTO-3 increases in the presence of Hg\(^{2+}\). Our data from fluorescence polarization spectroscopy and capillary electrophoresis with laser-induced fluorescence detection confirm the formation of folded T33-Hg\(^{2+}\) complexes. Under optimum conditions, the TOTO-3/T33 probe exhibited a high selectivity (≥265-fold) toward Hg\(^{2+}\) over other metal ions, with a limit of detection of 0.6 ppb. We demonstrate the practicality of this TOTO-3/T33 probe through its use for the rapid determination of Hg\(^{2+}\) in pond water and in batteries. This approach offers several advantages, including rapidity (<15 min), simplicity (label free), and low cost.
INTRODUCTION

Heavy metal pollution is an important environmental issue because of its adverse effect on human health. Mercury(II) is one of the most potently toxic metal ions; it affects many different areas of the brain and their associated functions, resulting in symptoms such as tremors, vision problems, deafness, and losses of muscle coordination, sensation, and memory. In addition to the brain, inorganic mercury can damage the heart, kidney, stomach, and intestines. The US Environmental Protection Agency (EPA)’s estimate of annual total global mercury emission from all sources—both natural and human-generated—is ca. 7500 tons per year. Thus, techniques for the detection and/or removal of Hg$^{2+}$ are required to protect our environment and health.

Although many techniques—such as atomic absorption/emission spectroscopy, inductively coupled plasma mass spectrometry (ICP-MS), and selective cold vapor atomic fluorescence spectrometry—have been applied widely to detect Hg$^{2+}$ in environmental samples, they require expensive and sophisticated instrumentation and/or complicated sample preparation processes, and their complexity makes them unsuitable for use in in-field Hg$^{2+}$ analyses. Alternative techniques based on fluorescent probes using small molecules, DNAzymes, oligonucleotides, polymer–protein complexes, and nanoparticles have been demonstrated for the detection of Hg$^{2+}$. Nevertheless, each of these approaches exhibits some feature that limits its practical use, be it poor aqueous solubility, cross-sensitivity toward other metal ions, short emission wavelengths, and/or weak fluorescence intensities.

In this study, we developed a simple and rapid fluorescence approach—using the polythymine oligonucleotide T$_{33}$ and a double-strand-chelating dye TOTO-3—for the sensitive and selective detection of Hg$^{2+}$ in aqueous solutions. TOTO-3 is a weakly fluorescent unsymmetrical cyanine dye that exhibits a more than 8.3-fold enhancement in its fluorescence upon binding to double-stranded DNA, with excitation and emission wavelengths centered at 620 and 660 nm, respectively. Our approach toward sensing Hg$^{2+}$ is based on the fluorescence increase that occurs as a result of the strong interaction between TOTO-3 and the folded T$_{33}$ structure induced by Hg$^{2+}$. 


EXPERIMENTAL SECTION

Chemicals. Tris(hydroxymethyl)aminomethane (Tris), the metal salts, and all the other reagents were purchased from Aldrich (Milwaukee, WI). OligoGreen, TOTO-3, YOYO-3 were obtained from Molecular Probes (Portland, OR). The N,N-dimethyl-2,7-diazapyrenium dication was synthesized and purified according to an established procedure. All of the DNA samples (6-FAM-T7, -T15, -T33, -T50, -T80, and -T33) were purchased from Integrated DNA Technology (Coralville, IA).

TOTO-3/T33-Based Sensor for Hg$^{2+}$. A stock solution of TOTO-3 (0.2 $\mu$M) was prepared in DI water. Aliquots of this TOTO-3 solution (0.2 $\mu$M, 50 $\mu$L) were added separately to 5 mM Tris-HCl (pH 7.4) solutions containing Hg$^{2+}$ (0–10 $\mu$M) and the unmodified T33 oligonucleotide (10 nM) to give final volumes of 500 $\mu$L. After equilibration at ambient temperature for 15 min, the fluorescence intensities of the mixtures were measured using a Cary Eclipse fluorescence spectrophotometer (Varian, CA, USA), with excitation at 620 nm.

