Supplementary Information

Label-Free Fluorescent Assays Based on Aptamer-Target Recognition

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1. Experimental Section

Fluorescence experiments

The fluorescence spectra were recorded using SPEX FL3 (Jobin Yvon Horiba) with excitation at 497 nm and an emission range from 500 to 650 nm. A 350 μL micro quartz cuvette was used for the experiments and the sample volume was 200 μL.

Determine the fluorescence response of ATP in the design

The samples were prepared in 10 mM Tris-HCl buffer (pH = 8.0) containing 6 mM $MgCl_2$. The constant concentrations of aptamer-probe and SG were 0.25 μM and 1× respectively in the aptamer probe design (Figure S1). The ATP concentration range in the titration experiment was from 0.375 mM to 18 mM. In the control experiments, the concentration for all NTPs was 1.0 mM and for all mutant aptamers was 0.25μM. The fluorescence spectra for all samples were measured at 25 ºC.

Determine the fluorescence response of thrombin in the design

The samples were prepared in 20 mM Tris-HCl buffer (pH = 8.0) containing 5 mM KCl, 1mM $MgCl_2$ and 140 mM NaCl. The thrombin concentration range in samples was from 12.5 nM to 0.6 μM. The fluorescence spectra of all samples were

measured at 25 ºC.

DNA concentration determination

Fluorescence intensity was recorded at 520 nm with an excitation wavelength of 497 nm. The fluorescence signal enhanced via 0-0.5 μM DNA aptamer at constant concentration of SG (1×). We chose the DNA concentration at 0.25 μM which assured excessive SG and suitable fluorescence intensity in the aptamer probe design.

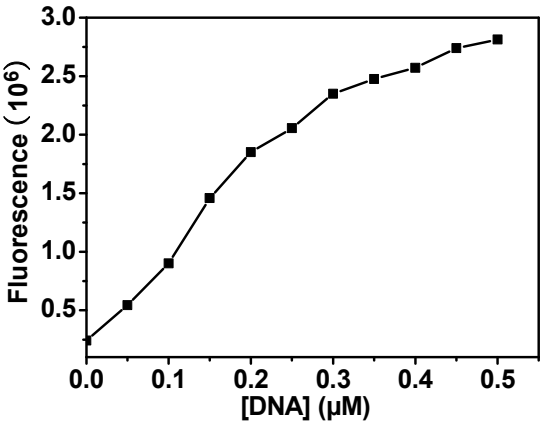

Figure S1. Fluorescence intensity of SG (1×) in the presence of 0-0.5 μM DNA aptamer.

2. Results

ATP assays

Mutant ATP-aptamers

Three mutant aptamers DNA1-3 were tested. Fig. S2 showed a relative high selectivity of A1 aptamer for ATP against the three mutant aptamers.

Table S1. Three mutant aptamers binding with ATP

No	Sequence
A1	5′-CACCTGGGGGAGTATTGCGGAGGAAGGTT CAGGTG-3′
DNA1	5′-CACCTAGGAGAGTAATGCAGACGAAGGTT TAGGTG -3′
DNA2	5′-CACCTGGGGCAGTATTGCGGAGCAAGGTT CAGGTG -3′
DNA3	5′-CACCTAAAGGAGTAATGCAAAGGAAGGTT TAGGTG -3′

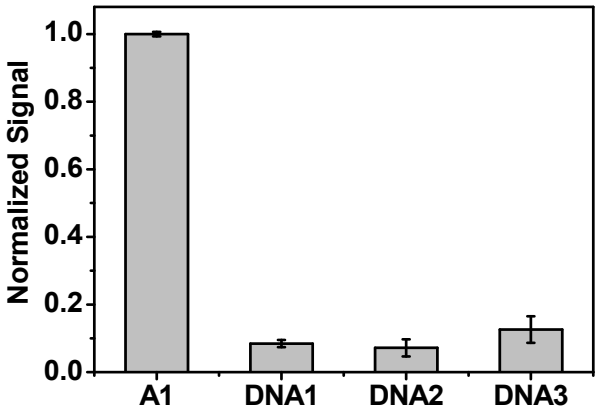

Figure S2. The specificity of aptamer-ATP system using three mutant aptamers. The fluorescence signal decreases were normalized to A1 sequence.

