Supporting Information

Highly Sensitive and Selective Detection of Mercury Ions by Using Oligonucleotides, DNA Intercalators and Conjugated Polymers

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1. Materials: All the materials were obtained from commercial sources and used without further purification. PFP was obtained from American Dye Source Inc. The molecular weight is 10000 – 15000. YOYO-1 was purchased from Invitrogen and the thymine oligonucleotide, T_{24}, was from Sigma.

2. The detailed results of the same scheme using TOTO-1 to substitute YOYO-1 as the DNA intercalator and two-photon excitation results.

We have also performed the same experiments using another DNA intercalator, TOTO-1 to substitute YOYO-1. TOTO-1/T_{24} is less fluorescent than YOYO-1/T_{24}. The maxima absorption wavelength of TOTO-1 is located at ~510 nm, red shifted compared to that of YOYO-1 (~490 nm). The overlap between the emission spectrum of PFP and the absorption spectrum of TOTO-1 is smaller than that of PFP/YOYO-1 pair. The energy transfer from the conjugated polymer, PFP, to TOTO-1 is thus not as efficient as that between PFP and YOYO-1, as indicated by our experimental results.

Addition of Hg^{2+} into TOTO-1/T_{24} resulted in a fluorescence enhancement of 9.6 times (Figure S1). The use of TOTO-1 also showed exceptional selectivity for Hg^{2+} over other metal ions (Figure S2). Further addition of PFP results in additional enhancement

factor of 7.9 (Figure S3). The overall limit of detection was estimated to be about 0.38 nM.

Comparison of the data in Figure 3 and Figure S3 shows that the acceptor/donor intensity ratios are quite different when TOTO-1 or YOYO-1 was used as the intercalator, even though the enhancement factors for TOTO-1 (7.9) and YOYO-1 (12) are only slightly different after conjugated polymers was used to improve the detection sensitivity. The large difference in their acceptor /donor intensity ratios is mainly due to different fluorescence efficiencies of the two acceptors. Upon intercalation, the fluorescence yield of YOYO-1 is about four times higher than that of TOTO-1, which will result in a larger difference in their acceptor/donor intensity ratios in their fluorescence spectra.

Our results also suggest that Hg^{2+} will significantly affect the interactions between the intercalator and DNA. The interactions between Hg^{2+} and thymine will result in formation of a stable T-Hg^{2+}-T complex that leads to a "dsDNA-like" or partially folded structure. The DNA intercalators (YOYO-1 and TOTO-1) will bind strongly to the "dsDNA-like" hairpin structure, and consequently, intercalators will exhibit a large increase in their fluorescence quantum yields. It has been widely known that the fluorescence intensities of these DNA intercalators increase significantly upon binding to dsDNA. But, on the other hand, the existence of Hg^{2+} will also introduce steric and electrostatic hindrance for the intercalators binding to DNA. Because of this, the fluorescence intensity of YOYO-1 only increased about 17 times in total (2.6-fold increase due to addition of T_{24} and another 6.6-fold increase when mercury ions were added), which is much lower than the previously reported up to 460-fold fluorescence

enhancement when YOYO-1 was intercalated into the normal dsDNA. These results suggested that the interaction between YOYO-1 and T_{24}/Hg is much weaker than that between YOYO-1 and the normal dsDNA.