Capillary Electrophoresis and Fluorescence Polarization Spectroscopy. A homemade capillary electrophoresis (CE) separation system was employed to monitor the Hg$^{2+}$-induced conformational changes of T33. Briefly, a high-voltage (HV) power supply (Gamma High Voltage Research, Ormond Beach, FL) was used to drive the electrophoresis process. The entire detection system was enclosed in a black box equipped with an HV interlock. The HV end of the separation system was housed in a Plexiglass box for safety. A 10.0-mW laser having an output at 475 nm (B&W TEKINC, Newark, DE, USA) was used for excitation. The emission light was collected with a 10× objective (numeric aperture 0.25). A 520 nm long-pass filter was used to block scattered light before the emitted light reached the phototube (R928, Hamamatsu Photonics K. K., Shizuoka-Ken, Japan). The fluorescence signal was transferred directly through a 10-k$\Omega$ resistor to a 24-bit A/D interface at 10 Hz (Borwin, JMBS Developments, Le Fontanil, France) and stored in a PC. Capillaries (i.d.: 75-$\mu$m; o.d.: 365-$\mu$m; total length: 40 cm; effective length: 30 cm; Polymicro Technologies, Phoenix, AZ) were dynamically coated overnight with 5.0% poly(vinyl pyrrolidone) (PVP; Mw 1.3 $\times$ 10$^6$) and then with 0.5% poly(ethylene oxide) (PEO; Mw 8.0 $\times$ 10$^6$) for 12 h prior to use in DNA separations. Before conducting CE
separations, aliquots of Hg$^{2+}$ (0–1.0 μM) were added separately to 5 mM Tris-HCl solutions (pH 7.4) containing 6-FAM-T$_{33}$ (10 nM) and fluorescein (30 nM) and equilibrated for 10 min. The mixtures were injected hydrodynamically at the cathode end into the capillary at a 20-cm height for 10 s; separations were conducted at −5 kV. After each run, the capillary was sequentially washed and filled with 5 mM Tris-HCl (pH 7.4) containing Hg$^{2+}$ (0–1.0 μM). For measurement of the fluorescence polarization spectra, 5 mM Tris-HCl solutions (pH 7.4) containing 6-FAM-T$_{33}$ (50 nM) and Hg$^{2+}$ (0–120 μM) were reacted for 15 min and then the anisotropy of each solution was recorded using a Cary Eclipse fluorescence spectrophotometer (Varian, CA, USA) equipped with a manual polarizer accessory (Varian, CA, USA). Circular dichroism (CD) spectra measurement were made using a Jasco J-815 spectropolarimeter (JASCO Inc., Easton, MD Easton, MD).

**Analysis of Real Samples.** A pond water sample from the NTU campus was filtered through a 0.2-μm membrane and analyzed using ICP-MS. Aliquots of the pond water (250 μL) were spiked with standard solutions (50 μL) containing Hg$^{2+}$ at concentrations over the range 0.01–1.0 μM. Next, 50 mM Tris-HCl solution (pH 7.4, 50 μL), T$_{33}$ solution (100 nM, 50 μL), TOTO-3 solution (200 nM, 50 μL) and water (50 μL) were added to the mixtures to give final volumes of 500 μL. The mixtures were equilibrated for 15 min. The samples from three button-type alkaline manganese batteries were prepared according to the standard method published by the National Electrical Manufacturers Associations.$^{9}$ Briefly, these samples were digested in a mixture of HCl and HNO$_3$ (2:1, v/v) for 18 h and then directly basified through the addition of 0.5 N NaOH and filtered through a 0.2-μm membrane. The solutions (10 μL) were then diluted to 10 mL with 5 mM Tris-HCl solution (pH 7.4) prior to analysis using both ICP-MS and the TOTO-3/T$_{33}$ probe. The quantitation of Hg$^{2+}$ in these samples was obtained by applying a standard addition method.

**RESULTS AND DISCUSSION**

**Sensing Strategy.** Scheme 1 depicts the mechanism underlying this TOTO-3/T$_{33}$ probe’s approach to sensing Hg$^{2+}$. In the absence of Hg$^{2+}$, T$_{33}$ exists in aqueous solution in a random-coil
structure. Because the interactions between the randomly coiled T₃³ and TOTO-3 are weak, the fluorescence of such a mixture is weak. In the presence of Hg²⁺, however, T–Hg²⁺–T bonding results in T₃³ changing its random coil conformation to that of a folded structure. Because TOTO-3 has a high affinity ($K_b = \text{ca. } 10^9 \text{ M}^{-1}$) for double-stranded DNA (dsDNA), TOTO-3-DNA complexes form preferably in the presence of Hg²⁺.¹⁰ As a result of reduced collision and forming stiffer structures, TOTO-3-DNA complexes fluoresce more strongly than does free TOTO-3. Thus, the fluorescence intensity of the complexes formed between TOTO-3 and T₃³ increases in the presence of Hg²⁺.

