Supplementary Data

DNAzyme-based fluorescent microarray for highly selective and sensitive detection of lead (II)

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Materials and Methods

Materials

Silyated slides (aldehyde) were purchased from CEL Associates (Pearland, USA). 2×spotting solution was purchased from TeleChem International Inc. The probe (1) (5'-NH_2-T_{12}-CATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT-3'), and probe (2) (5'-Cy5-ACTCACTATrAGGAAGAGATG-3') were synthesized and purified by Takara Biotechnology Co. (Dalian, China). Concentrated Pb^{2+} solutions were prepared using $Pb(OAc)_2$ salt in 10 mM acetic acid to assist in solubility. Working solutions of lower concentration were prepared by serial dilution of the concentrated solution with 50 mM Tris-HCl buffer, pH 7.2, containing 50 mM NaCl. All reagents were analytical grade or higher. And all solutions were prepared with Milli–Q water (18.2 MΩ cm^{-1}) from a Millipore system.

Preparation of DNAzyme-based microarray

5'-amine-modified oligonucleotide, probe (1) or 3'-amine-modified oligonucleotide, probe (3), was dissolved in 1×spotting solution to make 20 μM solutions. The solution was spotted onto the aldehyde-coated glass slides with a commercial arrayer (Cartesian, Pixsys 7500). After the spotting process, the oligonucleotide arrays were fixed at 25 °C for 48–72 h. Then, the oligonucleotide-arrayed slides were immersed in 0.2% SDS solution for 2 min and rinsed with Milli–Q water for 2 min. Subsequently, the slides were treated with aldehyde blocking solution (1 g $NaBH_4$, 300 ml phosphate buffer saline, pH 7.4, 100 ml 99% ethanol) for 15 min, and rinsed sequentially with 0.2% SDS solution, Milli–Q water for 2 min each, followed by air-drying for 30 s.

Cy5-labeled DNA/RNA chimer substrate, probe (2), or probe (4), was immobilized onto the chip via hybridization between probe (2) and probe (1) that was functionalized on the chip. In brief, probe (2)

or probe (4) was dissolved in 50 mM Tris-HCl buffer (pH 7.2) and 1M NaCl to make a 5 μM solution. Hybridization on (1)-arrayed slide was accomplished by placing probe (2) solution onto the probe (1) spots, followed by covering a coverslip and leaving overnight in a humid chamber at 4°C or room temperature, respectively.

Detection of Lead (II) in Solution

Prior to the lead reaction, the DNAzyme-arrayed slide was soaked in 50 mM Tris-HCl buffer, pH 7.2, 50 mM NaCl for 5 min in order to remove physically adsorbed probe (2). Aliquots of various concentrations of Pb^{2+} were prepared in 50 mM Tris-HCl buffer, pH 7.2, 50 mM NaCl from one concentrated Pb^{2+} stock solution. The Pb^{2+} solutions were added to the six-well hybridization chamber assembled with the DNAzyme-arrayed slide and reacted for 1 h at 4 °C for probe (1)/(2), or room temperature for probe (3)/(4), respectively. The chamber was then disassembled, and the slide was rinsed with 50 mM Tris-HCl buffer, pH 7.2, 50 mM NaCl for 5 min. Finally, the slide was imagined by a fluorescence scanner (General Scanning, Scanarray 3000) at 635 nm and fluorescence intensity was calculated from the image using software written in-house. To investigate the selectivity of the assay, other metal ions (Cu^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, Hg^{2+}) at 10 μM were tested in a similar way.

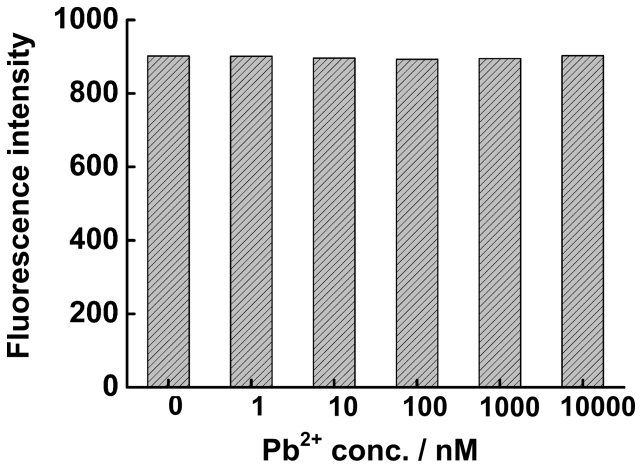

Fig. S1 The single-wavelength fluorescence intensities of 1 μM Cy5-labeled DNA/RNA chimer substrate (2) with different concentration of Pb^{2+}. The measurements were conducted with excitation wavelength at 650 nm and emission at 670 nm.

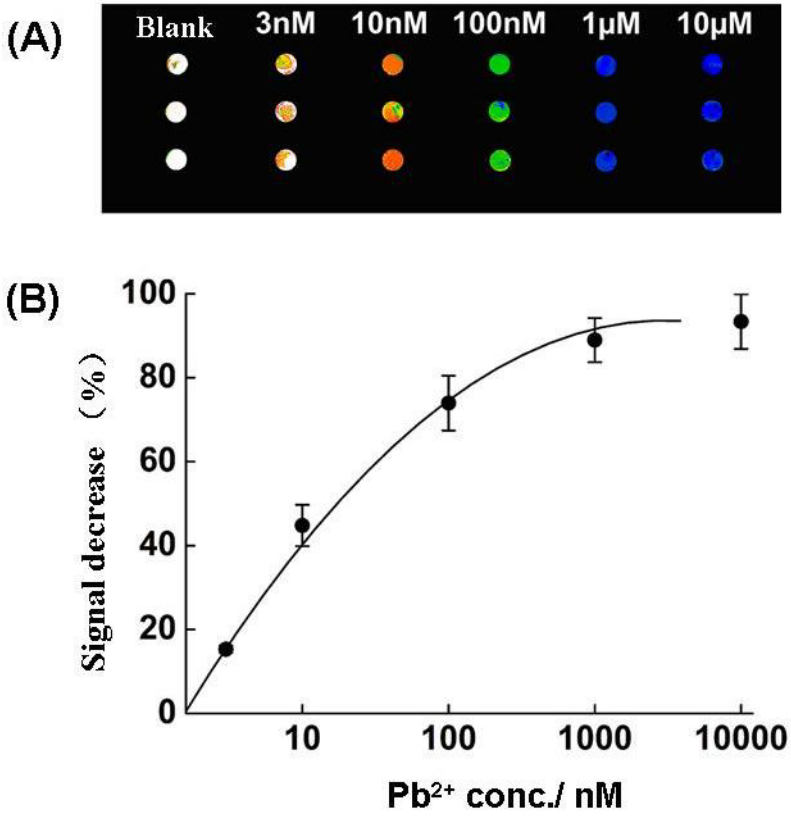

Fig. S2 (A) Scanometric images of the microarray chip in the presence of various concentration of Pb^{2+}. (B) Relative fluorescence change (%) as a function of the Pb^{2+} concentration. The sensor comprises of probe (3) and probe (4) as described in the text. The illustrated error bars represent the standard deviation obtained from 8 data points.

Electronic Supplementary Material (ESI) for Analyst