Electronic Supplementary Material (ESI) for Chemical Communications
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Electronic Supplementary Information (ESI) for

Dual polarisation interferometry for real-time, label-free detection of interaction of mercury(II) with mercury-specific oligonucleotides

Yong Wang, [a,b] Yu Zheng,[a,b] Fan Yang[a] and Xiurong Yang[*a]

[a] *State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China*

[b] *Graduate School of the Chinese Academy of Sciences, Beijing 100039, China*

** Corresponding author. Telephone: +86 431 85262056. Fax: +86 431 85689278.*

E-mail: xryang@ciac.jl.cn.

Experimental Section

Reagents and Chemicals. Poly(ethylenimine) (PEI, MW = 750000), 50% (w/v) aqueous solution was purchased from Sigma-Aldrich (St Louis, MO, USA). Tris(hydroxymethyl)aminomethane (Tris) was obtained from A brand of EMD Biosciences, Inc. (La Jolla, CA, Germany). Mercury(II) perchlorate hydrate was purchased from Sigma-Aldrich (Milwaukee, WI, USA). Sodium acetate anhydrous (NaAc) was purchased from Beijing Chemical Reagent Co. (Beijing, China). Tris-HAc buffer solution was prepared with 10 mM Tris-HAc (pH 7.4) and 150 mM NaAc. The buffer solution was filtered and degassed before use. 21-mer oligonucleotides containing mercury-specific sequence (MSO, 5'-TTC TTT CTT CCC TTG TTT GTT-3') and control sequence (cMSO, 5'-AAC AAA CAA GGG AAG AAA GAA-3') were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Before use, oligonucleotide stock solutions were prepared with 10 mM

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Tris-HAc buffer (pH=7.4, with 150 mM NaAc). The concentrations of oligonucleotide solutions were quantified by measuring the 260 nm UV absorbance. Unless otherwise mentioned, all other reagents were of analytical reagent grade and used without further purification or treatment. Ultrapure water (Milli-Q plus, Millipore Inc., Bedford, MA) was used throughout.

Instrumentation. All real-time dual polarisation interferometry (DPI) measurements were conducted on an AnalLight Bio200 dual polarisation interferometer (Farfield Group Ltd., Manchester, U.K.). Circular dichroism (CD) spectral data were collected on a Jasco J-810 spectropolarimeter (Tokyo, Japan) attached with a Peltier temperature control unit in a rectangular optical chamber (10 mm path length, 0.7 mL volume) at room temperature. The optical chamber was deoxygenated with dry purified nitrogen (99.99%) prior to use and kept the nitrogen atmosphere through experiments. The reported spectrum was the average of three successive scans at a scan rate of 200 nm/min keeping a band width of 2 nm at a standard sensitivity. The background of the buffer solution was automatically subtracted from the CD data. Atomic force microscopy (AFM) measurements were performed on a Digital Instruments Nanoscope V (DI, Santa Barbara, CA) in tapping mode. Silicon (Si) cantilevers with spring constants of 0.6-6.0 N/m below their resonance frequency (typically, 67-150 kHz) were used in the measurements. The AFM images were obtained at room temperature in air under ambient conditions. For AFM study, a silicon wafer was first dipped into the PEI solution (1 mg/mL) for 10 min. After adsorption, the PEI-coated film was rinsed by continuously dipping it five times for 8 min each in water and was carefully dried in a stream of nitrogen at temperature room to remove the water from the surface and avoid contamination with dust. Next, the film was dipped into the MSO (18 μM) or cMSO (18 μM) solution for 10 min, respectively. Then, the MSO/PEI or cMSO/PEI film followed a rinsing and drying procedure similar to that of the PEI film.

