

Supplementary Information

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DNAzyme Catalytic Beacon Sensors that resist Temperature-dependent Variations.

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Experimental procedures

All oligonucleotides were synthesized and HPLC purified by Integrated DNA Technologies Inc. (Coralville, Iowa). Ultrapure HEPES-sodium salt, Trizma® acetate and PIPES were purchased from Sigma-Aldrich or USB corporation while ultrapure hydrochloric acid, puratronic® sodium chloride and puratronic® lead acetate were purchased from Alfa-Aesar. The latter was used for preparation of lead stock solutions in 0.5% of acetic acid. Buffers were brought up to pH using ultrapure hydrochloric acid.

The substrate strand was labeled with FAM on the 5' end and a black hole quencher (BHQ1®) on the 3' end while all the different enzyme strands used were labeled with a

DABCYL quencher on the 3' end. The enzyme substrate complex was prepared with 1 μ M each of +5_17E (A_{16.7}-G) or +5_17E (T_{16.5}-C) and +5_17S in either 50 mM HEPES buffer, pH 7.2 or a mixed buffer of 50 mM Tris acetate/PIPES at pH 6.8, both containing 100 mM of NaCl. The sample was heated to 80 °C for 3 min and cooled to 23 °C over 45 min for annealing the enzyme and substrate strands. Sensors made in the mixed buffer solution at pH 6.8 could be air-dried in small aliquots and stored at room temperature for over one month without significant loss of activity. The sensors were then rehydrated in 50 mM HEPES, pH 7.2 containing 100 mM NaCl and used for fluorescence measurements.

Fluorescence experiments were carried out on a Fluoromax-2 fluorometer (HORIBA Jobin Yvon inc., Edison, NJ) using the Constant Wavelength Analysis (CWA) mode. The temperature of the sample was adjusted using a temperature controller connected to the fluorimeter. (LSI-3751 from Wavelength Electronics) The performance of the sensors was monitored by studying the overall change in the fluorescence intensity at 520 nm, at 10 sec time intervals upon Pb²⁺ addition. The initial rate of fluorescent increase, attributable to release of the substrate arm was measured by plotting the change in fluorescence intensity for 16 seconds, at a 2 sec time interval, after Pb²⁺ addition using the equation: $y = y_0 + V_{\text{fluor}}x$; where V_{fluor} is the initial rate of release of the substrate arm.

