Electronic Supporting Information

A Selective Oligonoucleotide-Based Luminescent Switch-On Probe for the Detection of Nanomolar Mercury(II) ion in Aqueous Solution†

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Experimental section

Materials. Calf thymus DNA (ct DNA) was purchased from Sigma Chemical Co. Ltd. and purified by the literature method.[1a] The DNA per base pair concentration was determined by UV/Vis absorption spectroscopy using the following molar extinction coefficients at the indicated wavelengths: calf thymus DNA, ε_{260} = 13200 bp cm^{-1} M^{-1}.[1b] Two complementary oligonucleotides, 5'-GCTCCCCTTTCTTGCGGAGATTCTCTTCCTCTG and 5'-CAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGC were obtained from DNAgency (Malvern, USA) and annealed to give a double-stranded 33-bp DNA. The purity of the 33-bp DNA was checked by electrophoresis in a 20% polyacrylamide gel. A plasmid DNA, pDR2 (10.7 kb), was purchased from Clontech Laboratories Inc. (Palo Alto, USA). Unless otherwise stated, DNA binding experiments were performed in aerated Tris buffer solutions (5 mM Tris, 50 mM NaCl, pH 7.2) at 20 ^{o}C. K_2PtCl_4 and $^{t}Bu_3$terpy (4,4′,4″-tri-*tert*-butyl-2,2′:6′,2″-terpyridine) were obtained from Aldrich. Reagents and solvents were reagent grade. 4-Amino-4-phenylamino-6-(2-pyridyl)-1,3,5-triazine (4-appt),[2] 6-phenyl-2,2'-bipyridine (HCNN),[3] [Pt(CNN)Cl],[4] and [Pt(terpy)Cl]$^{+}$ (**2**)[5] were prepared according to reported procedures. The stock solutions (10 mM) of Pt^{II} complexes for titration studies were prepared in either DMSO or MeCN, and further dilution to designated concentrations was made using deionised water. All the stock solutions were kept at –20 ^{o}C in the dark between experiments.

HepG2 (hepatocellular) cell line[6] was provided by Prof. W. F. Fong of City University of Hong Kong, Hong Kong SAR. Cell proliferation Kit I (MTT) from Roche was used for cytotoxicity evaluation.

Physical measurement. Absorption spectra were recorded on a Perkin-Elmer Lambda 19 UV/Visible spectrophotometer. Emission spectra were recorded on a SPEX Fluorolog-2 Model fluorescence spectrophotometer. Emission lifetime measurements were performed with a Quanta Ray DCR-3 pulsed Nd:YAG laser system (pulse output 355 nm, 8 ns). Error limits were estimated: λ (±1 nm); τ (±10%); ϕ (±10%). ^{1}H NMR spectra were recorded on a Bruker DPX-300 NMR spectrometer. Positive-ion mass spectra were recorded on a Finnigan MAT95 mass spectrometer. Elemental analysis was performed by the Institute of Chemistry at the Chinese Academy of Sciences, Beijing. Infrared spectra were recorded as Nujol mulls on a Bio-Rad FT-IR spectrometer.

Spectroscopic titration. Solutions of the platinum(II) complexes (50 μM) were prepared in Tris buffer solutions (5 mM Tris, 50 mM NaCl, pH 7.2). Aliquots of a millimolar stock ct DNA solution (0–500 μM) were added. Absorption spectra were recorded in the 200–600 nm range, after equilibration at 20.0 °C for 10 min. Emission spectra were recorded in the 400–800 nm range, after

equilibration at 20.0 °C for 10 min. The intrinsic binding constant, K, was determined from a plot of $D/\Delta\varepsilon_{ap}$ vs D according to equation (1):[7]

$$D/\Delta\varepsilon_{ap} = D/\Delta\varepsilon + 1/(\Delta\varepsilon \times K) \quad (1)$$

where D is the concentration of DNA, $\Delta\varepsilon_{ap} = |\varepsilon_A - \varepsilon_F|$, $\varepsilon_A = A_{obs}/[\text{complex}]$, and $\Delta\varepsilon = |\varepsilon_B - \varepsilon_F|$; ε_B and ε_F correspond to the extinction coefficients of DNA–complex adduct and unbound complex, respectively.

