

A sensitive and selective DNzyme-based flow cytometric method for detecting Pb²⁺ ions

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Supporting information

Experimental Section

Chemicals and materials

Streptavidin-functionalized Dynabeads (M-270) were purchased from Beijing Search Biotech Co., Ltd. The characteristics and properties of streptavidin-functionalized Dynabeads (M-270) provided by the vendor are as following: superparamagnetic, hydrophilic bead surface, 2.8 μm in diameter, size distribution (CV<3%), no blocking proteins used, isoelectric point of pH 4.5, highly charged (-50 mV at pH 7), iron content (ferrites) of 14% (20%), low aggregation of beads in high salt solutions. Pb²⁺ standard solution (1000 μg ml⁻¹) was purchased from General Research Institute for Nonferrous Metals of China. Tris(hydroxymethyl)aminomethane (Tris) was bought from Sinopharm Group Co. Ltd (Shanghai, China). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was bought from Xiamen Tagene Biotechnology Co., Ltd. DNase- and RNase-free water treated by 0.1% diethylpyrocarbonate (DEPC) and the HPLC-purified fluorescent biotinylated enzyme strands were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China). The HPLC-purified quencher-labeled substrate strands were synthesized by Takara Biotechnology (Dalian) Co., Ltd. All other chemicals were of analytical grade. The water used was purified by Millipore Milli-Q (18 MΩ·cm). The sequences of the enzyme strands and substrate strands were as following.

Enzyme strand:

5'(FAM)-ATCATCTCTGAAGTAGCGCCGCGTATAGTGAGACAGACTTTT
T-(Biotin)3'

Substrate strand:

5'(Dabcyl)-GTCTGTCTCACTATrAGGAAGAGATGAT-(Dabcyl)3'

10 μM stock solutions of enzyme strands and substrate strands were prepared by DNase- and RNase-free water to prevent the degradation of DNA or RNA by enzyme. Working solutions of enzyme strands and substrate strands were diluted from the stock solutions with water. 1 \times B&W (bind and wash) buffer (10 mM Tris-HCl, pH 7.5, 1 M NaCl) and 2 \times B&W buffer (10 mM Tris-HCl, pH 7.5, 2 M NaCl) were used to wash the magnetic beads (MBs) during the immobilization of DNAzyme onto MBs. HEPES buffer (10 mM HEPES, pH 7.2, 150 mM NaCl, 50 mM KCl) was used during the fluorescence analysis.

Immobilization of DNAzyme and detection of Pb^{2+} .

Before the MBs were taken out of the stock vial, the vial was vortexed to ensure that the MBs were homogeneously dispersed. 1.0 μL MBs (10 mg/mL) were washed three times by 1 \times B&W buffer to remove the preservative and then were dispersed in 2 μL 2 \times B&W buffer. 2.0 μL enzyme strands (1.0 μM) were added and the mixture was incubated at room temperature for 30 min to allow the immobilization of enzyme strands onto MBs via biotin-streptavidin interactions. The enzyme strand-immobilized MBs were separated and washed by 10 μL 1 \times B&W buffer to remove the unbound enzyme strands in the supernatant with the help of a magnet and then re-dispersed in 5 μL 2 \times B&W buffer. The unbound enzyme strands were collected and analyzed by a fluorospectrophotometer. 4.0 μL substrate strands (1.0 μM) were added to hybridize with the enzyme strands on the MBs by incubating the mixture at room temperature for 60 min [in B&W buffer](#). Finally, the DNAzyme-immobilized MBs were separated and washed consecutively by 10 μL 1 \times B&W buffer twice and 10 μL HEPES buffer once.

For Pb^{2+} detection, various amounts of Pb^{2+} were added to react with the DNAzyme-immobilized MBs in 50 μL HEPES buffer at 35 $^{\circ}\text{C}$ for 30 min and the MBs were then separated and washed by 30 μL HEPES buffer for three times. Each sample was then diluted to 300 μL with HEPES buffer for cytometry analysis. The fluorescence was measured with a Beckman EPICS-XL/MCL flow cytometer (with MBs) or a Varian Eclipse fluorospectrophotometer (without MBs). For flow

cytometry assays, normal operation conditions with phosphate buffered saline-based sheath fluids were used and at least 2000 events were counted for each sample. For assays with the fluorospectrophotometer, the unbound enzyme strands collected were diluted to 470 μL with HEPES buffer and the solutions were excited at 473 nm.

