Supplementary Material (ESI) for Chemical Communications

Supplementary Information
Submitted to Chemical Communications

A Highly Selective Lead Sensor Based on a Classic Lead DNAzyme.

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Experimental procedures

Materials: All DNA was synthesized and HPLC purified by Integrated DNA Technologies, Inc. Ultrapure NaHEPES, NaMES and Tris were purchased from Sigma-Aldrich. Ultrapure hydrochloric acid and metal salts (puratonic®) were purchased from Alfa Aesar. T4 polynucleotide kinase was purchased from Invitrogen.

Biochemical assays: the substrate was labeled with [γ-^{32}P]-ATP on the 5' termini using T4 polynucleotide kinase (Invitrogen). The enzyme and substrate were first heated to 80 °C and slowly cooled to room temperature over the course of about 45 mins. Before starting the activity assay, 2.5 μL DNA solution were added to 17.5 μL of stop solution (50 mM EDTA, 8 M urea, 90 mM boric acid, 0.05% xylene cyanol, and 0.05% bromophenol blue). To initiate the reaction, an equal volume of 2X metal solution was mixed with the enzyme-substrate complex. At each time point, 5 μL of DNA was taken out and added into the stop solution. The final conditions for the activity assay were: 5 μM enzyme, 5 nM substrate, 0.5 M NaCl, 0.5 M KCl, 50 mM $MgCl_2$, and 50 mM HEPES at pH 7.0. The reaction was then resolved on an 8 M urea 20% polyacrylamide gel and quantified on a Molecular Dynamics Storm 430 phosphorimager (Amersham Biocsciences). The percent cleavage vs. time was fitted to an exponential decay function in Origin to obtain the k_{obs}.

Fluorescence measurements: Fluorescence measurements were carried out on a Fluoromax-2 (HORIBA Jobin Yvon inc., Edison, NJ) fluorometer using the constant wavelength analysis mode with excitation at 473 nm and emission at 520 nm.

To observe the Pb^{2+} dependence, the fluorescence signal at 520 nm was monitored when various concentrations of Pb^{2+} were added to 10 nM of the sensor for duration of six minutes. The total volume of the sensing solution was 500 μL. Data was collected at ten second intervals. A trace of F/F_0 vs. time was generated for each concentration of Pb^{2+} addition. Pb^{2+} concentration tested for the GR-5 DNAzyme-based sensor were 0 nM, 10 nM, 25 nM, 50 nM, 75 nM, 100 nM, 250 nM, 500 nM, 1 μM and 2 μM. For the 8-17 sensor, Pb^{2+} concentrations at 0 nM, 50 nM, 100 nM, 200 nM, 300 nM, 500 nM, 700 nM, 1 μM and 2 μM were tested.

For the selectivity, the background fluorescence before addition of metal ions was monitored for 10s (2 data acquisitions per second), followed by the addition of various metal ions (5 μL) at different concentrations (with a total volume of 500 μL). After six minutes, the fluorescence was measured the same way as the background fluorescence. The final F/F_0 was plotted for each metal ion. Metal ions tested included Zn^{2+}, Mn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Ba^{2+}, Cu^{2+}, Fe^{3+}, Sr^{2+} and Hg^{2+} with concentrations at 4 μM, 40 μM, 400 μM and 1mM. Pb^{2+} was tested at 25 nM, 50 nM, 1 μM and 2 μM.

For the initial rate measurements, the fluorescence was monitored for 30s after the addition of various concentrations of Pb^{2+}. Data was collected at 2 s intervals. The initial rates were obtained by the linear fitting of data in Excel.