The emission titration data were analyzed by the neighbour exclusion model, and fitting the data with the McGhee-von Hippel equation[8] (equation 2) by the least-squares method.

$$r/c_F = K(1 - nr)[(1 - nr)/(1 - (n - 1)r)]^{n-1} \quad (2)$$

Restriction endonuclease fragmentation assay. Digestion of a plasmid pDR2 DNA (10.7 kb) with a restriction enzyme, ApaI (Boehringer Mannheim), was performed by mixing the DNA (21 nM bp^{-1}) in 1× SuRE/Cut Buffer A with ApaI (1 unit/L), followed by incubation at 37 °C for 1 h.[7] A mixture of ethidium bromide (4 M), Hoechst 33342 (200 M), cisplatin (*cis*-$[Pt(NH_3)_2Cl_2]$) (200 μM), **1** (4 μM) and pDR2 (10.7 kbp, 21 nM bp^{-1}) in digestion buffer was first incubated at room temperature for 5 min followed by addition of restriction enzyme (1 unit/μL). Two controls of pDR2 in the absence and presence of restriction enzyme in digestion buffer were prepared. All the solutions were incubated at 37 °C for 1 h; after restriction enzyme digestion the samples were analysed by agarose gel electrophoresis.

UV melting study. UV melting studies on 33-bp DNA were performed with a Perkin-Elmer Lambda 19 UV/Vis spectrophotometer equipped with a Peltier temperature programmer PTP-6. Solutions of DNA in the absence and presence of the platinum complex (DNA base pair:metal complex=1:1) were prepared in a Tris buffer solution. The temperature of solution was increased at a rate of 1 $^oC\ min^{-1}$, and the absorbance at 260 nm was continuously monitored. The Tm values were determined graphically from the plot of absorbance versus temperature.

Viscosity experiments. Viscosity experiments were performed on a Cannon-Manning Semi-Micro Viscometer immersed in a thermostatically controlled water bath maintained at 27 ^oC.[9] Titrations were performed by addition of small volume of concentrated stock solutions of metal complex to a solution of calf thymus DNA in BPE buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, and 1 mM Na_2EDTA, pH 7.0) in the viscometer. Mixing of the solutions in the viscometer was achieved by bubbling with nitrogen gas. The concentration of DNA was approximately 1 mM (in base pairs).

Gel mobility shift assay. A 400-bp PCR product (15.2 μM bp^{-1}) was mixed with the metal complex at 25–200 μM. The mixture was analysed by gel electrophoresis (Pharmacia Biotech GNA-200 submarine unit with Power Pac 300 power supply, Bio-Rad) using a 2% (w/v) agarose gel and 1× Tris-acetate-EDTA (TAE) buffer (40 mM Tris-acetate, 1 mM EDTA, 50 mM NaCl).

Cytotoxicity test (MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium bromide) assay). Cells were seeded in a 96-well flat-bottomed microplate at 20000 cells/well in 150 μL of growth medium solution (10% fetal calf serum (FCS, Gibco), 1% Sigma A-7292 Antibiotic and Antimycotic Solution in minimal essential medium (MEM-Eagle, Sigma)). Complex **1** and cisplatin (positive control) were dissolved in DMSO (dimethyl sulfoxide) and mixed with the growth medium (final concentration ≤ 4% DMSO). Serial dilution of each complex was added to each well. The microplate was incubated at 37 °C, 5% CO_2, 95% air in a humidified incubator for 48 h. After incubation, 10 μL MTT reagent (5 mg/mL) was added to each well. The microplate was re-incubated at 37 °C in 5% CO_2 for 4 h. Solubilization solution (10% SDS in 0.01 M HCl) (100 μL) was added to each well. The microplate was left in an incubator for 24 h. Absorbances at 550

nm were measured by a microplate reader. The IC_{50} values of **1** (concentration required to reduce the absorbance by 50% compared to the controls) were determined by the dose-dependence of surviving cells after exposure to the metal complex for 48 h.