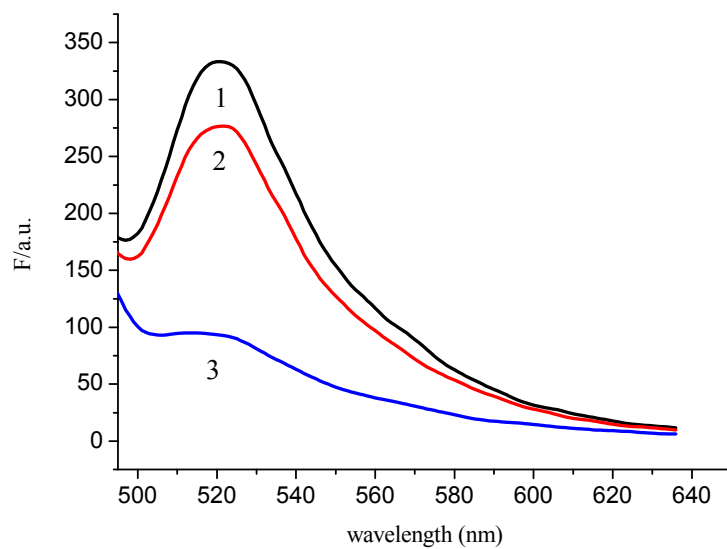


Fig. S1 Fluorescence spectra of (curve 1) the enzyme strands (0.02 μM), (curve 2) the DNAzyme hybridized by the enzyme strands (0.02 μM) and single Dabcyl-labeled substrate strands (0.04 μM) and (curve 3) the DNAzyme hybridized by the enzyme strands (0.02 μM) and double Dabcyl-labeled substrate strands (0.04 μM).