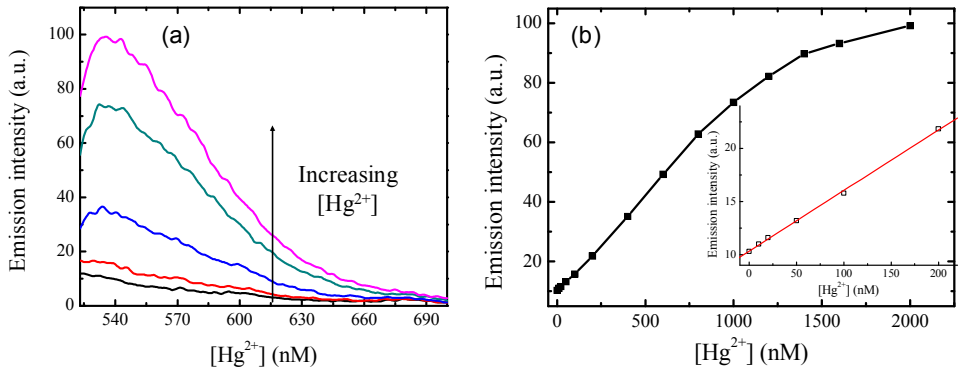

Figure S1. (a) Emission spectra of TOTO-1/T_{24} after addition of different amounts of Hg^{2+} (0, 100, 400, 1000 and 2000 nM): [TOTO-1] = 75 nM; [T_{24}] = 50 nM; λ_{ex}=510 nm; (b) Emission intensities of TOTO-1/T_{24} at 535 nm with titration of Hg^{2+}.

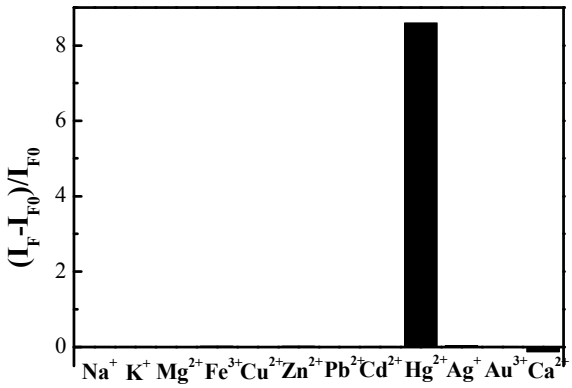

Figure S2. Relative fluorescence increases [(I_F-I_{F0})/ I_{F0}] at 535 nm of T_{24}/TOTO-1/metal ions in 50 mM (pH=7.4) PBS buffer solution: [T_{24}] = 50 nM; [TOTO-1] = 75 nM; [metal ions] = 2.0 μM; λex = 510 nm. I_{F0} and I_F are fluorescence intensities of T_{24}/TOTO-1 complex at 535 nm in the absence and presence of metal ions respectively.

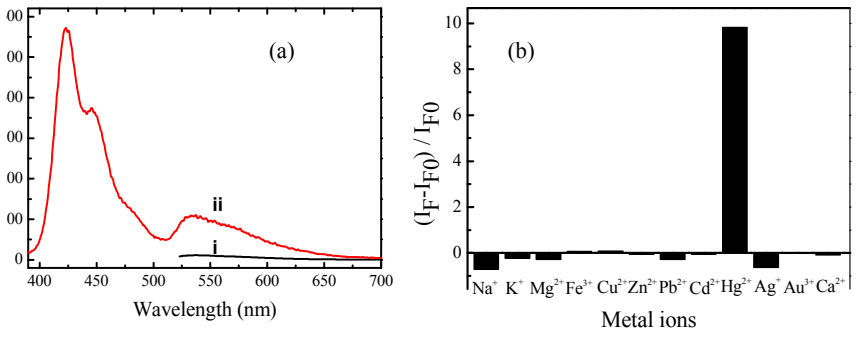

Figure S3. Emission spectra of T_{24}/TOTO-1/ Hg^{2+} in the absence (i) and presence (ii) of PFP; (b) Relative fluorescence intensity increases $[(I_F-I_{F0})/ I_{F0}]$ at 535 nm of PFP/T_{24}/TOTO-1/metal ions: [PFP] = 1.24 μM; [T_{24}]=50 nM; [TOTO-1]=75 nM; [metal ions]= 2.0 μM; λ_{ex}=380 nm. I_{F0} and I_F are fluorescence intensities of PFP/T_{24}/TOTO-1 complex in the absence and presence of metal ions, respectively.

3. The two-photon excitation fluorescence measurements

The experimental setup for two-photon excitation fluorescence measurements were described in ref 15. The excitation source for two-photon excitation fluorescence measurement was a femtosecond Ti:sapphire oscillator from Spectra Physics (Tsunami). The output laser pulses have pulse duration of 40 fs, a repetition rate of 76 MHz, and a center wavelength at 800 nm. The total output energy was 320 mW. The samples were excited by directing a tightly collimated, high intensity laser beam onto the sample. The emission from the sample was collected at a 90^o angle by a pair of lens and optical fibres and directed to the spectrometer, which was a monocromator (Acton, Spectra Pro 2300i) coupled CCD (Princeton Instruments, Pixis 100B) system. To avoid internal filter effects, the excited volume was located near the cell wall on the collection optics side. This configuration minimizes the fluorescence path inside the sample cell and thus reduces

self-absorption. A short pass filter with cut-off wavelength at 700 nm was paced before the spectrometer to minimize the intensity of pumping light scattering.