Emission measurement. Solutions of the platinum(II) complex (9.74 μM) with T_{14} or T_{33} oligonucleotide (285.7 nM) were prepared in Tris buffer. Aliquots of a millimolar stock Hg^{2+} solution (0–8.6 μM) or various metal ions (3.4 μM) were then added. Emission spectra were recorded in the 400–800 nm range, after equilibration at 20.0 °C for 10 min. Excitation wavelength = 320 nm.

Luminescence quantum yields were determined using the method of Demas and Crosby[10] $[Ru(bpy)_3]Cl_2$ in degassed acetonitrile as a standard reference solution ($\Phi_r = 0.062$) and calculated according to the following equation: $\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r)$, where the subscripts s and r refer to sample and reference standard solution respectively, n is the refractive index of the solvents, D is the integrated intensity, and Φ is the luminescence quantum yield. The quantity B was calculated by $B = 1 - 10^{-AL}$, where A is the absorbance at the excitation wavelength and L is the optical path length. The radiative (k_r) and nonradiative (k_{nr}) rate constants were calculated by $k_r = \Phi_0/\tau_0$ and $k_{nr} = k_r(1/\Phi_0 - 1)$, respectively.

[Pt(C^N^N)(4-appt)](ClO$_4$) (1). A suspension of 4-appt (0.06 g, 0.22 mmol) and [Pt(C^N^N)Cl] (0.10 g, 0.22 mmol) in acetonitrile-methanol mixture (1:1, 40 mL) was refluxed for 24 h under a nitrogen atmosphere to give a clear orange solution. Addition of a methanolic solution of $LiClO_4$ (0.10 g, 0.93 mmol in 1 mL) gave a yellow precipitate, which was washed with diethyl ether. Recrystallization by diffusion of diethyl ether into an acetonitrile solution afforded a yellow crystalline solid. Yield: 0.13 g, 76%. FAB-MS: *m/z* 690 [M^+], 426 [M^+ – (4-appt)]. ^{1}H NMR (300 MHz, CD_3CN): 6.02 (m, 1H), 6.36 (m, 1H), 7.07 (m, 4H), 7.36 (m, 4H), 7.63 (m, 1H), 7.74 (m, 4H), 7.94 (m, 2H), 8.13 (m, 3H), 8.45 (m, 2H) 8.99 (m, 1H). IR (Nujol/cm^{-1}): 3369 (w, N–H), 1603 (s, C=N). Anal. Calcd for $C_{30}H_{23}N_8O_4ClPt$: C, 45.53; H, 2.93; N, 14.16. Found: C, 45.66; H, 3.04; N, 14.45.

References

1 (a) J. Sambrook, E. F. Fritsch, T. E. Maniatis, *Molecular Cloning, A Laboratory Manual, 2nd Ed.,* **1989**, E.3 and E.10; (b) G. Felsenfeld, S. Z. Hirschman, *J. Mol. Biol.,* **1965**, *13*, 407.

