Electronic Supplementary Information (ESI)

Engineering DNAzymes Cascade for Signal Transduction and Amplification

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Table S1. All oligonucleotides sequences were used in experiment (from 5' to 3').

Bare Dz1:	**CATCTCTTCTCCGAGCCGGTCGAAATACTGTCA**
S1:	CCACCACTGACAGTAT/rA/GGAAGAGATGTGGTGGTCCGAGCC GGTCGAAATAGTG
Bare Dz2:	GTGGTGGTCCGAGCCGGTCGAAATAGTG
S2:	FAM-CACTAT/rA/GCCACCAC-BHQ1
L-S1:	FAM-CCACCACTGACAGTATrAGGAAGAGATGTGGdTBHQ1GGTCCG
W1	AGCCGGTCGAAATAGTG
	CCACCACTGACAGTATA
Dz1:	TTGTATAGTTCATCTCTTCTCCGAGCCGGTCGAAATACTGTCA
Inhibitor1:	GAAGAGATGTAACTATACAACCTACTA
Trigger1:	TAGTAGGTTGTATAGTTACATC
Dz2:	TTGGAAGAGATGTGGTGGTCCGAGCCGGTCGAAATAGTG
Inhibitor2:	CCACCACATCTCTTCCAACCTACTAC
Trigger2:	GTAGTAGGTTGGAAGAGATGTGG
Cleaved S2:	FAM-CACTATA
W2*:	GCCACCACTTTTTTTTTTTTTT
Let-7a:	TGAGGTAGTAGGTTGTATAGTT
Dz2*:	TTGTATAGTTGTGGTGGTCCGAGCCGGTCGAAATAGTG
Inhibitor2*:	CACCACAACTATACAACCTACTACC
Dz1*	GTATAGTTACATCTCTTCTCCGAGCCGGTCGAAATACTGTCAG

Inhibitor1*: AAGAGATGTAACTATACAACCTACTACCTCA

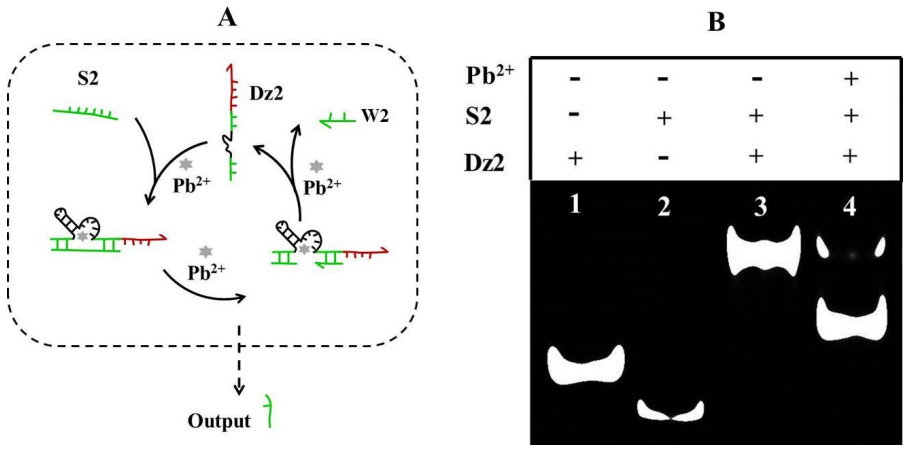

Fig. S1 Verification the cleavage feasibility of Dz2. (A) Schematic illustration of Dz2 cleavage. (B) 12% PAGE verified the feasibility of Dz2 cleavage. (+ denotes with it, -denotes without it).