Supplemental figures

Fig. S1

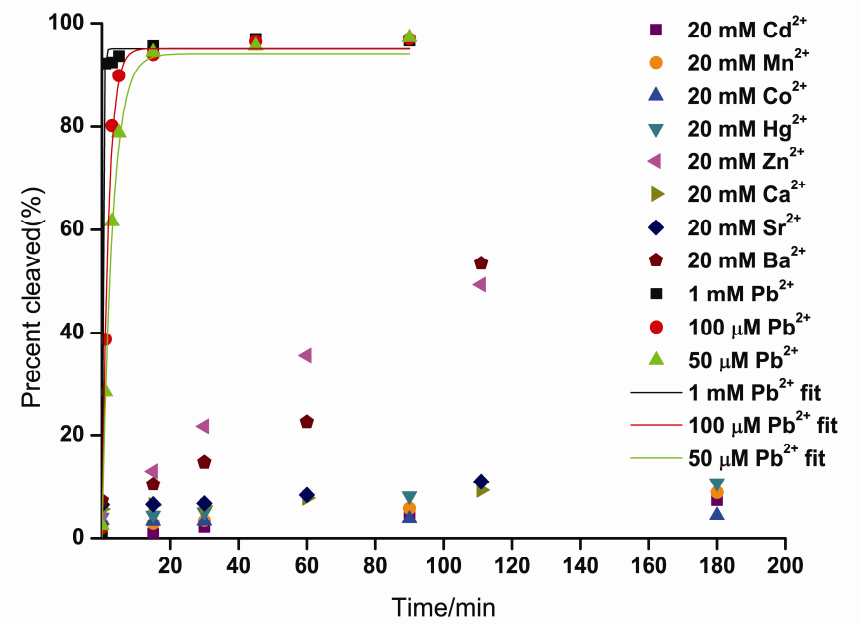

Fig. S1 Initial selectivity assay on the GR-5 Pb^{2+} DNAzyme. A DNA substrate containing an internal RNA base is 5′ labeled with γ-[^{32}P]-ATP. The enzyme (5 μM) substrate (5 nM) (ES) complex is prepared in 500 mM NaCl, 500 mM KCl, 50 mM $MgCl_2$, in 50 mM HEPES at pH 7. The complex is heated to 80 ºC and slowly cooled to room temperature over an approximately 45 minute time period. To initiate the activity assay, an equal volume of each respective metal ion (2X the target concentration) is mixed with the annealed ES complex. At each time point, a 5 μL aliquot is withdrawn and the reaction is stopped with 15 μL of stop solution (8M urea, 50 mM EDTA, 90 mM Tris, 90 mM boric acid, 0.05% xylene cyanol, and 0.05% bromophenol blue). The cleaved substrate is separated from the uncleaved by 20% PAGE and quantified by a Molecular Dynamics Storm 430 phosphorimager (from Amersham Biosciences). The rate of reaction was obtained by fitting the curves to an exponential decay function using Origin Pro 8.

Fig. S2

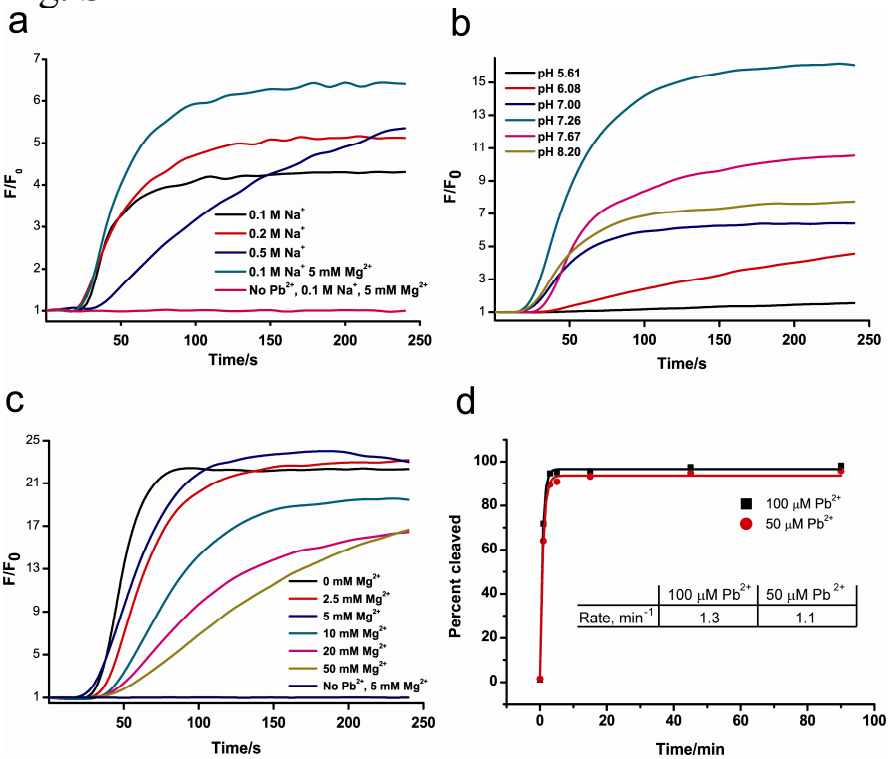

Fig. S2 Optimizations for the GR-5 lead sensor, in 50 mM NaHEPES. The salt concentration was investigated first because the original *in vitro* selection was carried out in a salt concentration (500 mM NaCl, 500 mM KCl, 50 mM $MgCl_2$) too high for most sensing applications. A 10 nM enzyme-substrate complex was prepared in four different salt concentrations in 50 mM NaHEPES at pH 7.0. The reaction was initiated by addition of 2 μM Pb^{2+} to the DNA solution, and the fluorescence signal was monitored. The fluorescence enhancement (F/F_o) was then determined. As shown in (a). Similar optimizations were carried out at five other pHs (b) with the above optimized salt condition. The result indicated that 7.26 is the optimal pH, allowing an F/F_o to exceed 15, which is among the best reported so far. The sub-optimal performance of the sensor at high pH may be partially due to the high tendency for Pb^{2+} to form precipitates, reducing the effective Pb^{2+} concentration in the solution. Finally, the concentration of Mg^{2+} present was tested with 0.1 M Na^+ at pH 7.26 (c). The activity of the DNAzyme under this sensing condition was also tested (d).