2 C.-W. Chan, D. M. P. Mingos, A. J. P. White, D. J. Williams, *Polyhedron* **1996**, *15*, 1753.

3 S.-W. Lai, M. C.-W. Chan, T.-C. Cheung, S.-M. Peng, C.-M. Che, *Inorg. Chem.* **1999**, *38*, 4046.

4 T.-C. Cheung, K.-K. Cheung, S.-M. Peng, C.-M. Che, *J. Chem. Soc. Dalton Trans.* **1996**, 1645.

5 H. K. Yip, L. K. Cheng, K. K. Cheung, C.-M. Che, *J. Chem. Soc., Dalton Trans*., **1993**, 2933.

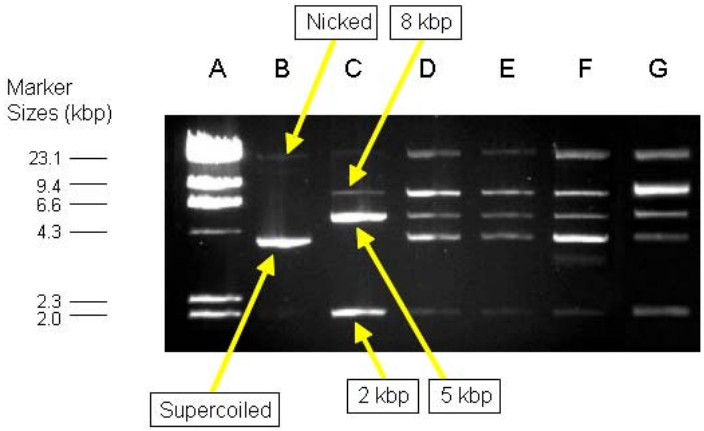
6 S. J. Busch, R. L. Barnhart, G. A. Martin, M. A. Flanagan, R. L. Jackson, *J. Biol. Chem*., **1990**, *265*, 22474.

7 C. V. Kumar, E. H. Asuncion, *J. Am. Chem. Soc.,* **1993**, *115*, 8547.

8 J. D. McGhee, P. H. Hippel, *J. Mol. Biol.* **1974**, *86*, 469.

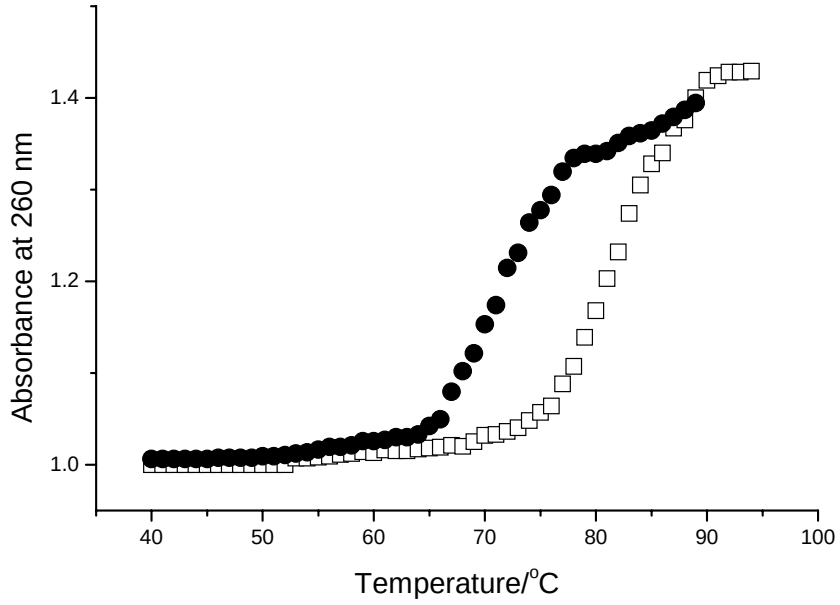
9 J. N. Demas, G. A. Crosby, *J. Phys. Chem.*, **1971**, *75*, 991.