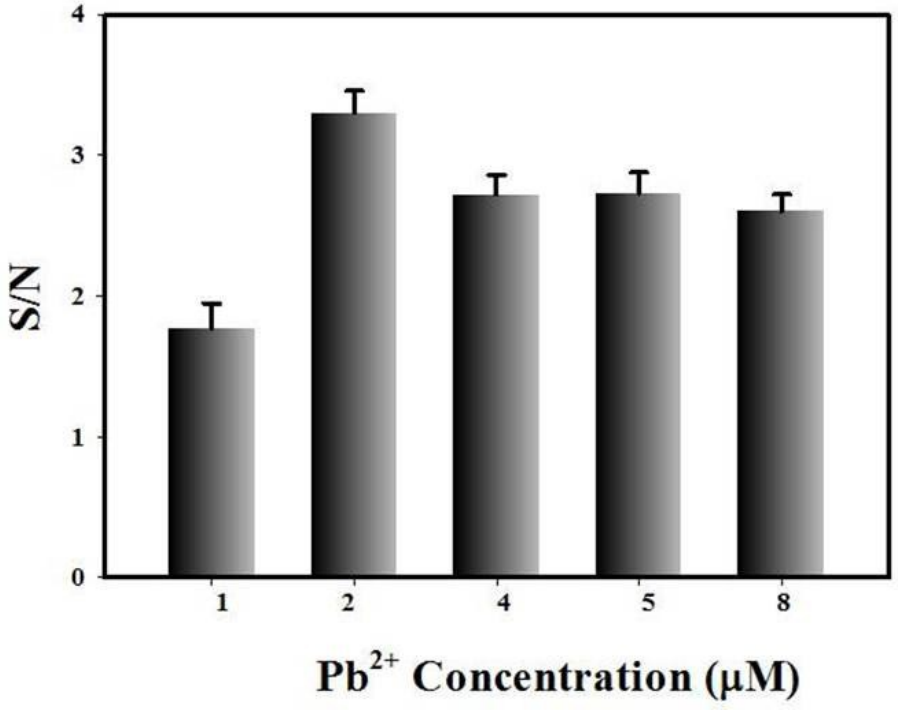

Fig. S2 Optimizing the concentration of Pb^{2+}. (A) The signal-to-noise ratio changed as a function of various Pb^{2+} concentrations (1 μM, 2 μM, 4 μM, 5 μM, 8 μM). The error bar represents the standard deviation of three independent experiments.

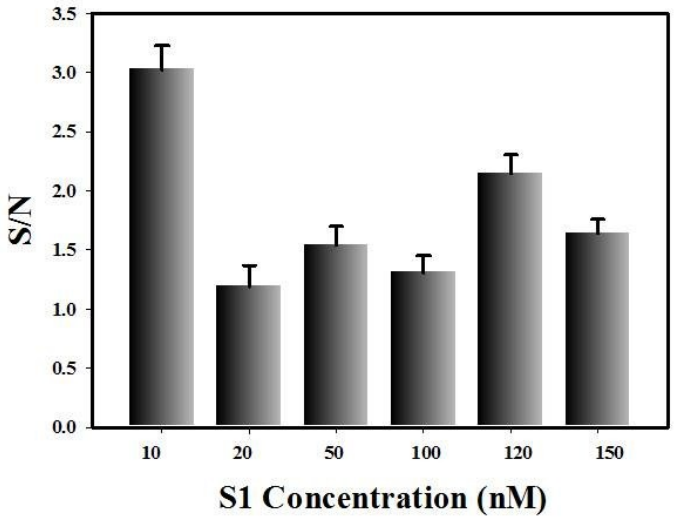

Fig. S3 Investigation of the concentration of S1. IDz1 and S2 were 100 nM in 50 mM tris-HCl for 3 h at 25°C. Error bars are standard deviation obtained from three independent experiments.