**Evidence for Folded T₃³ Forming in the Presence of Hg²⁺.** Curve a in Figure 1 indicates that the fluorescence at 660 nm of TOTO-3 (20 nM) in 5 mM Tris-HCl solution (pH 7.4) is very weak when excited at 620 nm. The fluorescence intensity of TOTO-3 in the presence of 10 nM T₃³ is slightly higher (curve b), supporting the notion that it interacts weakly with random-coil T₃³. After adding 1.0 μM Hg²⁺ to this mixture of TOTO-3 and T₃³, a rapid (<10 s) and significant increase (8.3-fold) in fluorescence intensity occurred (curve c). In contrast, in the absence of T₃³ we did not observe any change in the fluorescence spectrum of TOTO-3 (20 nM) after adding Hg²⁺ (1.0 μM). To confirm the specificity of T₃³ toward Hg²⁺, we tested a control DNA sample having the sequence GCC TTA ACT GCA GTA CTG GTG AAA TTG CT. We expected this control DNA to have difficulty folding in the presence of Hg²⁺, mainly because it has a less of a chance to form T–Hg²⁺–T bonds than does T₃³. We observed only slight changes (<10%) in the fluorescence intensity of the mixture of TOTO-3 and the control DNA after adding the same amount of Hg²⁺.

We further applied CE to support the notion of the formation of folded T₃³ in the presence of Hg²⁺. Because the fluorescence of mixtures of TOTO-3 and random-coil T₃³ is weak, we could not observe random-coil T₃³ when using CE in conjunction with laser-induced fluorescence (LIF) detection. In other words, the changes in the electrophoretic mobility of the complexes of T₃³ and TOTO-3 in the presence of Hg²⁺ were difficult to observe using CE-LIF. Thus, we use a 5´ end labeled 6-FAM-T₃³ for the CE experiment. Upon increasing the concentration of Hg²⁺, the migration time for 6-FAM-T₃³ decreased, while that for the internal standard (fluorescein) remained almost unchanged (Figure 2A).
We suspect that once 6-FAM-T33 interacted with Hg$^{2+}$, its conformation changed from a random coil to a folded structure and its charge-to-mass ratio decreased, leading to increased electrophoretic mobility (i.e., a decrease in migration time). Based on the plot in Figure 2B, we calculated the binding constant ($K_b$) for the interaction between T33 and Hg$^{2+}$ to be $6.1 \times 10^6$ M$^{-1}$. For comparison, we note that a value of $K_b$ of $4.2 \times 10^5$ M$^{-1}$ has been reported for Hg$^{2+}$ interacting with two oligonucleotides having two binding sites for Hg$^{2+}$ (i.e., each has two T units).

The formation of folded DNA molecules having many T residues in the presence of Hg$^{2+}$ has been proved using circular dichroism and nuclear magnetic resonance spectroscopy. To further support the formation of folded 6-FAM-T33 in the presence of Hg$^{2+}$, we recorded fluorescence polarization spectra of 6-FAM-T33 in the presence of various concentrations of Hg$^{2+}$ (Figure 2C). Because of the relatively low sensitivity of fluorescence polarization spectroscopy, we used higher concentrations of 6-FAM-T33 and Hg$^{2+}$ than those used in the CE-LIF experiments. The anisotropy of 6-FAM-T33 increased from 0.026 to 0.224 upon increasing the Hg$^{2+}$ concentration from 0 to 120 μM, consistent with the putative changes in the structure of 6-FAM-T33. The anisotropy in folded 6-FAM-T33 is higher than that in random-coil 6-FAM-T33 mainly because of the former’s stiffer structure.

To further support our reasoning, we conducted CD and melting temperature ($T_m$) measurements. The CD spectra of T33 indicated the conformation of T33 (500 nM) changed upon increasing the Hg$^{2+}$ concentration from 0 to 15 μM. The result support the formation of the folded structures from T33 in the presence of Hg$^{2+}$ (Figure S1A, Supporting Information). In $T_m$ study, we define the temperature at which the fluorescence of TOTO-3 reaches 50% of its original value as $T_m$. Upon increasing the temperature, fluorescence intensity of TOTO-3·poly-T complexes (10 nM) decreased as a result of breaking in the T–Hg$^{2+}$–T bonding (Figure S1B, Supporting Information). Upon increasing Hg$^{2+}$ concentration, $T_m$ increases and reaches a plateau at the concentration of Hg$^{2+}$ of 750 nM.