Principle of DPI technique. The underlying principle associated with the DPI technique has been described in detail elsewhere.[1] Briefly, the instrument is composed of the optical assembly, sensor chip, sample fluidic system and measurement system housed within a temperature controlled enclosure, which is linked to a computer for data collection. The core of this instrument is the sensor chip, consisting of a sandwich-like structure of two horizontally stacked waveguides, a sensing waveguide on top of a reference waveguide, separated by an opaque cladding material. The sensing and reference waveguides are fabricated with silicon oxynitride, i.e., nitrogen-doped silica. A gasket on top of the sensing waveguide gives two sensor flow cells with each volume of 1.7 μL

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(1 mm wide, 17 mm long and 100 μm thick). A fluidics system consisting of a Rheodyne HPLC injector valve and an external pump (Harvard Apparatus, PHD2000) enabled a controlled continuous fluid flow that could be directed over experimental microfluidic channels and also contained an injection-loop system to allow delivery of fixed volumes. The DPI chip is illuminated using a helium-neon laser emitting light at 632.8 nm, which is split equally into two beams and travels down both the sensing and reference waveguides before exiting the structure to form Young's interference pattern in the far-field that is captured by the camera and after A/D transformation recorded by a computer. The mode of polarisation of the light beam is switched between the transverse electric (TE) and transverse magnetic (TM) using a ferroelectric liquid crystal. When materials are introduced into the flow cell and attach to the sensing waveguide surface, the two propagated polarisation light (TE and TM) through the sensing waveguide suffers phase changes, resulting in a change in the interference pattern. The TE and TM phase changes are directly related to the optical properties of the adsorbed layer; the refractive index (RI), n, and optical thickness, d. The two values can be uniquely calculated by solving the electromagnetic equations of the system for TE and TM polarisations. The adsorbed mass is determined using the following de Feijter equation:[2]

$$\Gamma = d_L(n_L - n_{buffer})/(dn/dc) \quad (1)$$

where Γ is the layer mass per unit area (ng/mm^2), d_L is the layer thickness (nm), n_L and n_{buffer} are the refractive indices of the layer and the bulk solution, respectively, and dn/dc is the refractive index increment (cm^3/g). A dn/dc value of 0.175 was used here according to Lee et al.[3]

General Procedure for DPI measurements. Before the DPI experiments, the unmodified silicon oxynitride AnalChip was first cleaned with oxidizing piranha solution, a 7:3 mixture of concentrated sulfuric acid and 30% hydrogen peroxide for 15 min (Caution: piranha solution is highly corrosive, reactive, and potentially explosive. The solution should be handled using suitable personal protective equipment and proper safety procedures.). Next, the chip was rinsed sequentially by sonicating in ultrapure water and ethanol for 10 min. This process was repeated three times. Finally, the chip was dried under a stream of nitrogen gas to remove the water from the surface before loading into the DPI instrument.

In the work, all DPI experiments were performed at 20 °C in 10 mM Tris-HAc running buffer (150 mM NaAc, pH 7.4). Prior to each experiment, the sensor chip and the running buffer were calibrated by injecting 80% (v/v) ethanol/water solution and water, the refractive index of which

were known. After two calibrations, the flow rate was kept at a continuous rate of 100 μL/min. A PEI solution (1 mg/mL) was injected for 2 min and incubated for 5 min before returning to the running buffer. After a stable baseline was attained, a solution of MSO (18 μM) or cMSO (18 μM) was injected for 2 min and incubated for 5 min. The flow was then returned to the running buffer until reaching stability. After that, the Hg^{2+} solutions at different concentrations were injected for 2 min. Linearization was performed at the end of the experiment to enable maximum measurement accuracy. The running buffer was kept at a flow rate of 100 μL/min, and an 80% (v/v) ethanol/water solution was injected to flow over chip surface for 1.5 min. The flow rate of the running buffer was reduced to 15 μL/min after the stable baseline of the 80% (v/v) ethanol/water solution was obtained, to dilute the ethanol/water solution as it flowed over the chip surface. Analysis of refractive index, thickness and mass per unit area on the sensor chip surface could be achieved using the AnaLight Bio200 software (Farfield Sensors Ltd., Manchester, U.K.).

References

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Table S1. Layer structure for the MSO/PEI or cMSO/PEI layer (average ± standard deviation, n = 4)

Layer material	Refractive Index	Thickness/nm	Mass[b]/(ng/mm^2)
PEI	1.485±0.013	1.023±0.100	0.878±0.046
PEI+MSO	1.475±0.004[a]	2.346±0.158[a]	1.007±0.151
PEI	1.481±0.008	1.038±0.089	0.864±0.048
PEI+cMSO	1.406±0.006[a]	4.476±0.500[a]	0.958±0.129

[a] Refractive index or thickness of the whole MSO/PEI or cMSO/PEI layer. [b] Mass of each PEI or oligonucleotide (MSO or cMSO) layer.