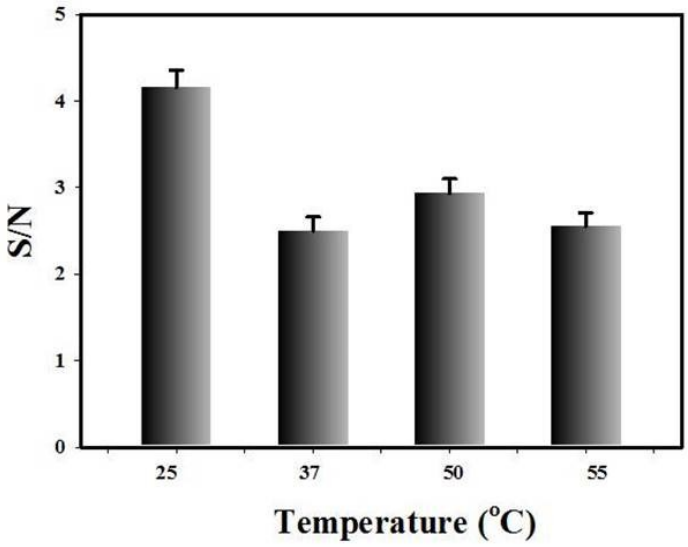

Fig. S4 Study the effect of temperature on reaction system. The different temperature points (25°C, 37°C, 50°C, 55°C) were tested to obtain the optimum reaction temperature. The error bar represents the standard deviation of three independent experiments.

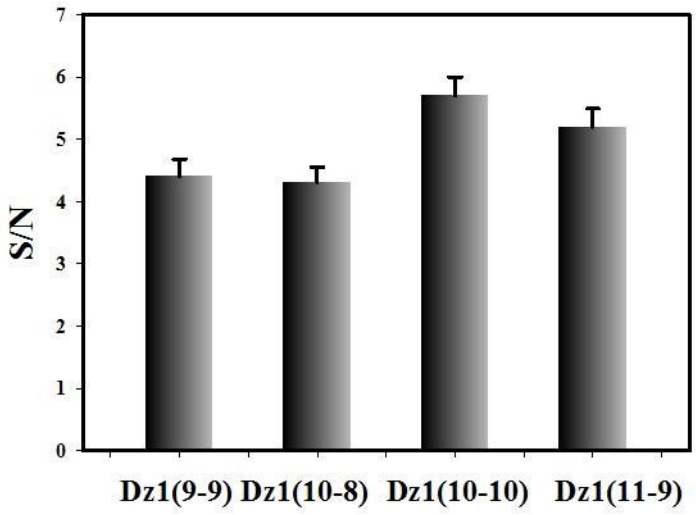

Fig. S5 Assays the binding arm length of Dz1. S2 were 100 nM in 50 mM tris-HCl for 3 h at 25^{o}C. The error bar represents the standard deviation of three independent experiments.

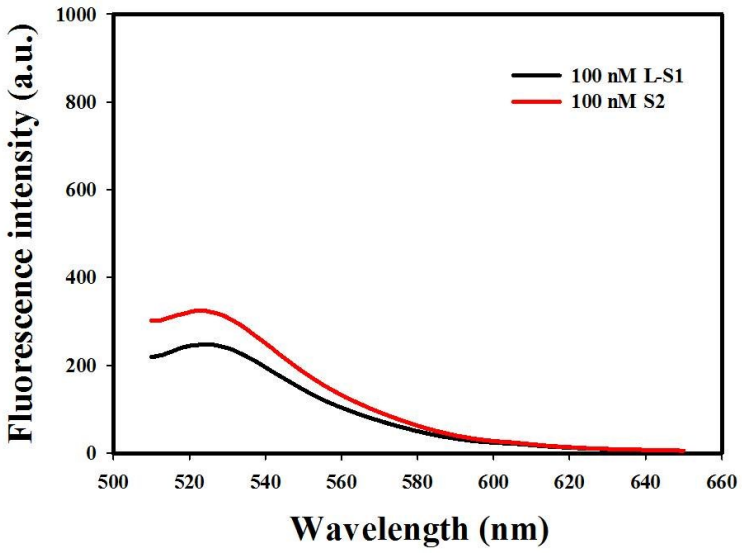

Fig. S6 Fluorescence intensity assays of the L-S1 and S2. 100 nM L-S1 and 100 nM S2 were dispersed in 50 mM tris-HCl and corresponding fluorescence intensity was measured on F-7000 at 25^{o}C, respectively.