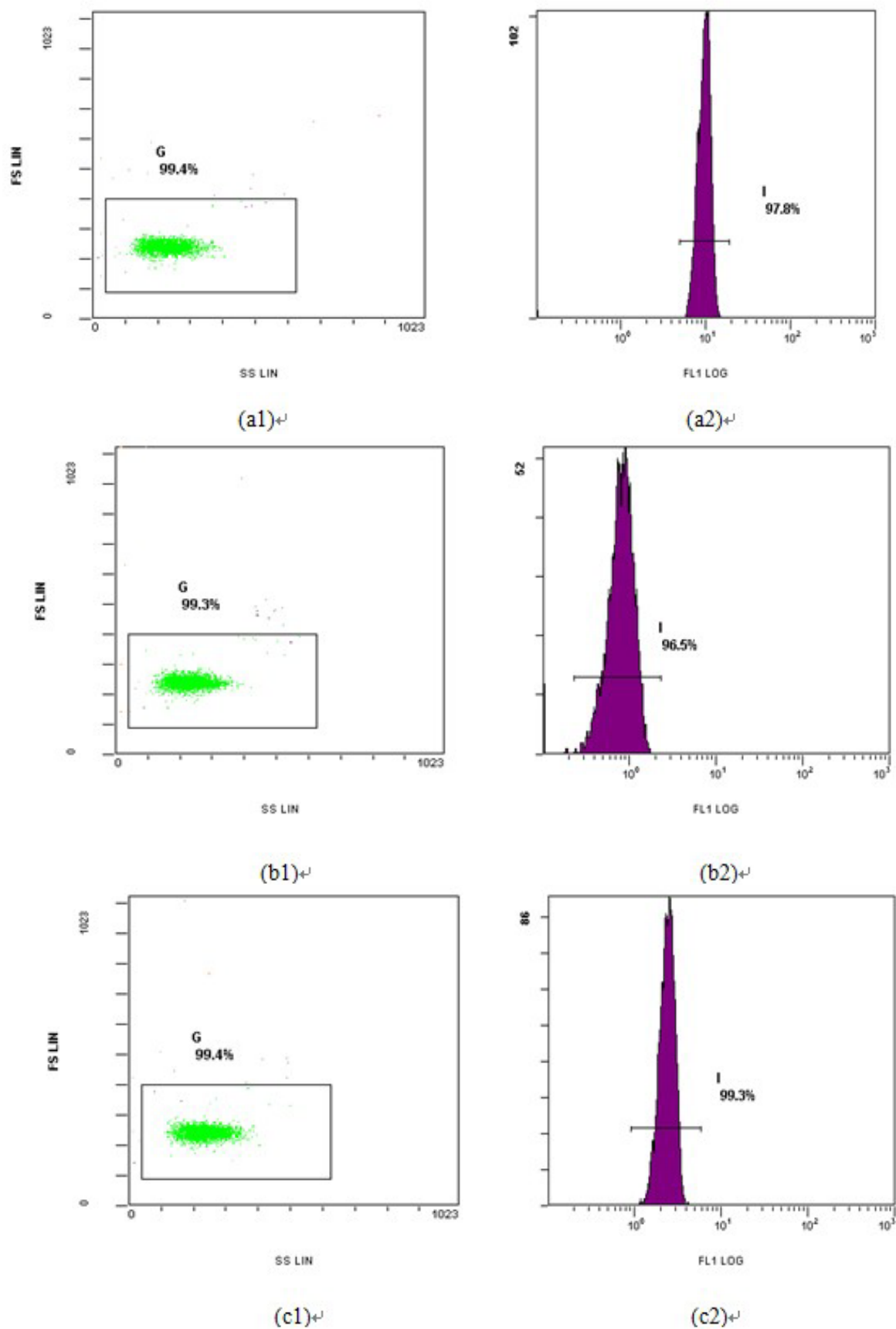


Fig. S2 Histograms of (a1, b1, c1) side (SSC) / forward (FSC) scatter, (a2, b2, c2) fluorescence of enzyme strand-immobilized MBs (a1, a2), DNAzyme-immobilized MBs in the absence of Pb²⁺ (b1, b2), and DNAzyme-immobilized MBs at the

presence of 50 nM Pb^{2+} (c1, c2) in HEPES buffer. The x axis is the fluorescence intensity in the FAM channel on the log scale, and the y axis is the number of MBs at each fluorescence intensity.

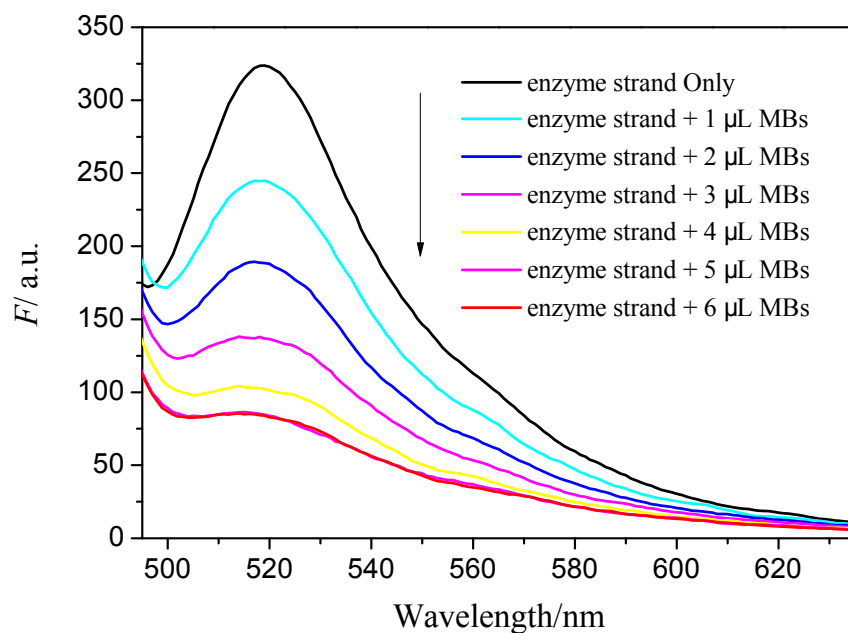


Fig.S3 Fluorescence spectra of biotinylated enzyme strands in the supernatant after incubation of 10 pmole enzyme strands with 1, 2, 3, 4, 5, 6 μL streptavidin-functionalized MBs, respectively at room temperature for 30 min..

