Supplementary Information

Dissecting metal ion-dependent folding and catalysis of a single DNAzyme

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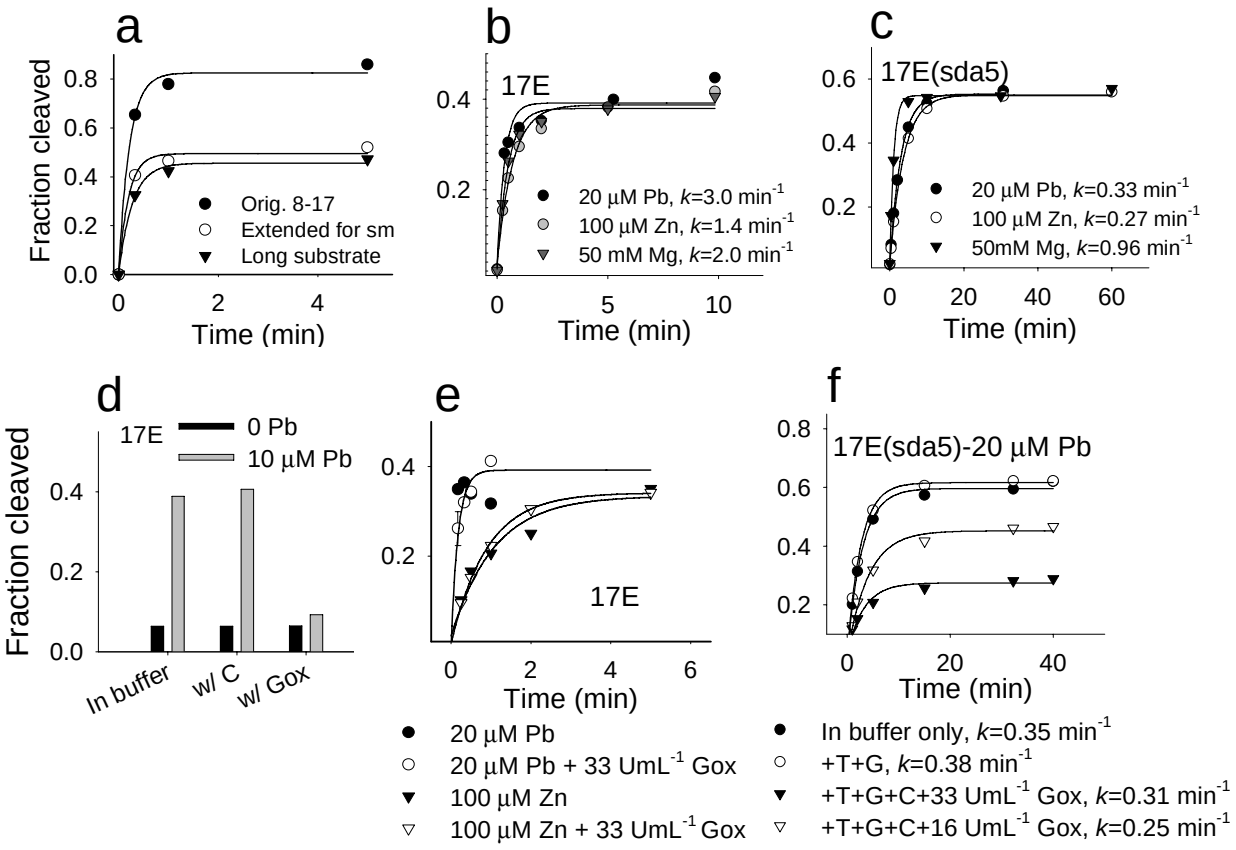
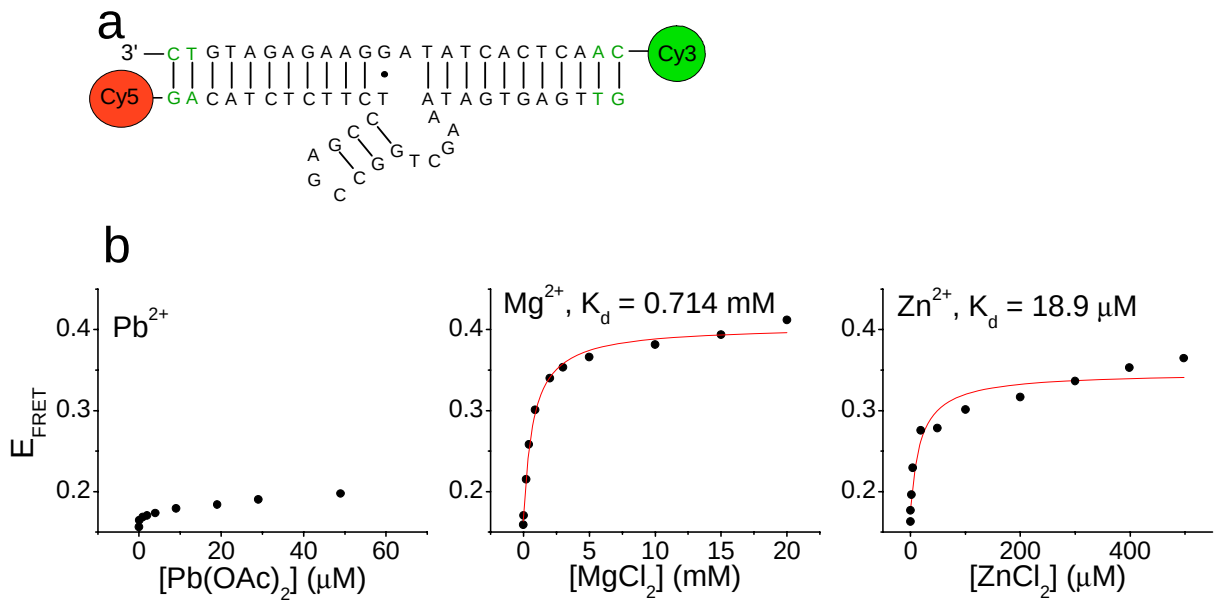
Supplementary Figure 1 Bulk activity assays of the modified enzyme–substrate complexes**.** We first characterized the activity of the modified constructs using bulk solution activity assays. The modified wild type enzyme–substrate complexes showed a similar reaction rate (k_{obs} = 5.1 ± 1.5 min^{-1} for the short substrate and k_{obs} = 3.7 ± 0.7 min^{-1} for the long substrate) as the original unextended 8–17 DNAzyme (k_{obs} = 4.7 ± 1.1 min^{-1}) but with a decrease in the final cleaved fraction from 80–90% in the original enzyme to 40–50% in the modified enzymes (**Supplementary Fig. 1** online). This result is consistent with the single molecule data showing that only 65% of the molecules showed folding and cleavage as described in the next section. The 17E(sda5) showed lower activities than the wild type in the presence of Pb^{2+} (k_{obs} = 0.33 vs 3.0 min^{-1}), Zn^{2+} (k_{obs} = 0.27 vs 1.4 min^{-1}), and Mg^{2+} (k_{obs} = 0.96 vs 2.0 min^{-1}) (**Supplementary Fig. 1** online). The final cleaved fraction also decreased to ~ 60% after the modifications.