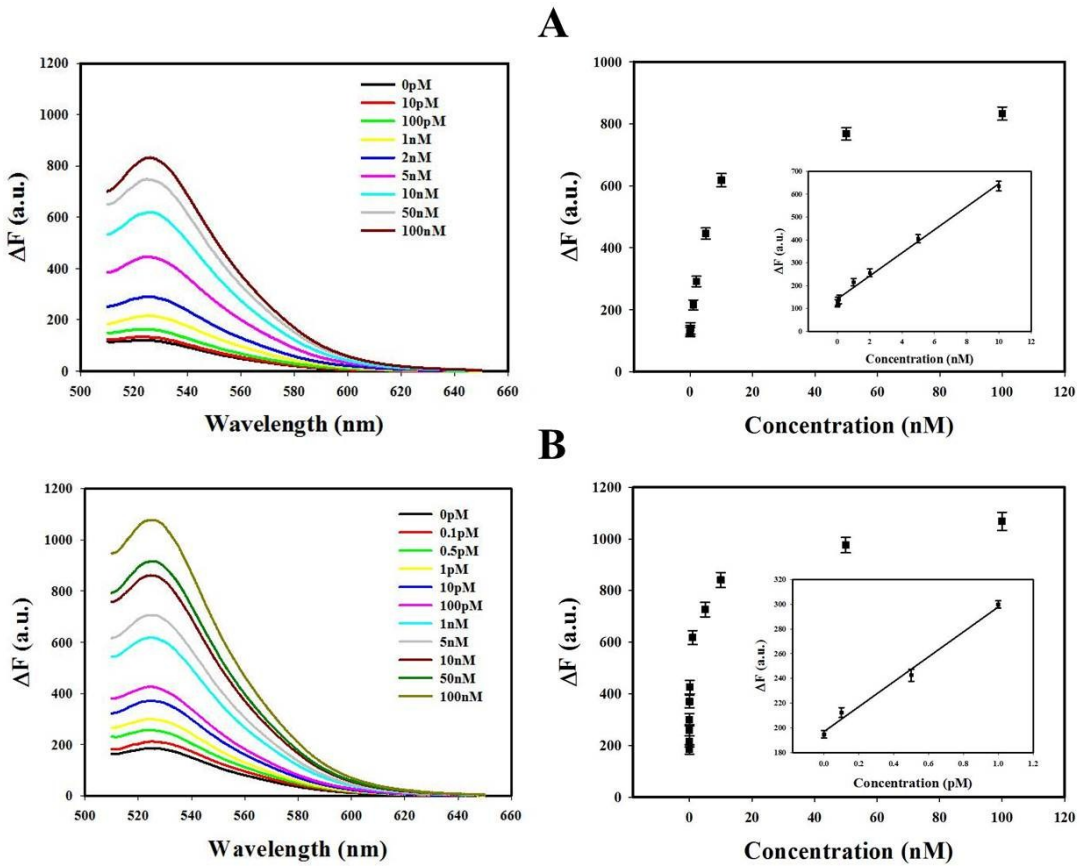

Fig. S7 Amplification efficiency of one step DNAzyme reaction (A) and multiple DNAzymes cascade (B). The fluorescence intensity changed as a function of trigger DNA concentrations and corresponding standard curve. The fluorescence experiments were performed on F-7000 exciting at 488 nm and recording emission from 520 nm to 650 nm. The error bar represents the standard deviation of three independent experiments.