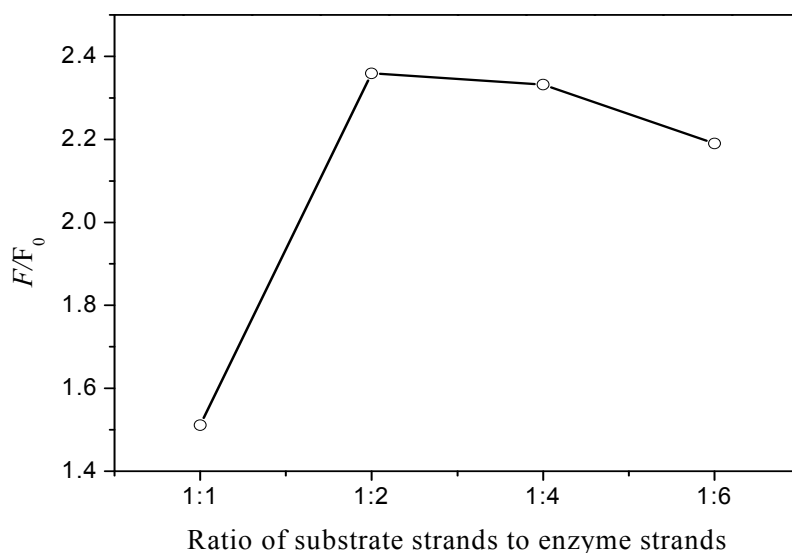


Fig. S4 Effect of ratio of substrate strand to enzyme strand for the hybridization on the fluorescence recovery at the presence of 500 nM Pb^{2+} . F and F_0 were the fluorescence intensity of DNAzyme-immobilized MBs in the absence and at the presence of Pb^{2+} , respectively.

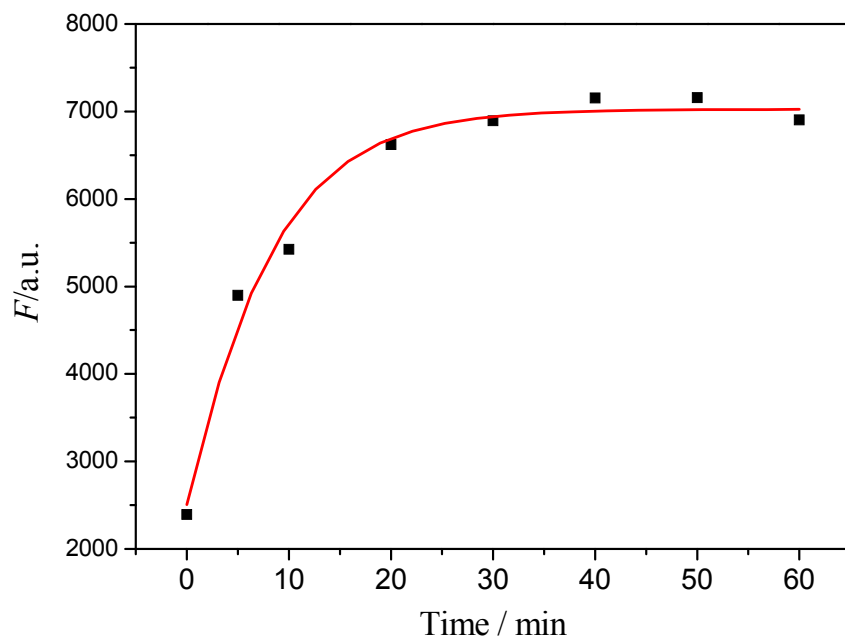


Fig. S5 Time-dependent fluorescence change of DNAzyme-immobilized MBs on addition of 500 nM Pb^{2+} .

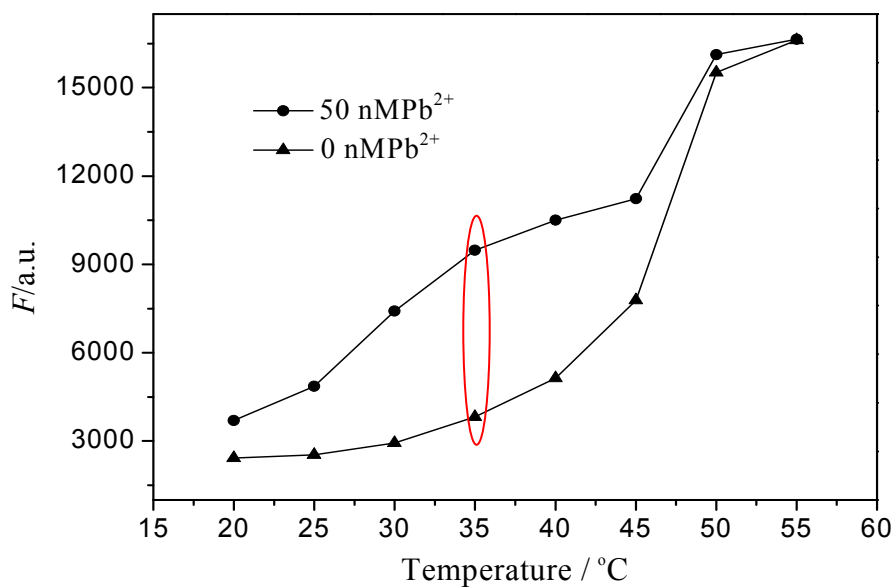


Fig. S6 Temperature-dependent fluorescence change of DNAzyme-immobilized MBs in the absence and at the presence of 50 nM Pb^{2+} .