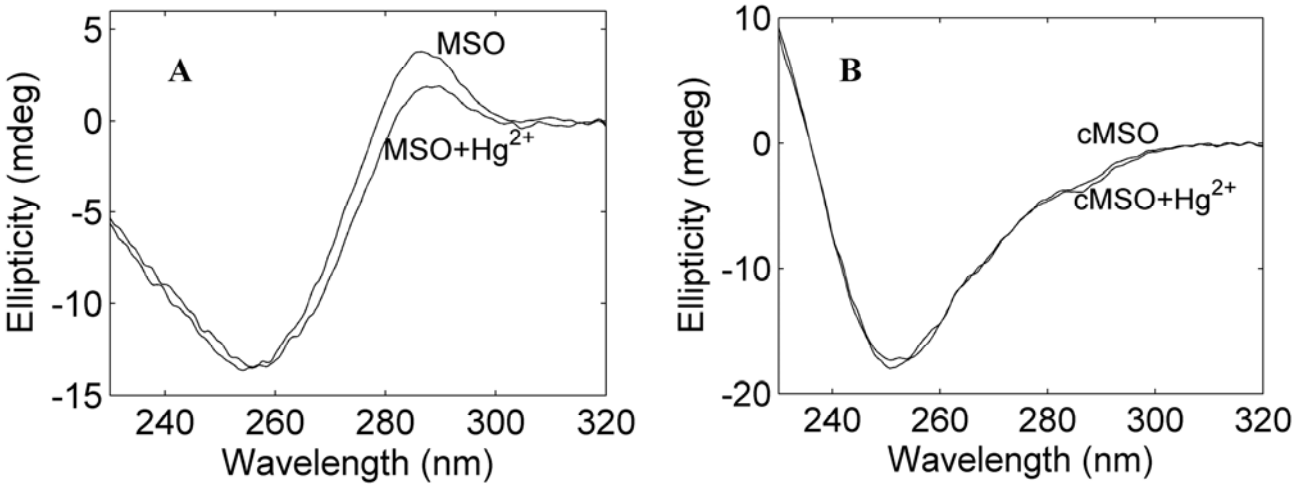

Fig. S1 CD spectra of (A) MSO and (B) cMSO in the absence and presence of 30 μM Hg^{2+} in Tris-HAc buffer of pH 7.4.

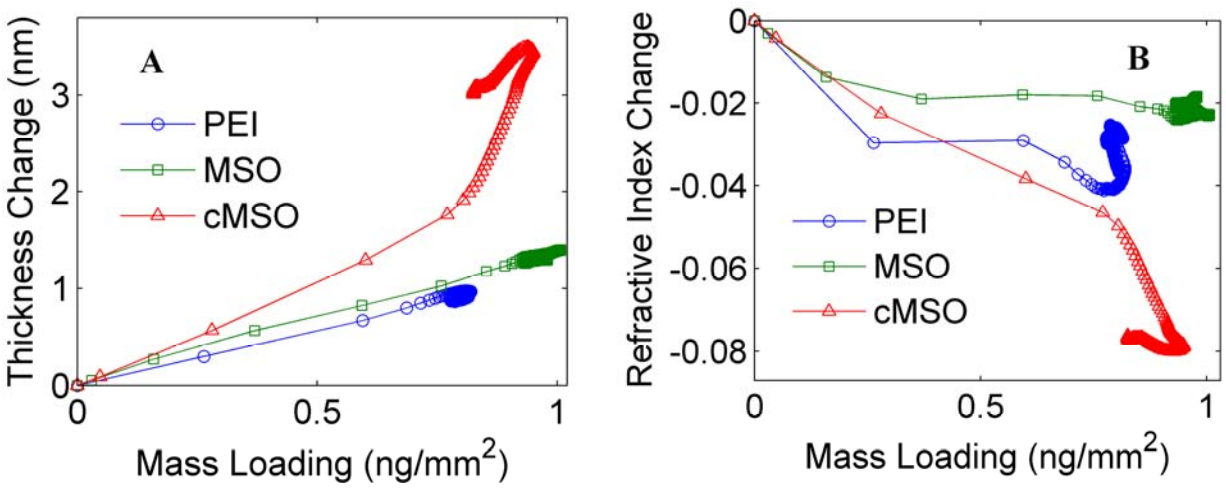

Fig. S2 (A) Layer thickness of PEI or MSO or cMSO as a function of each respective mass loading during the entire process of each adsorption from Fig. 1. (B) Layer refractive index of PEI or MSO or cMSO as a function of each respective mass loading during the entire process of each adsorption from Fig. 1.