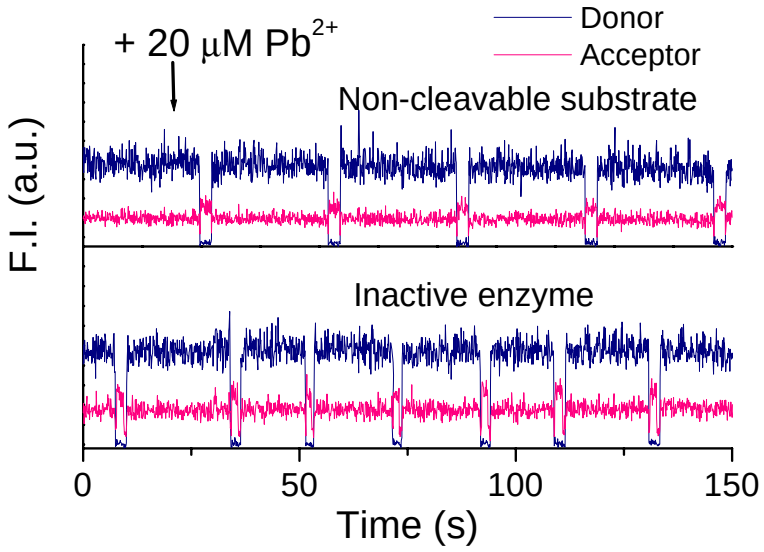
(**a**) Activities of the original 8–17 DNAzyme (**Fig. 1a**) and the extended variants (**Fig. 1b**) for the single molecule experiments in the presence of 10 μM Pb^{2+}. Comparison between (**b**) the wild type and (**c**) the 17E(sda5) in the presence of different metal ions. (Constructs are shown in **Fig. 1b**) (**d**) Activity of the wild type in the presence of 2170 U ml^{-1} catalase or 165 U ml^{-1} glucose oxidase. The reaction time was 5 minutes. (**e**) Activity comparison between the presence and the absence of the glucose oxidase in the presence of Pb^{2+} and Zn^{2+}. (**f**) Activity of the 17E(sda5) mutant under various glucose oxidase concentration conditions. T, G, C, and Gox denote saturated Trolox, 0.4% β–D–glucose, 2170 U ml^{-1} catalase, and glucose oxidase, respectively. *a* is the final cleaved fraction and *k* is the observed rate constant (k_{obs}) in min^{-1}. All the reactions were carried out in 50 mM Tris–HCl (pH 7.3) and 50 mM NaCl.

