Supplementary Information

Design of Controlled Multi-Probes Coupled Assay via Bioinspired Signal Amplification Approach for Mercury Detection

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Fig. S1. $^1$H NMR spectrum of compound 2

Fig. S2. IR spectrum of compound 2
Fig. S3. Mass spectrum (ESI+) of compound 2
Fig. S4. $^1$H NMR spectrum of AYF

Fig. S5. IR spectrum of AYF
**Fig. S6.** Mass spectrum (ESI+) of AYF

**Fig. S7.** Negative control of DPF$_1$. Fluorescence emission changes ($\lambda_{ex}=360$ nm) consisting of DPF$_1$ (50 μM) upon incubation with and without the present of Hg$^{2+}$ in 1% CBTP buffer (pH=7.1, 1 mM), 9% DMSO, 1% of Pyridine and 89% ACN for 3 h at 40°C.
Fig. S8. Negative control of DCC. Fluorescence emission changes ($\lambda_{ex}=500$ nm) consisting of DCC (20 μM) upon incubation with and without the present of Hg$^{2+}$ in 1% CBTP buffer (pH=7.1, 1 mM), 9% DMSO, 1% of Pyridine and 89% ACN for 3 h at 40°C.

**Preparation of tap water and bovine serum samples**

Tap water (90 μL) were spiked with 10 μL 0.1 M CBTP pH=7.1 with known quantities of Hg$^{2+}$. The final concentrations of spiked Hg$^{2+}$ are 10 ppb and 40 ppb. 5 μL of this mixed solution was then used for quantification of Hg$^{2+}$ (total volume of the sample assay for the 1st step is 100 μL). Calculated amount of Hg$^{2+}$ in final experimental medium is from first step which adds the dilution factor of 20.

Bovine serum (45 μL) were spiked with 5 μL H$_2$O with known quantities of Hg$^{2+}$ (1 ppm and 1.5 ppm), then the spiked bovine samples were dissolved in 0.1 M CBTP pH=7.1 (450 μL). The final concentrations of Hg$^{2+}$ in bovine sample are 100 ppb and 150 ppb. 5 μL of this mixed solution was then used for quantification of Hg$^{2+}$ (total volume of the sample assay for the 1st step is 100 μL). Calculated amount of Hg$^{2+}$ in final experimental medium is from first step which adds the dilution factor of 200.

The linear range curve with emission intensity and Hg$^{2+}$ concentration (Figure 3) plays the key role in this calculation. Final emission intensity was noted after completing real sample application with three probes assay. With reverse procedure, we can quantitatively estimate the amount of Hg$^{2+}$ concentration in simple volumetric calculation.