Fig. S3

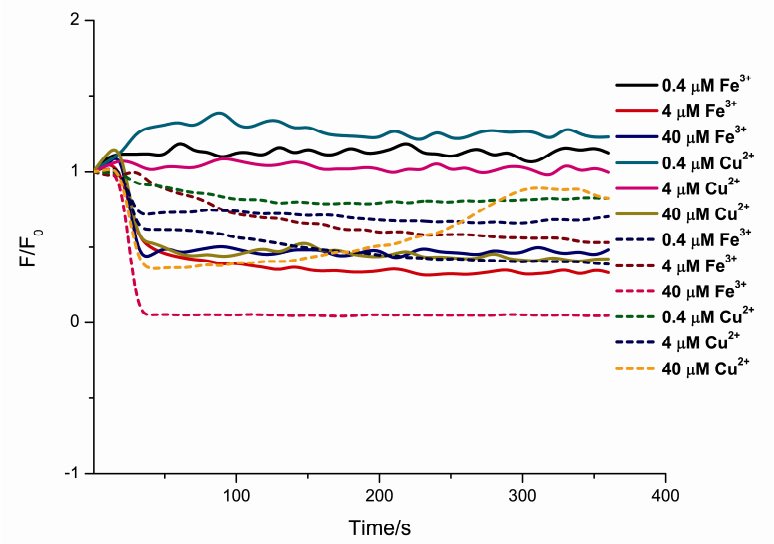

Fig. S3 Fluorescence response of the GR-5 DNAzyme Pb^{2+} sensor (solid) and 8-17 DNAzyme Pb^{2+} sensor (dash) to Cu^{2+} and Fe^{3+}.

Fig. S4

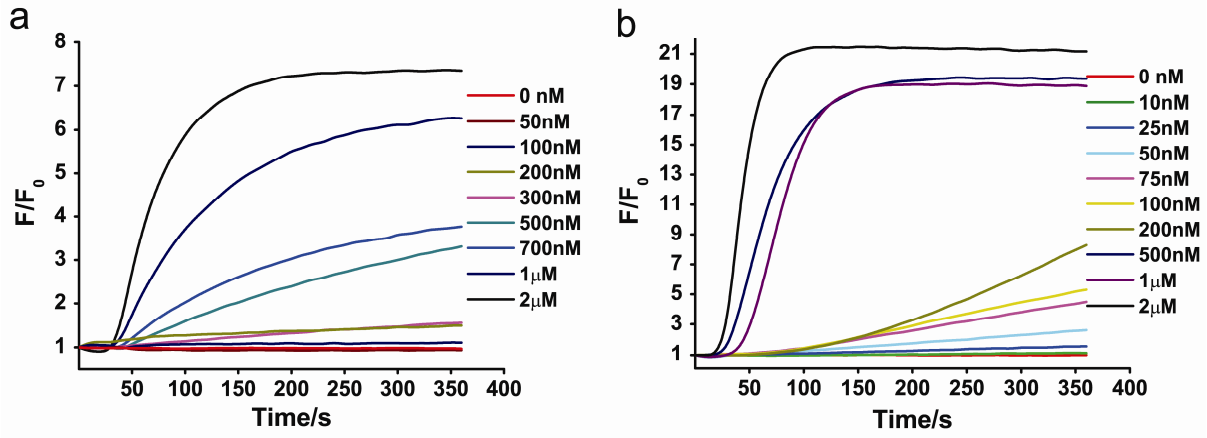

Fig. S4 Pb^{2+} response of the 8-17 DNAzyme sensor (a) and the GR-5 DNAzyme sensor (b).

Table-S1

Table S1: Rate constants of the GR-5 Pb^{2+} DNAzyme in the presence of various metal ions at pH 7.0

Metal ion	k_{obs} (min^{-1})
50 μM Pb^{2+}	0.3204±0.0157
100 μM Pb^{2+}	0.6369±0.0984
20 mM Zn^{2+}	0.0141±0.0029[a]
20 mM Ba^{2+}	0.0034[b]
20 mM Ca^{2+}	not detected
20 mM Sr^{2+}	not detected
20 mM Cd^{2+}	not detected
20 mM Mn^{2+}	not detected
20 mM Co^{2+}	not detected
20 mM Hg^{2+}	not detected