Figures

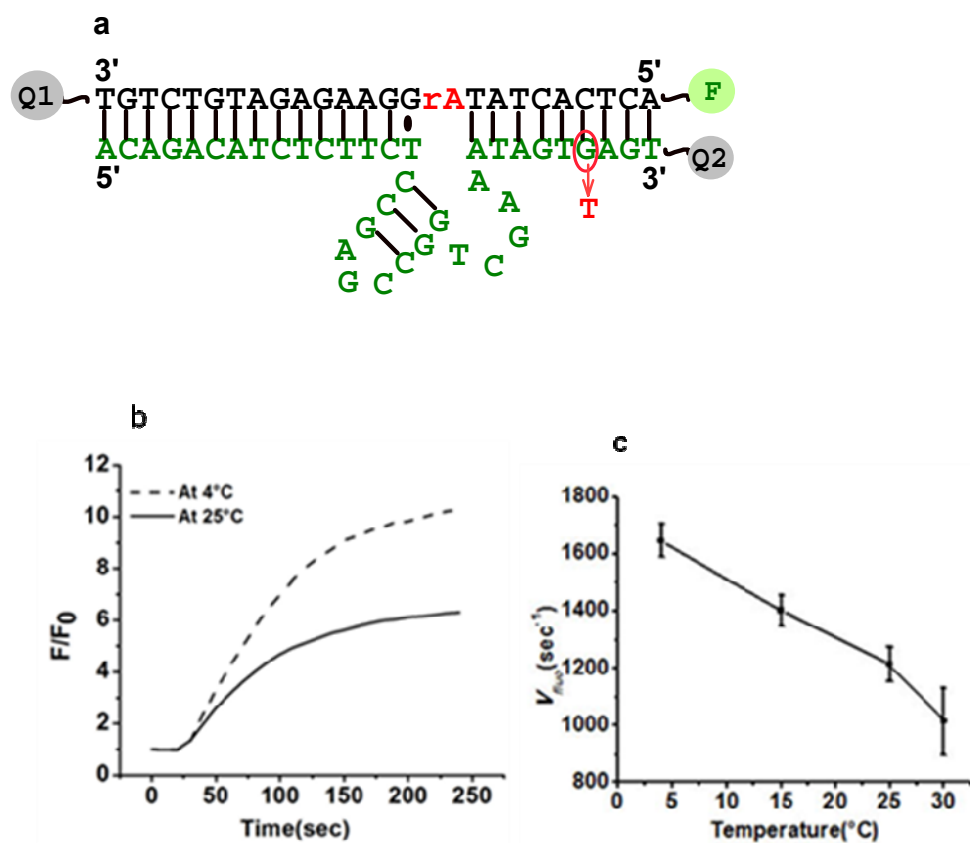


Figure S1. a) Predicted secondary structure of the lead sensor showing the position of the mismatch (17E) G^{16,6} T. b) Normalized fluorescence response and c) temperature dependence of the sensor with 2 μM Pb^{2+} in 50 mM HEPES at pH 7.2 with 100 mM NaCl.

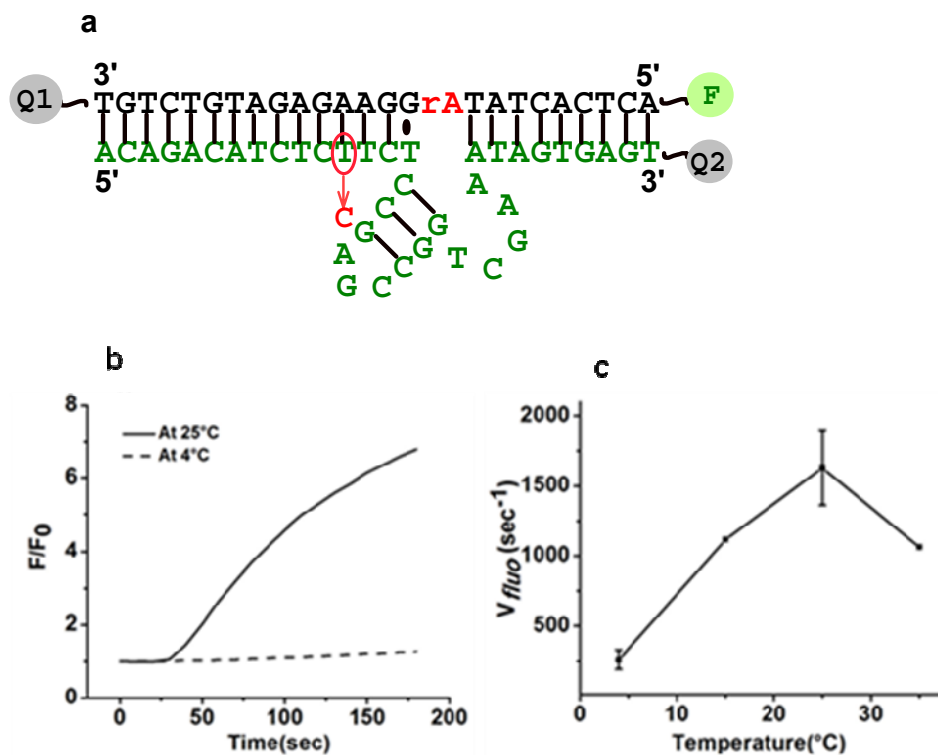


Figure S2. a) Predicted secondary structure of the lead sensor showing the position of the mismatch (17E) T²⁴ C. b) Normalized fluorescence response and c) temperature dependence of the sensor with 2 μM Pb^{2+} in 50 mM HEPES at pH 7.2 with 100 mM NaCl.

