Supplementary Information

Poly-adenine-based programmable engineering of gold nanoparticles for highly regulated spherical DNAzymes

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Study of lead-dependent enzyme activity

For DNAzyme in aqueous solution, we employed 50 nM S strand and 50 nM DNAzyme. For SNAzymes, in view of that the diffusivity of DNAzyme would be affected by the concentration of nanoparticles, the same amount of AuNPs was employed in each system. The concentration of S strand and AuNP-DNA was 50 nM and 2.5 nM, respectively. To compare the cleavage ability of each kind of DNAzyme,

the apparent cleavage velocity (k_{app}) of individual DNAzyme is calculated from equation 1.

$$k_{app} = \frac{F-F_0}{F_0 t} \cdot \frac{1}{[E]} \quad \textbf{1}$$

where F is the fluorescence signal during the detection process, F_0 is the initial fluorescence signal before the cleavage reaction, t is the reaction time and [E] is the concentration of DNAzyme in the system. $\triangle k_{app}$ is defined as $\triangle k_{app} = k_{app}(c) - k_{app}(0)$, where $k_{app}(0)$ is the k_{app} of the cleavage reaction in the absence of Pb^{2+} and $k_{app}(c)$ is the k_{app} of the cleavage reaction in the presence of certain amount of Pb^{2+}. We calculated the initial reaction velocity in the first 10 minutes corresponding to the fluorescence enhancements. At higher concentrations of Pb^{2+}, the rate of fluorescence increase reach to plateaus. The maximum apparent velocity of cleavage at the point of saturation of Pb^{2+} concentration ($\triangle k_{app,max}$) and the apparent dissociation constants ($\triangle k_{d,app}$) for Pb^{2+} binding to the DNAzyme/substrate complex in each system are calculated through equation 2.

$$\triangle k_{app} = \frac{\triangle k_{app,max} [Pb^{2+}]}{k_{d,app} + [Pb^{2+}]} \quad \textbf{2}$$

Supporting figures and tables

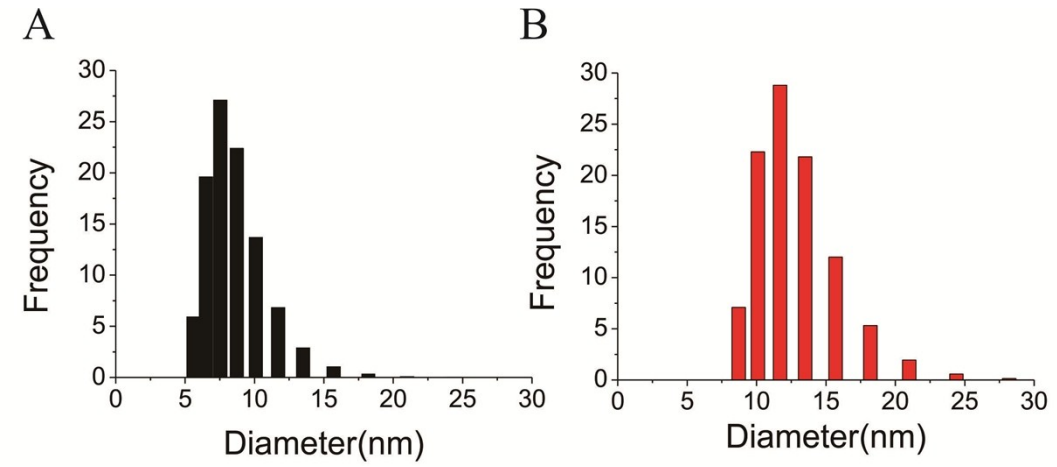

Fig. S1. The analysis of dynamic light scattering (DLS). The SNAzyme (B) with increased diameter than the AuNPs (A) indicated the successful immobilization of DNAzyme on AuNPs.