Supplementary Figure 2 Bulk FRET measurements on the wild type



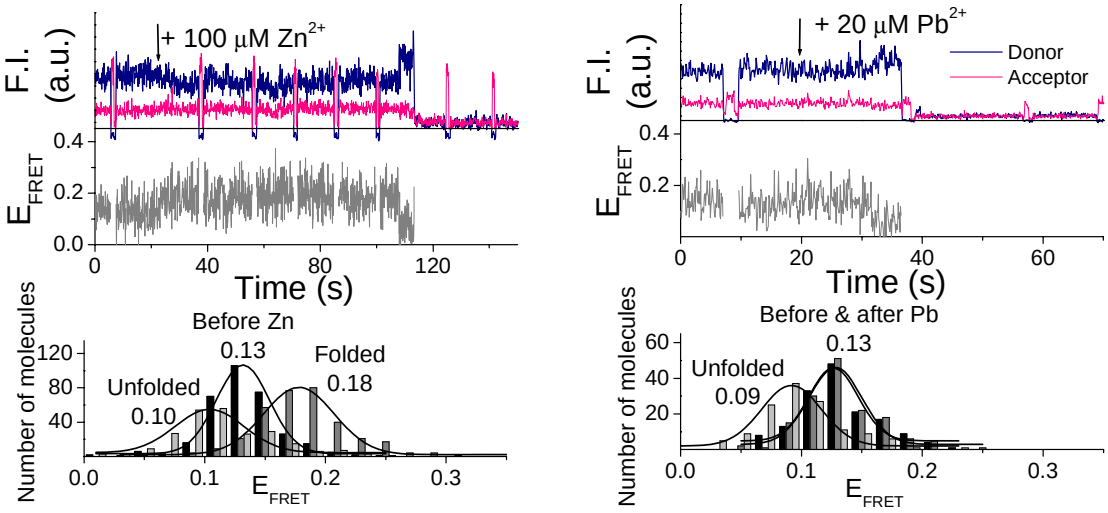
. (**a**) DNA construct. Enzyme and substrate were extended by two base pairs at both the 5′– and 3′–ends, and were labeled with Cy5 and Cy3 at the 5′–ends, respectively. Non-cleavable substrate was used in order to distinguish folding events from cleavage reaction. (**b**) FRET efficiencies of the DNAzyme as a function of Zn^{2+}, Mg^{2+}, or Pb^{2+}. Experiments were carried out at 10 °C in 50 mM Tris–HCl (pH 7.3) and 50 mM NaCl.

Supplemental Figure 3 Fluorescence time traces of control variants upon addition of 20 μM Pb^{2+}.



(**a**) The non–cleavable substrate was used instead of the cleavable substrate. (**b**) The inactive enzyme was used instead of the wild type.

Supplemental Figure 4 Zn^{2+}–and Pb^{2+}–dependent folding and cleavage reaction of the 17E(sda5) mutant.