**Effect of the Length of Poly-T and pH.** Having observed that Hg$^{2+}$ induced the formation of folded DNA structures, we suspected that the sensitivity of our analytical system would be dependent on the length of the DNA strand. We employed five poly-T ss-DNA samples of various lengths—T7,
T_{15}, T_{33}, T_{50}, \text{ and } T_{80} (each 10 \text{ nM})—to test our hypothesis. After plotting the values of $(I_{F_0} - I_F)/I_{F_0}$ for the TOTO-3-poly-T complexes against the concentration of Hg$^{2+}$ (Figure S2, Supporting Information), we found that Hg$^{2+}$ induced significant positive responses when the number of bases of poly-T was greater than 15. The degree of Hg$^{2+}$-induced fluorescence enhancement of the complex of TOTO-3 and T_{7} was very small, mainly because of the difficulty of forming a folded structure from T_{7}. The values of $(I_{F_0} - I_F)/I_{F_0}$ for the complexes formed between TOTO-3 and T_{80}, T_{50}, and T_{33} in the presence of Hg$^{2+}$ were similar: they all increased upon increasing the Hg$^{2+}$ concentration and reached a plateau at a concentration of 500 nM.

We also explore the effect of pH of TOTO-3-poly-T solution on the sensing Hg$^{2+}$. As indicated of Figure S3 (Supporting Information), the values $(I_{F_0} - I_F)/I_{F_0}$ for TOTO-3-poly-T the complexes is optimized at pH 7.4. The affinity of TOTO-3-DNA was reported optimized at pH 6–8. Otherwise, Hg$^{2+}$ binds directly to N3 of thymidine in place of the imino proton and bridges two thymidine residues to form the T-Hg$^{2+}$-T pair. Our results good agree with these reports, thus, the studies following this herein were performed by T_{33} in 5 mM Tris-HCl buffer (pH 7.4) for further study.

**Impact of Fluorophore.** We investigated the effect of the TOTO-3 concentration on the sensing of 1 \mu M Hg$^{2+}$ in the presence of 10 nM T_{33}. The values of $(I_{F_0} - I_F)/I_{F_0}$ for the complexes of TOTO-3, T_{33}, and Hg$^{2+}$ increased upon increasing the concentration of TOTO-3 over the range 0–20 nM (Figure S4A, Supporting Information). At TOTO-3 concentrations greater than 20 nM, the sensitivity decreased as a result of a higher fluorescence background. We also investigated the use of some other DNA binding dyes (YOYO-3, TOTO-3, ethidium bromide, OliGreen, acridin orange, methyl blue, and the $N,N$-dimethyl-2,7-diazapyrenium dication; each 20 nM) and DNA nonbinding dyes (fluorescein and rhodamine B; each 20 nM) on the determination of Hg$^{2+}$ using T_{33} (10 nM). Of these systems, the sensitivity for Hg$^{2+}$ was highest when using the TOTO-3/T_{33} probe (Figure S4B, Supporting Information). The values of $(I_{F_0} - I_F)/I_{F_0}$ for the best three dyes increased in the order TOTO-3 ($K_b = \text{ca. } 10^9 \text{ M}^{-1}$) > YOYO-3 ($K_b = \text{ca. } 10^7 \text{ M}^{-1}$) > ethidium bromide ($K_b = \text{ca. } 10^6 \text{ M}^{-1}$), suggesting that the strength of binding of the dyes to T_{33} was an important factor determining the sensitivity. We note that
the presence of Hg$^{2+}$ induced slight increases in the fluorescence of mixtures of T$_{33}$ and OliGreen, mainly because OliGreen is a dye that binds to single-stranded DNA. Fluorescein and rhodamine B did not bind to T$_{33}$; thus, their fluorescence intensities did not change significantly in the presence of Hg$^{2+}$.