Fig. S1 Inhibition of restriction endonuclease ApaI cutting sites by various small molecules. Lane A is size marker. Lanes B and C are undigested and ApaI (1 unit/μL) digestion products of pDR2 DNA (10.7 kbp, 21 nM bp^{-1}), respectively.[a] Lanes D–F are the digestion products of pDR2 DNA in the presence of DNA interacting molecules: ethidium bromide (4 M) (Lane D), Hoechst 33342 (200 M) (Lane E), cisplatin (200 M) (Lane F).[b] Lanes G–I are the digestion products of pDR2 DNA in the presence of **1** at 20 M (Lane G).[c]



[a] Two bands corresponding to the supercoiled and nicked DNA were observed for the undigested DNA (Lane B). After ApaI digestion of pDR2, three bands corresponding to DNA fragments with 8, 5 and 2 kbp were obtained and resolved by agarose gel electrophorsis (Lane C).
[b] In the presence of the classical intercalator ethidium bromide (4 μM), the minor groove binder Hoechst 33342 (200 μM), or the intrastrand crosslinker cisplatin (200 μM), DNA digestion was incomplete and bands attributed to the whole plasmid plus fragments were observed (Lanes D–F).
[c] Because complex **1** binds to DNA, treatment of pDR2 and ApaI with **1** at concentration 20 μM in 1× SuRE/Cut Buffer A inhibit the ApaI digestion and bands attributed to the whole plasmid plus fragments were observed (Lane G).

Fig. S2 Plots of A/A_0 vs. temperature of 33-bp DNA (20 μM) (●) and 33-bp DNA in the presence of **1** (□) with a 1:1 ratio of DNA base pair to **1** in Tris buffer solution.[a]



[a] The thermodynamic stability of the metal-bound DNA was studied by measuring the UV-vis absorption spectra at various temperatures. The melting temperature (T_m) of the untreated 33-bp DNA was found to be 71 °C, and this increased to 87 °C upon binding to **1**.

Fig. S3 UV-vis spectra of **1** (50 μM) in Tris buffer solution with [DNA]/[**1**] ratios of 0, 0.3, 0.5, 1.2, 1.5 at 20 ^{o}C. Inset: plot of $D/\Delta\varepsilon_{ap}$ vs D. Absorbance was monitored at 320 nm.