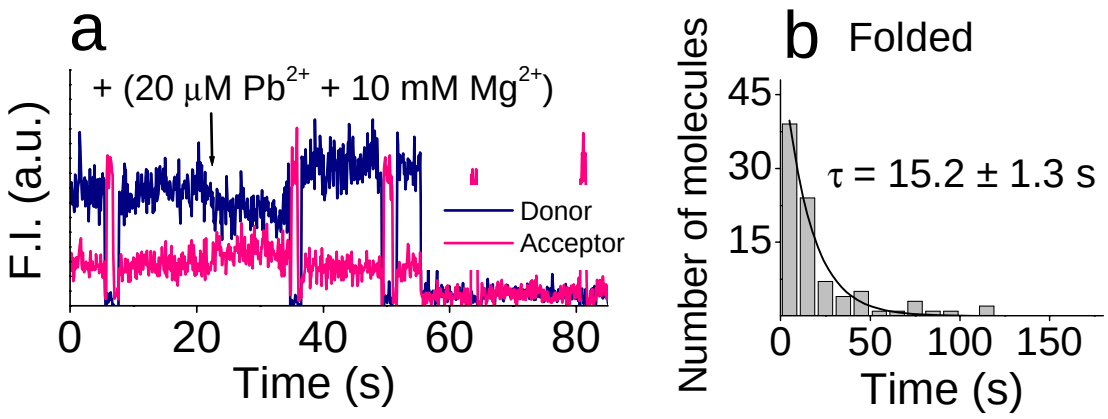


100 μM Zn	Number of molecules	Percentage
Reaction	361	53
Foled–no reaction	266	39
No folding–no reaction	50	7
Sum	677	100

20 μM Pb	Number of molecules	Percentage
Reaction	148	42
No reaction	207	58
Sum	355	100

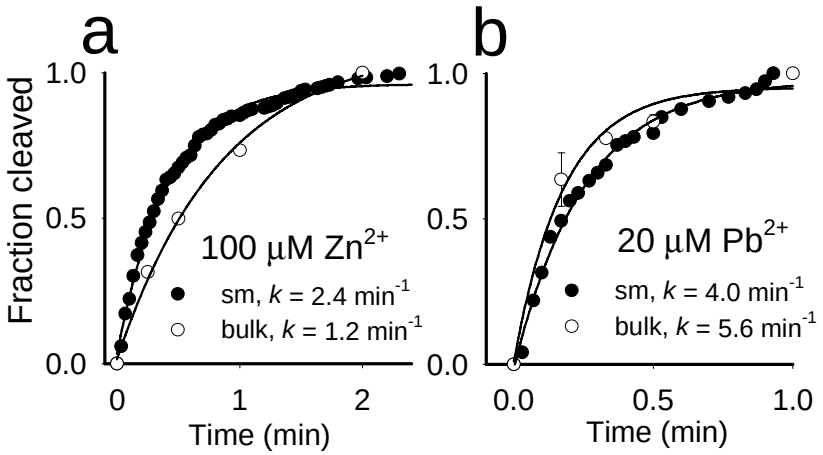
Top: time traces of fluorescence signal and FRET changes along 100 μM Zn^{2+}– and 20 μM Pb^{2+}–dependent reaction. Middle: histograms of the FRET at the different states obtained from the individual molecules. Bottom: Molecule counting.

Supplementary Figure 5 Folding and cleavage reaction of the wild type enzyme in the presence of 20 μM Pb^{2+} + 10 mM Mg^{2+} mixture.



(**a**) Fluorescence time traces of the donor and acceptor upon addition of the mixture. (**b**) Dwell time histograms of the folded state. The glucose oxidase concentration was 16 U ml^{-1}. Reaction rate was measured by fitting the histogram to a single exponential curve.

Supplementary Figure 6 Comparison of the cleavage activities measured by single molecule FRET and bulk activity assays in the presence of (**a**) 100 μM Zn^{2+} and (**b**) 20 μM Pb^{2+} ions.



To further confirm that our single molecule FRET assay recapitulates the solution behavior of the enzymes, we compared the cleavage time courses measured by single molecule FRET with those measured in bulk. The fraction of the cleaved molecules measured by the bulk assay was normalized to the reaction amplitudes at 2 minutes for Zn^{2+} and at 1 minute for Pb^{2+}. For single molecule reactions, an accumulative histogram of the cleavage events vs. time is plotted . The data were fitted to an exponential function with three parameters, $y = y_0+a(1-e^{-kx})$, where k is the observed rate constant. The rate constant for the Pb^{2+}–dependent reaction measured from single molecules (4.0 min^{-1}) is very similar to that in the bulk solution (5.6 min^{-1}), considering the experimental errors and differences in conditions. The rate constant for Zn^{2+}-dependent reaction using single molecule FRET (2.4 min^{-1}) compares well with that of the bulk assay (1.2 min^{-1}). The faster rate in single molecules for Zn^{2+} may be due to photobleaching because our analysis included only those molecules that showed the acceptor survival until completion of the reaction. Such an effect would be more pronounced for Zn^{2+} whose reaction rate is significantly lower than that of Pb^{2+}. This data also support the assignment of the transition from the high FRET to the low FRET to the cleavage reaction.