The two-photon excitation (TPE) fluorescence of T_{24}/YOYO-1 in the absence and presence of PFP have also been measured using femtosecond laser pulses at 800 nm (Figure S4). Addition of conjugated polymers (PFP) can enhance the TPE emission of YOYO-1 by a factor of up to 37 times compared to that in the absence of PFP.

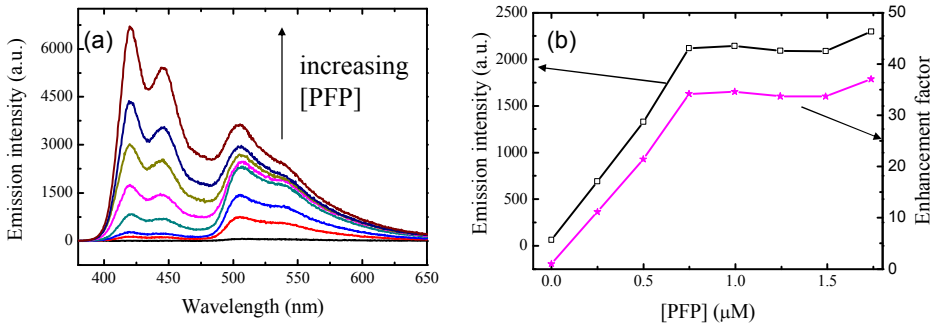

Figure S4. (a) Two-photon excitation emission spectra of YOYO-1/T_{24}/Hg^{2+} after addition of different amounts of PFP: [YOYO-1] = 75 nM; [T_{24}]=50 nM; [Hg^{2+}]=2.0 μM; λ_{ex}=800 nm; (b) Two photon excitation emission intensities (with contributions from PFP residue emission subtracted) of YOYO-1 at 510 nm in YOYO-1/T_{24}/Hg^{2+}/PFP and the corresponding enhancement factors.

The two-photon excitation (TPE) fluorescence of T_{24}/TOTO-1 in the absence and presence of PFP are shown in Figure S5. Addition of conjugated polymers (PFP) can enhance the TPE emission of TOTO-1 by a factor of up to 23 times compared to that in the absence of PFP.

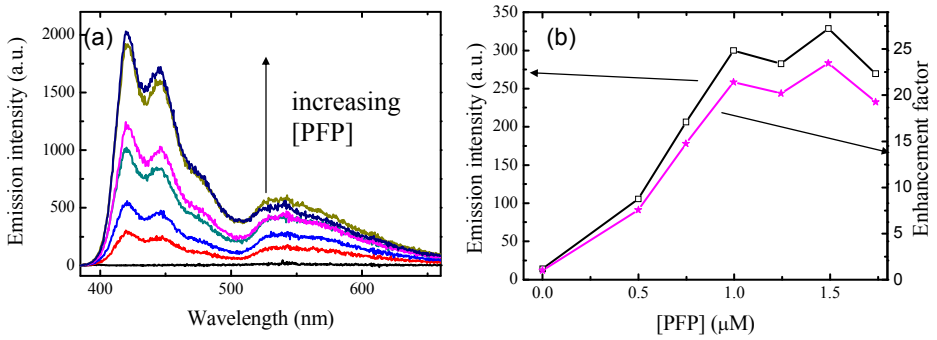

Figure S5. (a) Two-photon excitation emission spectra of TOTO-1/T_{24}/Hg^{2+} after addition of different amounts of PFP: [TOTO-1] = 75 nM; [T_{24}] = 50 nM; [Hg^{2+}]=2.0 μM; λ_{ex}=800 nm; (b) Two photon excitation emission intensities (with contributions from PFP residue emission subtracted) of TOTO-1 at 535 nm in TOTO-1/T_{24}/Hg^{2+}/PFP and the corresponding enhancement factors.