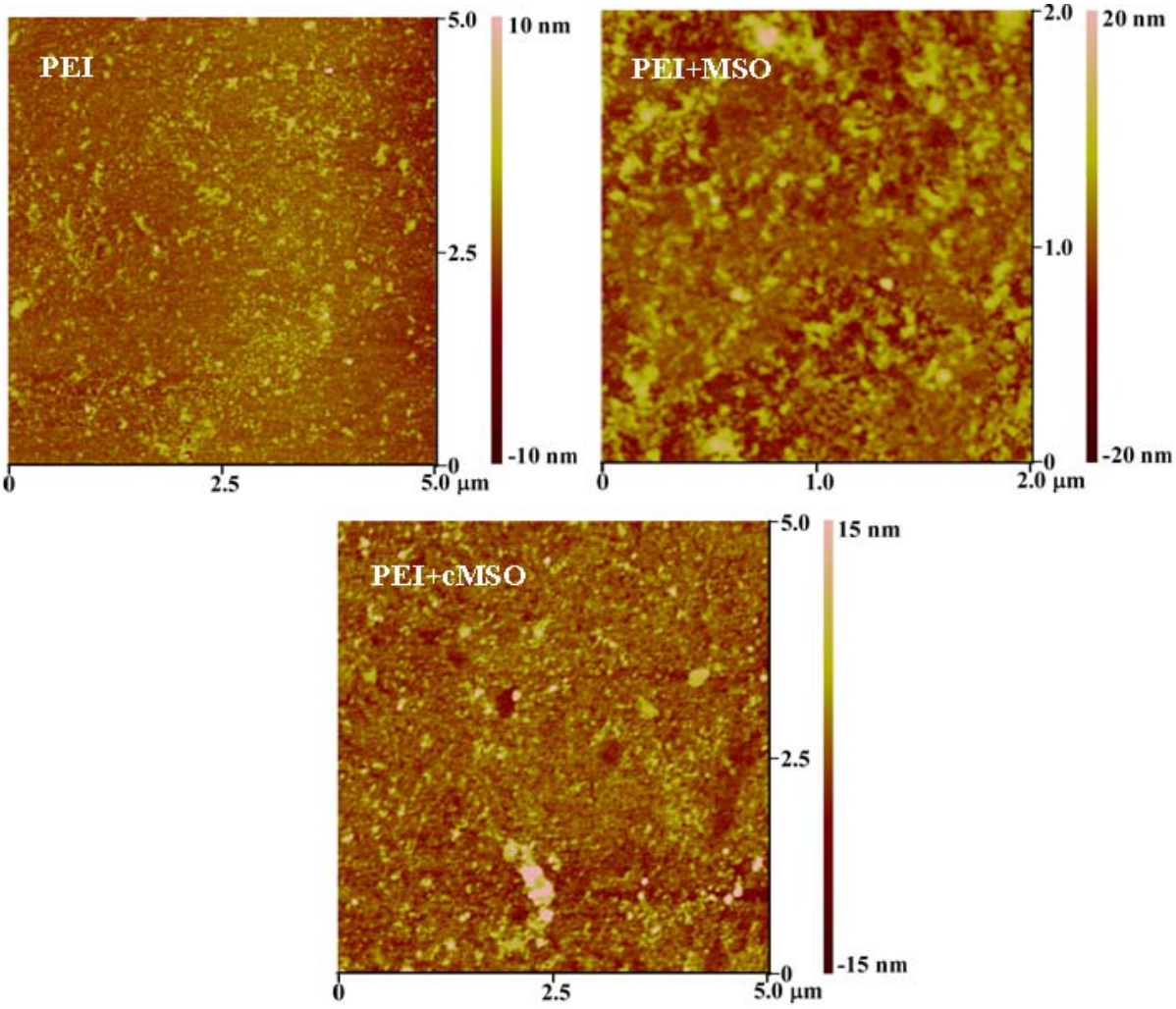

Fig. S3 AFM images of PEI or MSO/PEI or cMSO/PEI.