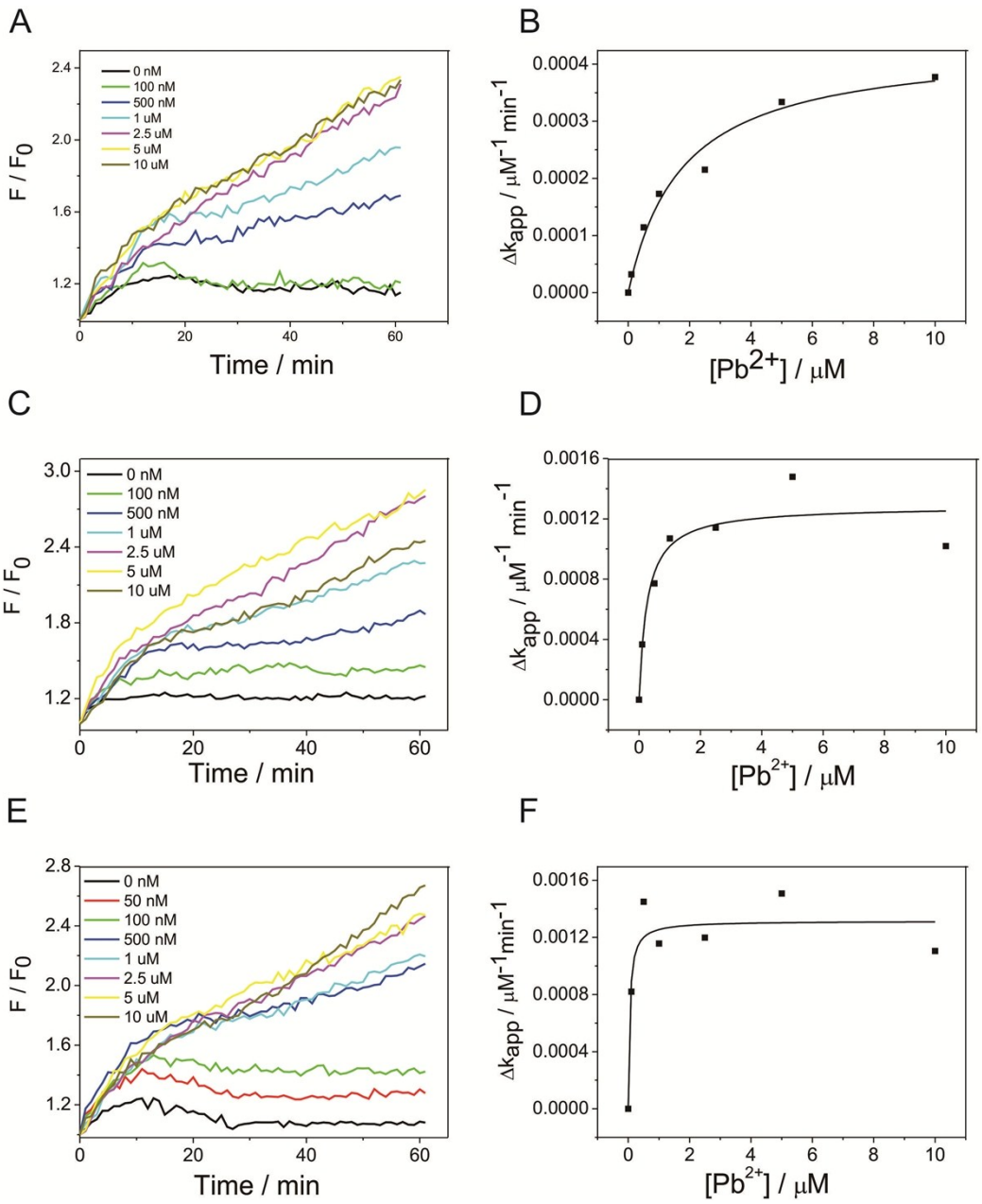

Fig. S2. Fluorescence enhancement with time and the apparent cleavage rate ($\triangle k_{app}$) for the cleavage of substrate at different Pb^{2+} concentrations by 8-17 DNAzyme in three polyA tailed SNAzyme systems: (A and B) polyA10 SNAzyme; (C and D) polyA20 SNAzyme; (E and F) polyA30 SNAzyme. The black curves in (B, D and F) are fit to the equation 2 to calculate $\triangle k_{app,max}$ and $\triangle k_{d,app}$.