**Sensitivity and Selectivity for Hg$^{2+}$**. Under optimum conditions (20 nM TOTO-3, 10 nM T$_{33}$, and 5 mM Tris-HCl; pH 7.4), we observed that the value of \((I_F - I_{F0})/I_{F0}\) increased linearly \((R^2 = 0.98)\) upon increasing the Hg$^{2+}$ concentration over the range 10–200 nM (Figure 3A). From a plot of \((I_{F0} - I_F)(I_F - I_{Fs})\) versus the Hg$^{2+}$ concentration, we calculated the formation constant of Hg$^{2+}$ and T$_{33}$ to be $5.8 \times 10^6$ M$^{-1}$, which is close to the value we calculated from the CE-LIF data. The limit of detection (LOD) at a signal-to-noise ratio (S/N) of 3 was 3 nM (0.6 ppb), which is below the maximum level of mercury permitted by the US EPA for drinking water. Thus, our present approach provides a sensitivity toward Hg$^{2+}$ that is one order of magnitude better than the reported previously for a T–Hg–T-mediated sensor.$^7$d

To evaluate the resistance of our sensor to endogenous nuclease degradation, we conduct TOTO-3·T$_{33}$ (10 nM) to sensing Hg$^{2+}$ ions (0–1.2 μM) in the presence DNAase I (100 nM). The plot of signal enhancement ratios \((I_{F0} - I_F)/I_{F0}\) of TOTO-3·T$_{33}$ complexes against the concentration of Hg$^{2+}$ are exhibited in Figure S5 (Supporting Information). The plot of signal enhancement ratios increased linearly \((R^2 = 0.98)\) upon increasing the Hg$^{2+}$ concentration over the range 50–200 nM. This result indicated Hg-mediated folded structure can resist DNAse I digestion.

Next, we investigated the selectivity of our new approach for Hg$^{2+}$ over other metal ions (Li$^+$, Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Sr$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Al$^{3+}$, Cr$^{3+}$, Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Zn$^{2+}$, Pb$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Ag$^+$, and Au$^{3+}$; each 1.0 μM) under the optimum conditions. As indicated in Figure 3B, the TOTO-3/T$_{33}$ probe was highly selective (265-fold or more) for Hg$^{2+}$ over the other metal ions. We performed a series of competition experiments to test the practicality of our TOTO-3/T$_{33}$ sensor for the selective detection of Hg$^{2+}$. The tolerance concentrations of various metal ions (within a relative error of ±5%) for the sensing of Hg$^{2+}$ (100 nM) using the TOTO-3/T$_{33}$ probe were 1.0 μM for Au$^{3+}$, 5.0 μM for Ag$^+$, Cd$^{2+}$, and Pb$^{2+}$, and 10.0 μM for Cu$^{2+}$, Co$^{2+}$, Fe$^{3+}$, and Ni$^{2+}$. These results suggest that the metal ions we tested should not interfere with the determination of Hg$^{2+}$ when applying our developed probe.
On the other hands, we introduced EDTA into TOTO-3·T₃₃ complexes containing Hg²⁺ (200 nM), the fluorescence intensity for the TOTO-3·T₃₃ complexes decreased to 21% and 15% when the concentration of added EDTA was 200 nM and 2 μM.¹⁴ These results suppose the TOTO-3·T₃₃ sensor may be renewable when separated the extracted Hg-EDTA complex and TOTO-3·T₃₃ in our further work.

**Detection of Hg²⁺ in Real Samples.** As indicated in Figure S6 (Supporting Information), the intensity of the fluorescence of the TOTO-3·T₃₃ complexes increased upon increasing the spiked concentration of Hg²⁺ in pond water over the range 25–200 nM ($R^2 = 0.98$). The recoveries of these measurements were 97–108%. The LOD at an S/N ratio of 3 for Hg²⁺ in the presence of the complicated pond water matrix was 10 nM (2.0 ppb). We also applied our TOTO-3/T₃₃ probe to the detection of Hg²⁺ in battery samples. Table 1 lists the concentrations of Hg²⁺ that we determined in three different types of batteries using both our developed probe and ICP-MS. On the basis of an F-test, the results using our present approach are in good agreement with those obtained using ICP-MS. Note, however, that the sample preparation and analysis time when using the TOTO-3/T₃₃ probe was less than 15 min; this assay provides the additional advantages of simplicity, low cost, and high throughput.

**CONCLUSION**

We have developed a homogenous Hg²⁺ assay using TOTO-3 and T₃₃. Upon interaction with Hg²⁺, T₃₃ changes its conformation from a random coil to a folded structure, leading to an increase in the fluorescence intensity, electrophoretic mobility, and fluorescence anisotropy for the TOTO-3·T₃₃ complex. This probe is highly sensitive and selective for Hg²⁺. Although we have demonstrated the detection of Hg²⁺ ions only, we suspect that this probe strategy—using DNA samples of various lengths and sequences—will also be applicable to systems for the detection of metal ions such as Pb²⁺ and Cu²⁺.
ACKNOWLEDGMENT This study was supported by the National Science Council of Taiwan under contract NSC 96-2627-M-002-014.