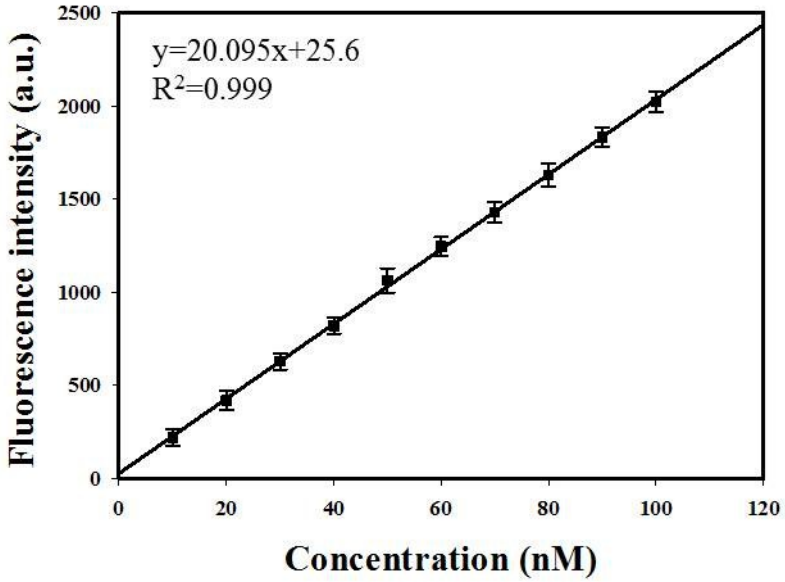

Fig. S8 Standard plot of cleaved S2 labeled with FAM. A standard curve of fluorescence intensity as a function of various given concentrations of cleaved products of S2 labeled with FAM. The error bar represents the standard deviation of three independent experiments.

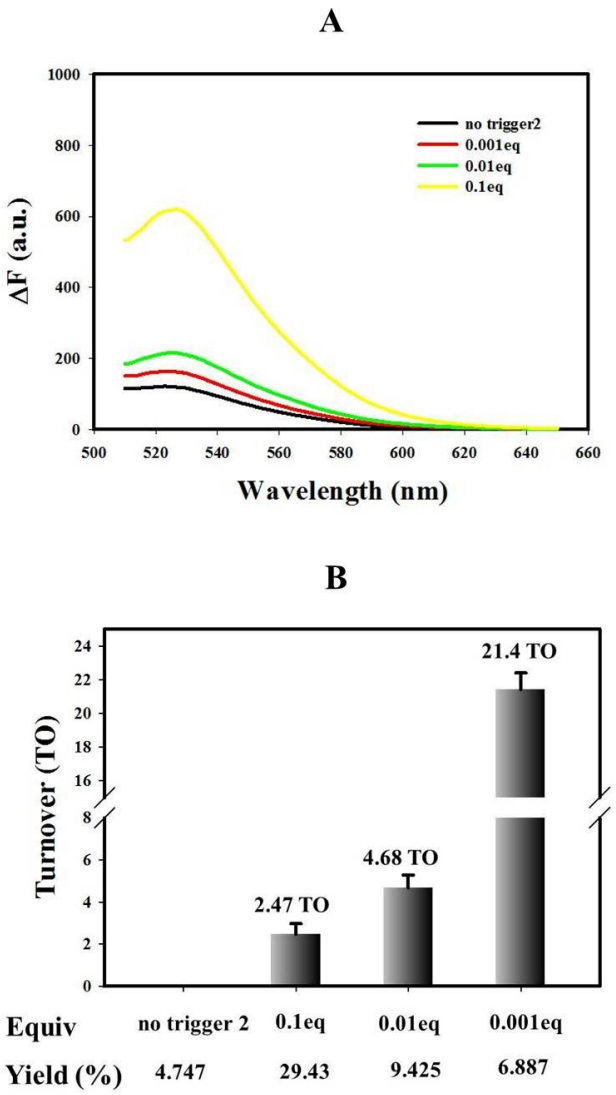

Fig. S9 Turnover experiment of one step DNAzyme reaction. (A) The fluorescence intensity changed with various trigger DNA concentrations (from 10 nM to 10 pM) and probe concentrations were 100 nM. Equiv = trigger DNA concentration/probe concentration (0.1eq, 0.01eq, 0.001eq). It was used for obtaining the concentration of fluorescent products by corresponding standard curve. (B) The histogram denotes the calculated turnover number of one step DNAzyme reaction with corresponding equiv and yields according to the formula of turnover number. The error bar represents the standard deviation of three independent experiments.

Table S2. **Analytical recoveries of one step DNAzyme reaction in detecting let-7a in human serum samples**

Sample	Added (nM)	Found (nM)	Recovery (%)	RSD (%), n=3
1	0.1	0.0996	99.6	1.35
2	2	2.083	104.2	2.37
3	5	4.912	98.2	0.62

Recovery= (Found/Added) × 100%