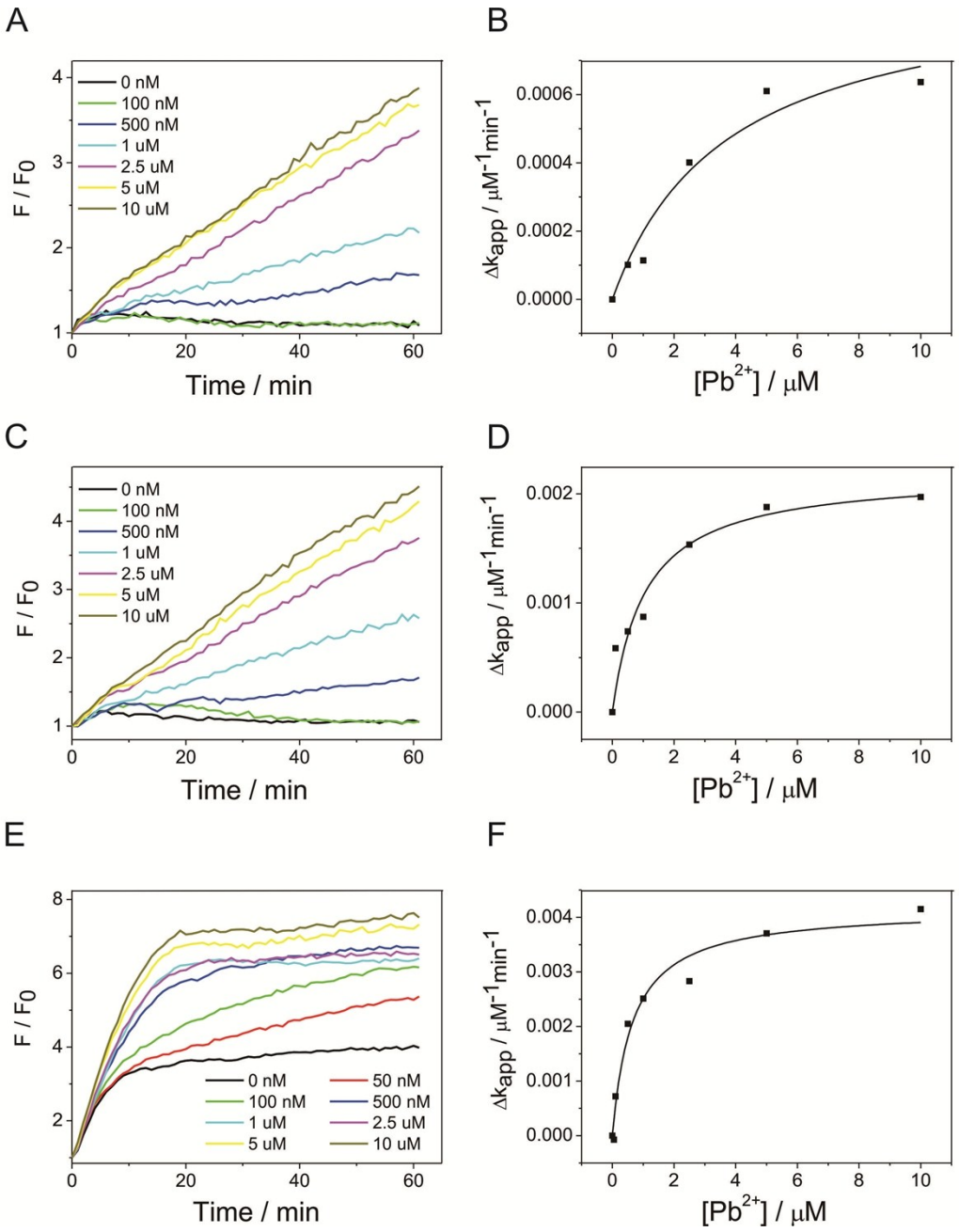

Fig. S3. Fluorescence enhancement with time and the apparent cleavage rate ($\triangle k_{app}$) for the cleavage of substrate at different Pb^{2+} concentrations by 8-17 DNAzyme: (A and B) thiolated SNAzyme; (C and D) MCH treated thiolated SNAzyme; (E and F) individual DNAzyme. The black curves in (B, D and F) are fit to the equation 2 to calculate $\triangle k_{app,max}$ and $\triangle k_{d,app}$.