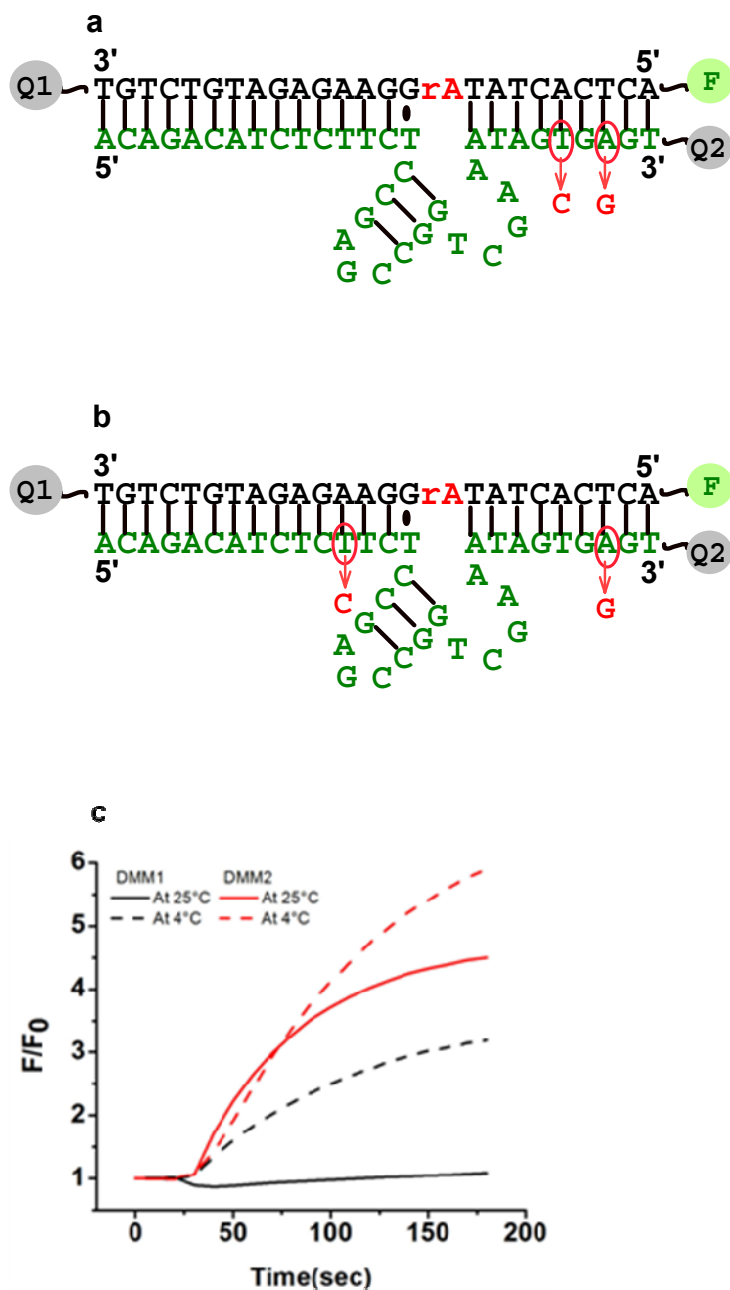


Figure S3. a) Predicted secondary structure of the lead sensors a) DMM1, showing the position of the mismatches (17E) T^{16,5} C, A^{16,7} G and b) DMM2, showing the position of the mismatches (17E) T^{2,4} C, A^{16,7} G . c) Normalized fluorescence response of the sensors with 2 μM Pb²⁺ in 50 mM HEPES at pH 7.2 with 100 mM NaCl.

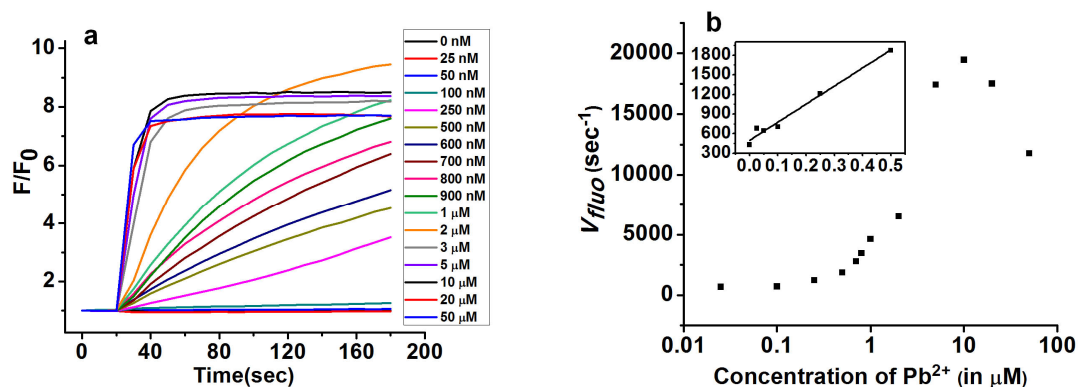


Figure S4. Sensitivity of the MM2 sensor. a) Kinetics of fluorescence increase relative to the background, with varying concentrations of Pb^{2+} at 25 °C. b) Variation of the initial rate with Pb^{2+} concentrations at 4°C. The inset presents the linear range in submicromolar concentrations of Pb^{2+} . The rates were measured in buffer containing 50 mM HEPES with 100 mM NaCl at pH 7.2.