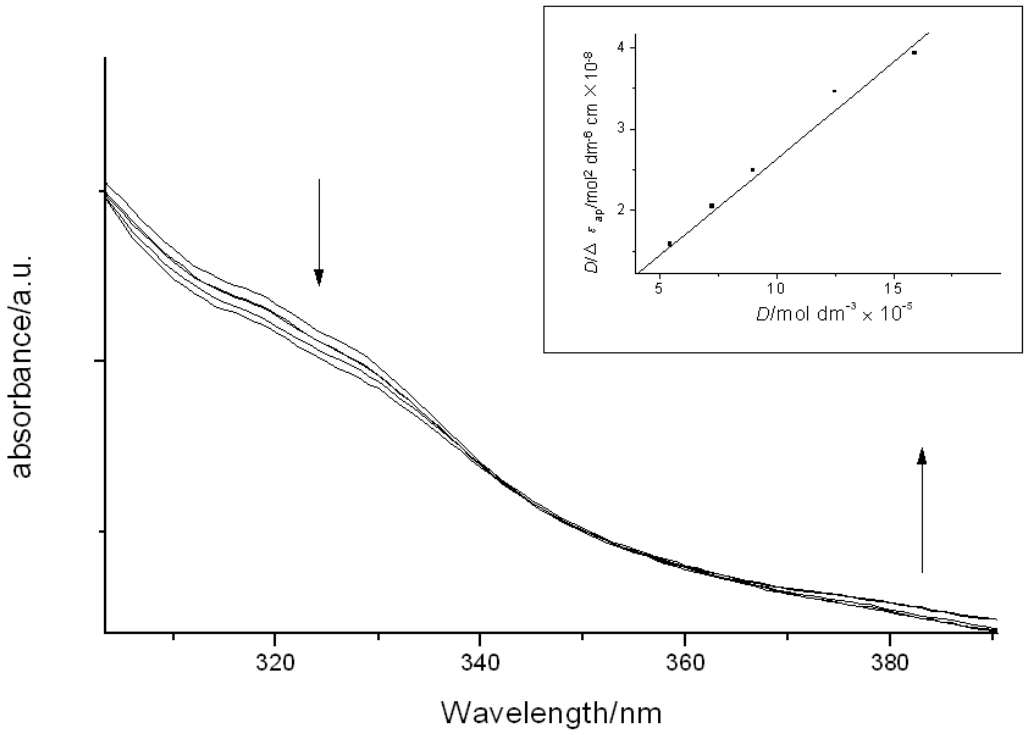


Fig. S4 Emission spectral traces of **1** (50 μM) in Tris buffer solution with [DNA]/[**1**] ratios of 0.53, 2.70, 10.9, 18.4, 22.2, 27.8, 34.4, 40.9, 49.5, 55.0 at 20 ^{o}C. Inset: Scatchard plot for the binding of **1** to ct DNA. The titration was used to generate the solid line in the figure, which was determined by using McGhee-von Hippel equation and a nonlinear least-squares fit for the data points.

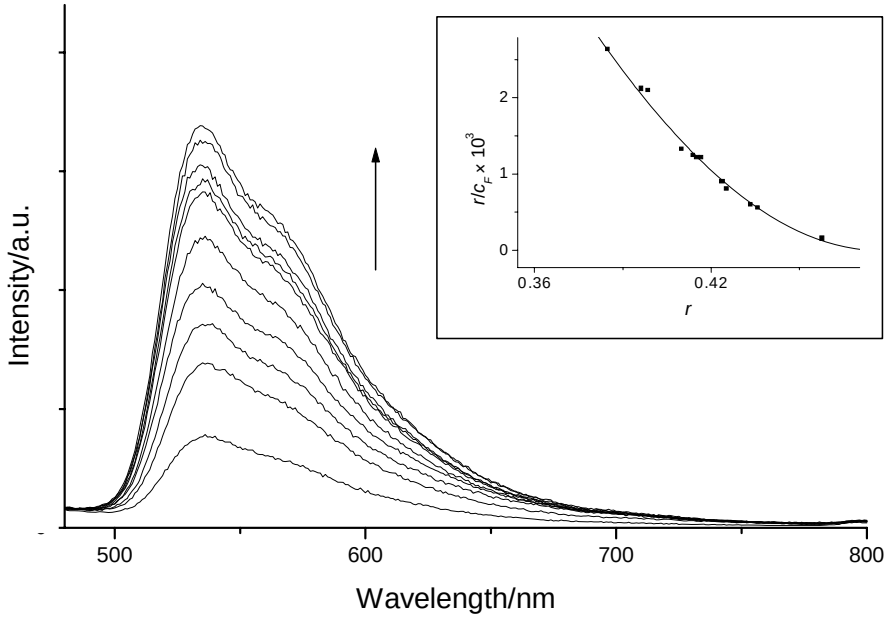


Fig. S5 Gel electrophoresis of a 400-bp DNA ladder in 2 % (w/v) agarose gel showing the mobility of DNA. Lane A is the 400-bp DNA. Lanes B–F are the 400-bp DNA in the presence of **1** at 25 μM (Lane B), 50 μM (Lane C), 100 μM (Lane D), 150 μM (Lane E) and 200 μM (Lane F).

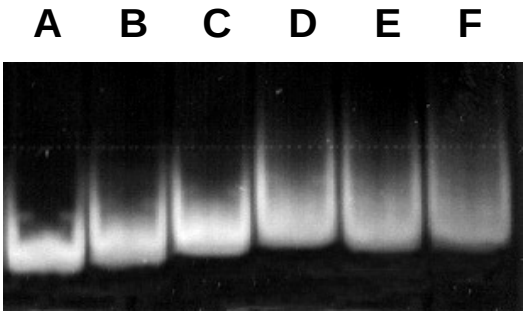


Fig. S6 Relative specific viscosity of calf thymus DNA in the presence of ethidium bromide (◆), Hoechst 33342 (●), and **1** (○) shown as a function of the binding ratio.