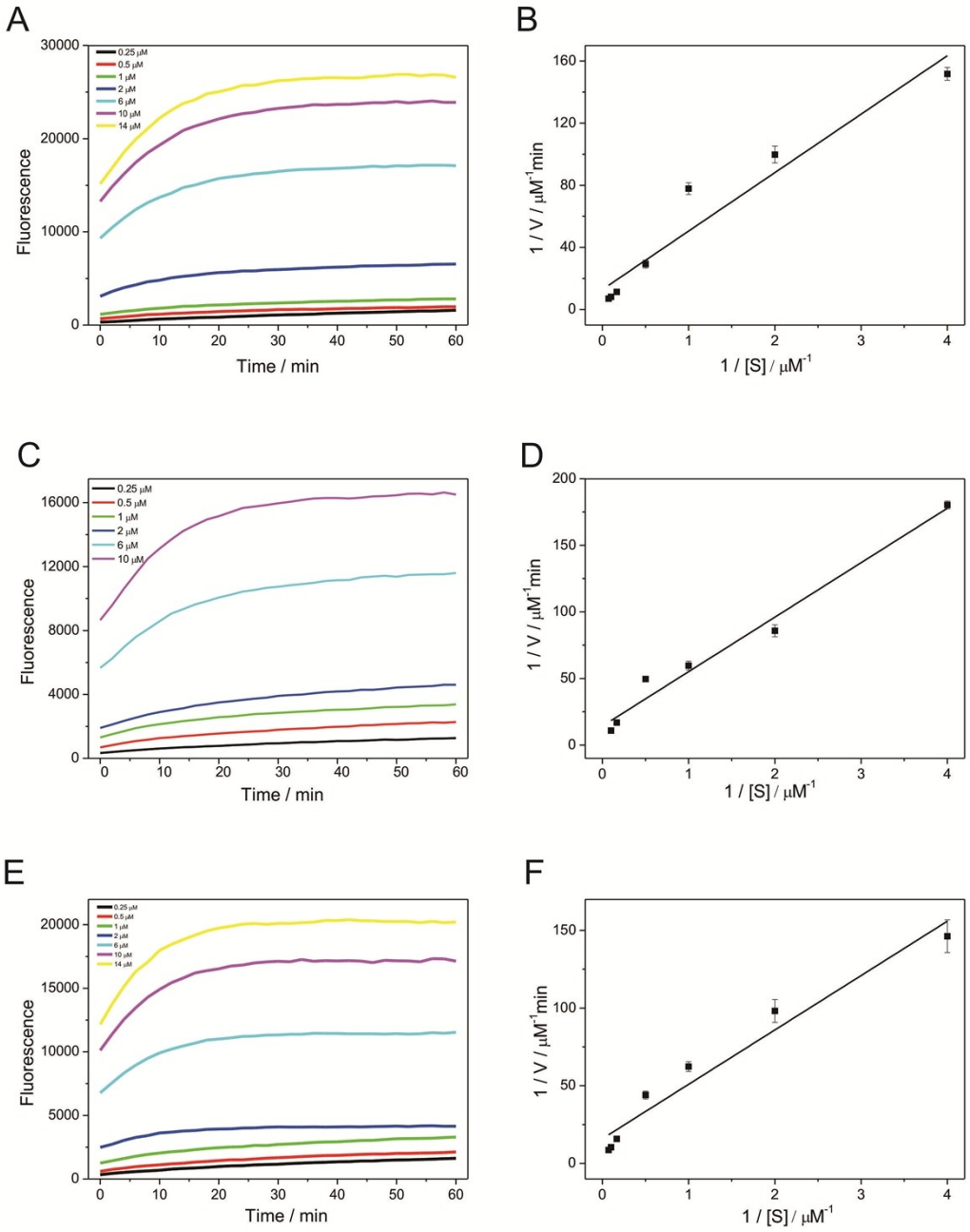

Fig. S4. Fluorescence-based progress curves of the SNAzyme-catalyzed reaction as a function of time and Lineweaver-Burk plot of the initial cleavage velocity as a function of substrate concentration in systems: (A and B) polyA10 SNAzyme; (C and D) polyA20 SNAzyme; (E and F) polyA30 SNAzyme.