Supporting Information Available: CD spectra of the T₃₃ (500 nM) and TOTO-3 (1 μM) complexes after the addition of Hg²⁺ ions (0–20 μM) and plot of the Tₘ of Hg²⁺·T₃₃ as a function of the concentration of Hg²⁺ (0–1.2 μM) (Figure S1). Fluorescence responses of mixtures of TOTO-3 (20 nM) and poly-T (10 nM) after the addition of Hg²⁺ ions (Figure S2). Fluorescence responses of mixtures of TOTO-3 (20 nM) and T₃₃ (10 nM) after the addition of Hg²⁺ ion (200 nM) in various pH value buffers (5 mM) (Figure S3). Values of relative fluorescence [(Iₐ – I₀)/I₀] of 5 mM Tris-HCl solutions (pH 7.4) containing T₃₃ (10 nM) and a selection of fluorophores (Figure S4). Fluorescence responses of the T₃₃·TOTO-3 complexes in the presence of DNAase I (100 nM) and Hg²⁺ ions (0–750 nM) (Figure S5). Fluorescence responses of mixtures of T₃₃ and TOTO-3 after standard addition of Hg²⁺ ions (0–200 nM) into diluted pond water (Figure S6).
**FIGURE CAPTIONS**

**Figure 1.** Fluorescence spectra of (a) TOTO-3 solution (20 nM) and (b, c) mixtures of TOTO-3 (20 nM) and T₃₃ (10 nM) in the (b) absence and (c) presence of Hg²⁺ (1.0 μM). Inset: Temporal change in the fluorescence intensity (660 nm) of the mixture of T₃₃ and TOTO-3 after the addition of Hg²⁺. The arrow indicates the initial time at which Hg²⁺ was added. Background solution: 5 mM Tris-HCl (pH 7.4); excitation wavelength: 620 nm. The fluorescence intensities (Iₓ) are plotted in arbitrary units (a. u.).

**Figure 2.** (A) Electropherograms of a solution containing 6-FAM-T₃₃ (10 nM), fluorescein (30 nM), and Hg²⁺ at concentrations of (a) 0, (b) 50, (c) 100, (d) 250, and (e) 500 nM. (B) Plot of the relative mobility difference (μₓ – μₓ)/μₓ versus the concentration of Hg²⁺, where μₓ, μₓ, and μₓ are the electrophoretic mobilities of 6-FAM-T₃₃ (10 nM) in the absence of Hg²⁺, in the presence of 1.0 μM Hg²⁺, and in the presence of 10–100 nM Hg²⁺, respectively. Background electrolytes: 5 mM Tris-HCl (pH 7.4) containing (a) 0, (b) 50, (c) 100, (d) 250, and (e) 500 nM Hg²⁺ ions. Total capillary length: 40 cm; effective length: 30 cm. The separations were performed at −5 kV. The fluorescence intensity (Iₓ) is plotted in arbitrary units (a. u.). (C) Values of anisotropy of 6-FAM-T₃₃ (50 nM) in 5 mM Tris-HCl solution (pH 7.4) plotted as a function of the Hg²⁺ concentration (0–120 μM). Other conditions were the same as those described in Figure 1.

**Figure 3.** (A) Fluorescence responses of the T₃₃·TOTO-3 complexes after the addition of Hg²⁺ ions (0, 25, 50, 100, 150, 200, 250, 500 nM). Inset: linearity of the relative fluorescence [(Iₓ – Iₓ₀)/Iₓ₀] at 660 nm with respect to the Hg²⁺ concentration over the range 10–200 nM. (B) Relative fluorescence increases [(Iₓ – Iₓ₀)/Iₓ₀] at 660 nm of 5 mM Tris-HCl solutions (pH 7.4) containing T₃₃ and TOTO-3 upon the addition of 1.0 μM metal ions. The descriptors Iₓ₀ and Iₓ are the fluorescence intensities of the TOTO-3·T₃₃ complexes in the absence and presence of Hg²⁺, respectively. Other conditions were the same as those described in Figure 1.
Scheme 1. Schematic representation of the function of a Hg$^{2+}$ sensor that operates based on modulation of the fluorescence of the complex formed between TOTO-3 and T$_{33}$. 
REFERENCES


(9) EPBA, BAT, and NEMA. *Battery Industry standard analytical method for the determination of mercury, cadmium and lead in alkaline manganese cells using AAS, ICP-AES and cold vapor;* European Portable Battery Association (EPBA), Battery Association of Japan (BAT), and National Electrical Manufactures Association (NEMA): Brussels, Belgium, Tokyo, and Rosslyn, VA, respectively, April 1998.