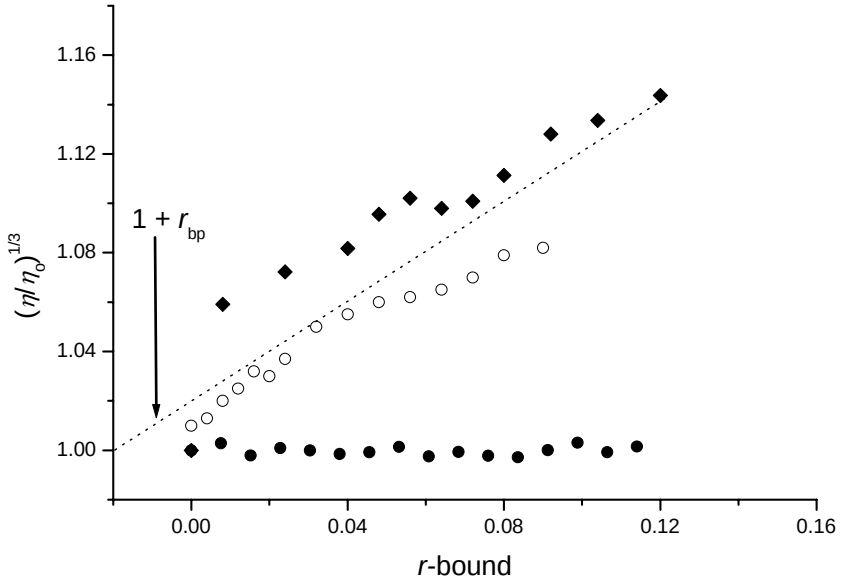


Fig. S7 Phosphorescence intensity at 536 nm of **1** (9.7 μM) and T_{33} (0.29 μM) when treated with (**1**) Mix = Zn^{2+}, Pb^{2+}, Mn^{2+}, Ag^{+} (each 3.4 μM), (**2**) Hg^{2+} (0.68 μM), and (**3**) Mix (each 3.4 μM) + Hg^{2+} (0.68 μM)

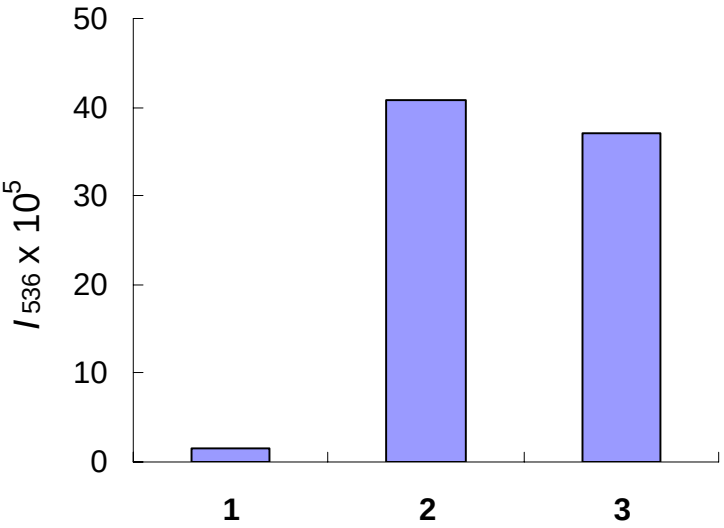


Table S1. UV-vis Absorption and Emission Data recorded in MeCN at 20 ^{o}C

Complex	UV-vis	Emission
	λ/nm (ε_{max}/dm^3 mol^{-1} cm^{-1})	λ_{max}/nm (τ/μs, ϕ_{em})
1	400–450 (1200), 320 (1.51×10^4), 268 (4.11×10^4)	537 (4.2, 0.034)

Scheme S1. Schematic representation of the function of a Hg^{2+} sensor that operates based on modulation of the luminescence of the complex formed between metallointercalator and DNA duplex of T_{33}.