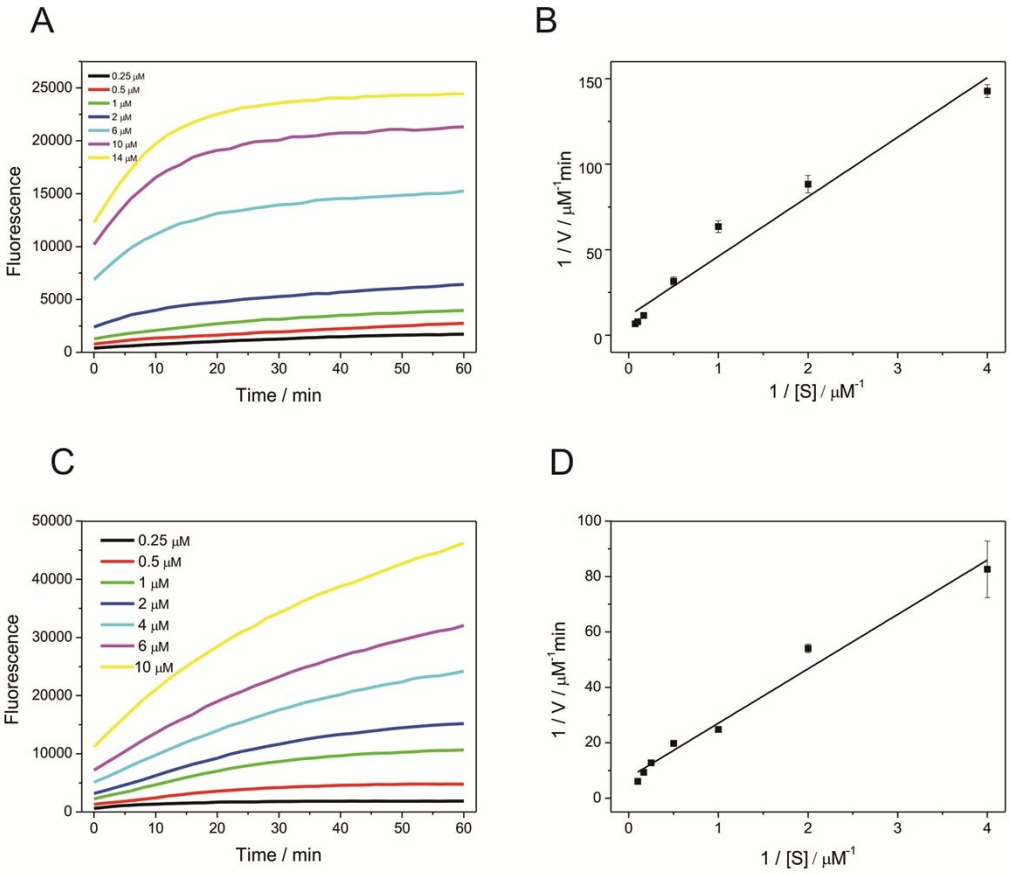

Fig. S5. Fluorescence-based progress curves of the SNAzyme-catalyzed reaction as a function of time and Lineweaver-Burk plot of the initial cleavage velocity as a function of substrate concentration in systems: (A and B) thiolated SNAzyme; (C and D) individual DNAzyme.

Table S1. Comparison of kinetic parameters of DNAzyme on AuNPs and in solution.

System	K_M (μM)	K_{cat} (min^{-1})	K_{cat}/K_M (μM^{-1} min^{-1})
DNAzyme in solution	2.64	1.35	0.51
polyA10-SNAzyme	2.91	0.49	0.17
polyA20-SNAzyme	2.87	0.76	0.26
polyA30-SNAzyme	2.20	1.09	0.49
Thiolated DNAzyme based SNAzyme	3.06	0.45	0.15

Table S2. DNA sequences and their labeling in the study.

DNA Name	**sequence (5'-3')**
poly A10-DNAzyme	AAAAAAAAAATTTTTCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT
poly A20-DNAzyme	AAAAAAAAAAAAAAAAAAAATTTTTCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT
poly A30- DNAzyme	AAAAAAAAAAAAAAAAAAAAAAAAAAAATTT

	TTCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT
Thiolated DNAzyme	SH-TTTTTCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT
ss- DNAzyme	CATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT
S strand	FAM-ACTCACTAT(rA)GGAAGAGATG-DABCYL
poly A10- DNAzyme -FAM	AAAAAAAAAATTTTTCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT-FAM
poly A20- DNAzyme -FAM	AAAAAAAAAAAAAAAAAAAATTTTTCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT-FAM
poly A30- DNAzyme -FAM	AAAAAAAAAAAAAAAAAAAAAAAAAAAAATTTTTCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT-FAM
SH- DNAzyme -FAM	TTTTTCATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT-FAM