Table 1. Concentrations of Hg\(^{2+}\) Determined in Three Different Types of Alkaline Manganese Batteries Using Two Different Methods

<table>
<thead>
<tr>
<th>Battery</th>
<th>TOTO/T(_{33}) probe, mean ± S.D. (mg/g; (n = 5))</th>
<th>ICP-MS, mean ± S.D. (mg/g; (n = 5))</th>
<th>F-test between the two methods(^a)</th>
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<tr>
<td>A</td>
<td>2.24 ± 0.07</td>
<td>2.19 ± 0.09</td>
<td>1.65</td>
</tr>
<tr>
<td>B</td>
<td>1.23 ± 0.06</td>
<td>1.26 ± 0.05</td>
<td>1.44</td>
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<tr>
<td>C</td>
<td>1.78 ± 0.10</td>
<td>1.82 ± 0.09</td>
<td>1.2</td>
</tr>
</tbody>
</table>

\(^a\) F-test value: 6.39 at 95% confidence.
Scheme 1.
Figure 1.
Figure 2.
Figure 3.

A.

B.
Supporting Information

Oligonucleotide-Based Fluorescence Probe for Sensitive and Selective Detection of Mercury(II) in Aqueous Solution

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Figure S1. (A) CD spectra of the T₃₃ (500 nM) and TOTO-3 (1 μM) complexes after the addition of Hg²⁺ ions (0, 5, 10, 15 and 20 μM). Inset: linearity of the ellipticity at 264 nm with respect to the Hg²⁺ concentration over the range 1–20 μM. (B) Plot of the Tₘ of Hg²⁺-T₃₃ as a function of the concentration of Hg²⁺ (0–1.2 μM). Other conditions were the same as those described in Figure 1.
Figure S2. Fluorescence responses of mixtures of TOTO-3 (20 nM) and poly-T (10 nM) after the addition of Hg$^{2+}$ ions. (a) T_{33}, T_{50}, or T_{80}; (b) T_{15}; (c) T_{7}. Other conditions were the same as those described in Figure 1.
Figure S3. Fluorescence responses of mixtures of TOTO-3 (20 nM) and T33 (10 nM) after the addition of Hg^{2+} ion (200 nM) in various pH value buffers (5 mM). (a) sodium acetate (pH 3.0); (b) sodium acetate (pH 5.0); (c) Tris-HCl (pH 7.4); (d) Tris-HCl (pH 9.0). Other conditions were the same as those described in Figure 1.
Figure S4. Values of relative fluorescence \([\left( I_F - I_{F0} \right) / I_{F0} \] of 5 mM Tris-HCl solutions (pH 7.4) containing T33 (10 nM) and a selection of fluorophores. (A) TOTO-3 at various concentrations; (B) other fluorophores (20 nM). (1) YOYO-3; (2) TOTO-3; (3) ethidium bromide; (4) OliGreen; (5) acridin orange; (6) methyl blue; (7) N,N-dimethyl-2,7-diazapyrenium dication; (8) fluorescein; (9) rhodamine B. Other conditions were the same as those described in Figure 1.
Figure S5. Fluorescence responses of the T₃₃-TOTO-3 complexes in the presence of DNAase I (100 nM) and Hg²⁺ ions (0, 100, 150, 250, 500, 750 nM). Inset: Relative fluorescence [(I_F – I_Fo)/I_Fo] at 660 nm with respect to the Hg²⁺ concentration (0–1.2 μM). Other conditions were the same as those described in Figure 1.
**Figure S6.** Fluorescence responses of mixtures of T33 and TOTO-3 after standard addition of Hg$^{2+}$ ions (0–500 nM) into diluted pond water. Inset: linear response of the relative fluorescence $[(I_F - I_{F0})/I_{F0}]$ at 660 nm with respect to the Hg$^{2+}$ concentration over the range 25–200 nM. The dilution factor was 2. Other conditions were the same as those described in Figure 1.