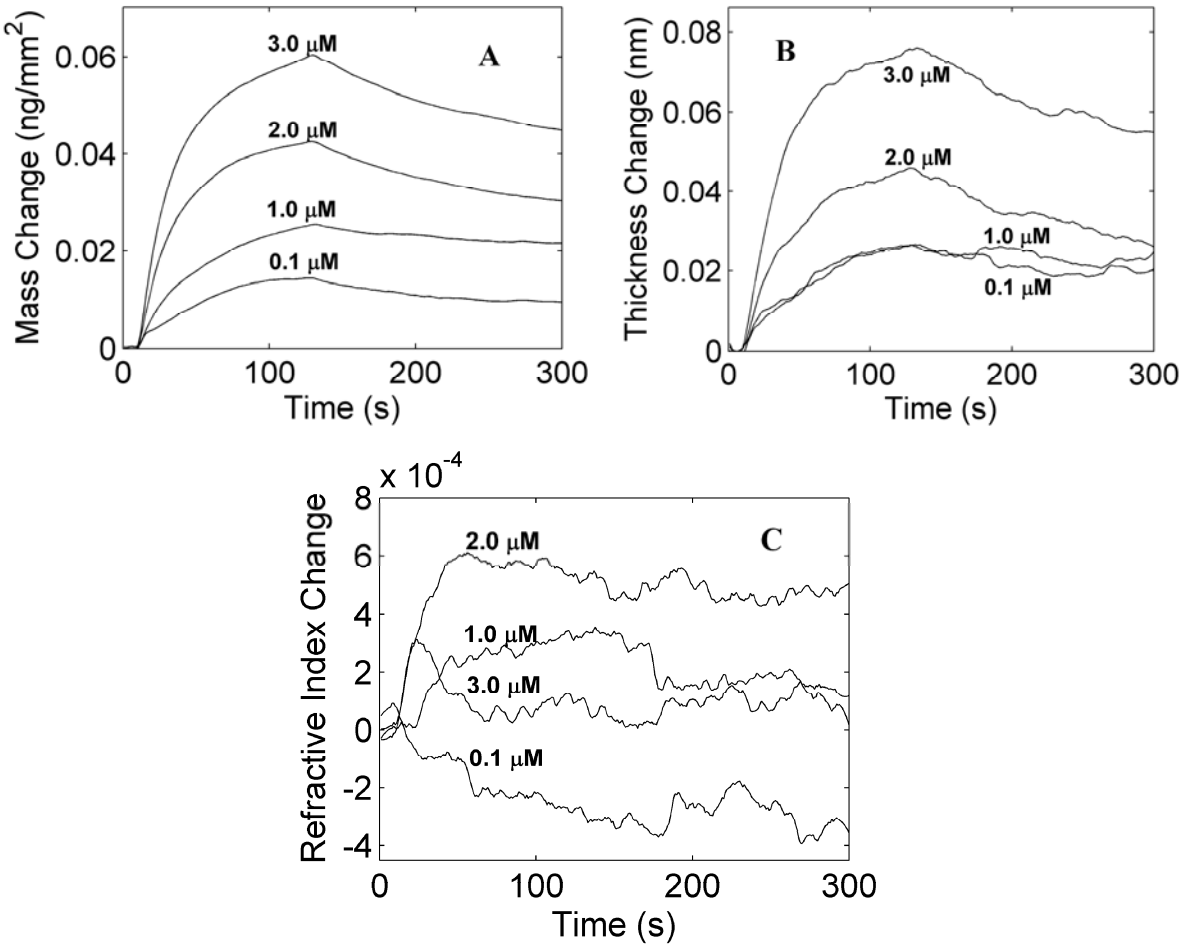

Fig. S4 Real-time changes in (A) mass, (B) thickness and (C) refractive index of MSO/PEI layer upon the injection of Hg^{2+} at different concentrations.

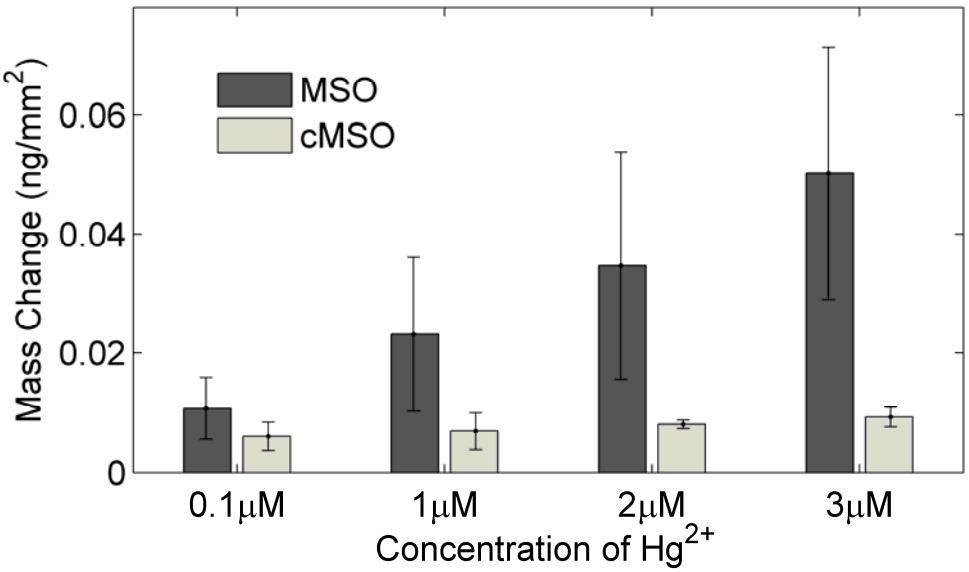

Fig. S5 Mass changes of MSO/PEI or cMSO/PEI layer after the addition of Hg^{2+} at different concentrations. The error bars represent the standard deviation of three measurements.

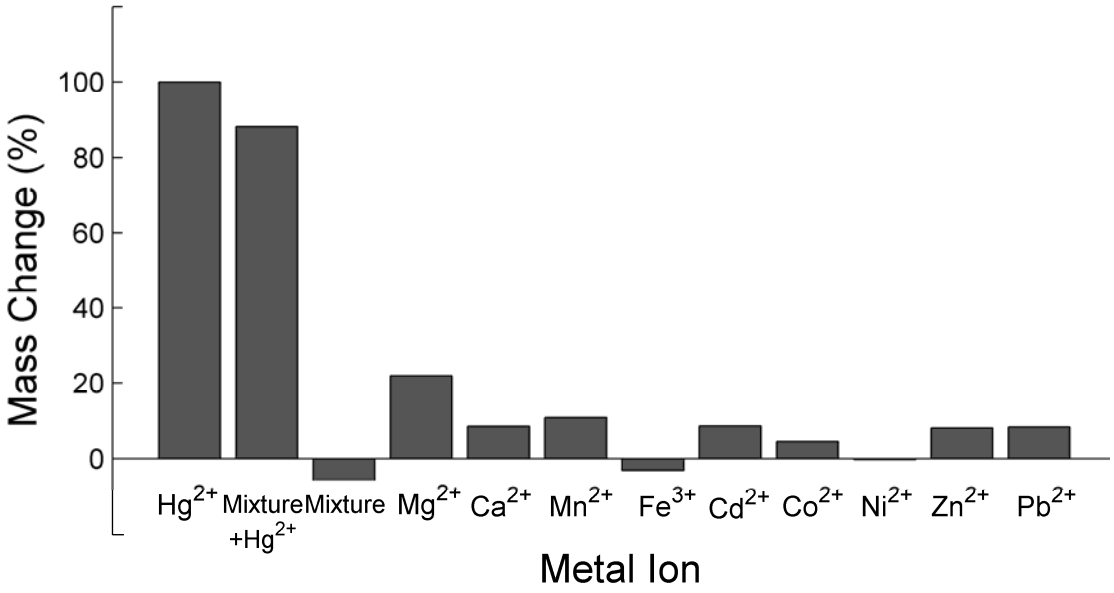

Fig. S6 Selectivity of the analysis of Hg^{2+} by comparing it to other individual metal ion and the mixture of various metal ions with and without Hg^{2+}. The concentration of all the metal ions is 3 μM.