Thrombin assays

The effect of sequences T1-T10 interacting with thrombin

The ten sequences T1-T10 based on 15nt anti-thrombin aptamer that 4 nt (T1-T5) and 5 nt (T6-T10) were added to 3' termini for intramolecular hybridization were used in studying interaction with thrombin.

Table S2. Aptamer probe sequences binding with thrombin in the design

No	Sequence
T1	5'-GGTTGGTGTGGTTGG **TTTT** AACC-3'
T2	5'-GGTTGGTGTGGTTGG **TTTTTT** AACC-3'
T3	5'-GGTTGGTGTGGTTGG **TTTTTTTT** AACC-3'
T4	5'-GGTTGGTGTGGTTGG **TTTTTTTTTT** AACC-3'
T5	5'-GGTTGGTGTGGTTGG **TTTTTTTTTTTT** AACC-3'
T6	5'-GGTTGGTGTGGTTGG **TTTT** CAACC-3'
T7	5'-GGTTGGTGTGGTTGG **TTTTTT** CAACC-3'
T8	5'-GGTTGGTGTGGTTGG **TTTTTTTT** CAACC-3'
T9	5'-GGTTGGTGTGGTTGG **TTTTTTTTTT** CAACC-3'
T10	5'-GGTTGGTGTGGTTGG **TTTTTTTTTTTT** CAACC-3'

The variable length poly (T) spacers were inserted into sequences. As shown as Fig. S3, there were better effects when the sequences contained more than 8mer ploy (T) spacer either 4 bases paired or 5 bases paired. To explain, by optimizing the length of the aptamer probe, this strategy can be theoretically adaptive to a wide range of targets.

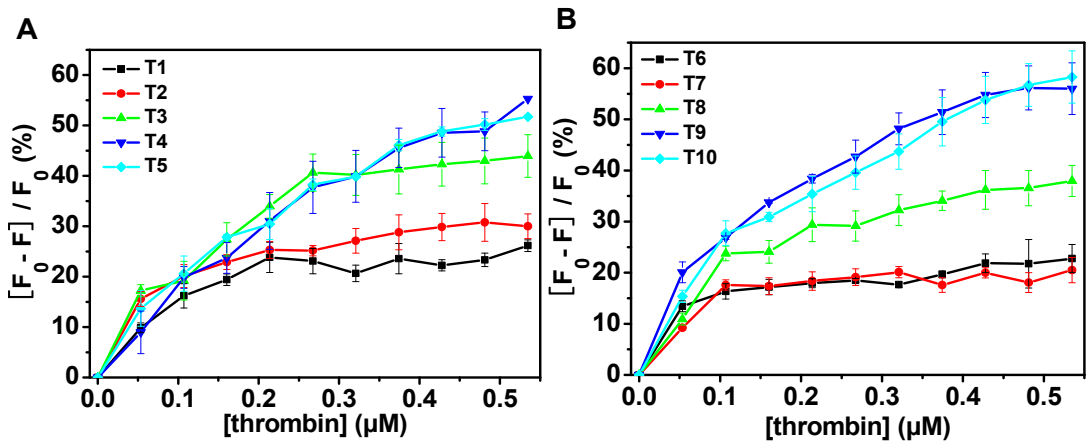

Figure S3. The effect of sequences T1-T10 interacting with thrombin in the tested system. (A) 3' extension of 4 nt (T1-T5) for intramolecular hybridization with variable lengths of poly(T) spacers. (B) 3' extension of 5 nt (T6-T10) for intramolecular hybridization with variable lengths of poly(T) spacers.

Selectivity studying of aptamer-thrombin

Control experiments were done to compare four proteins (IgG, lysozyme, BSA and cytochrome C) with thrombin at the concentration of 200 nM. Fig. S4 showed a relative high selectivity of the thrombin for aptamer against other similar proteins.

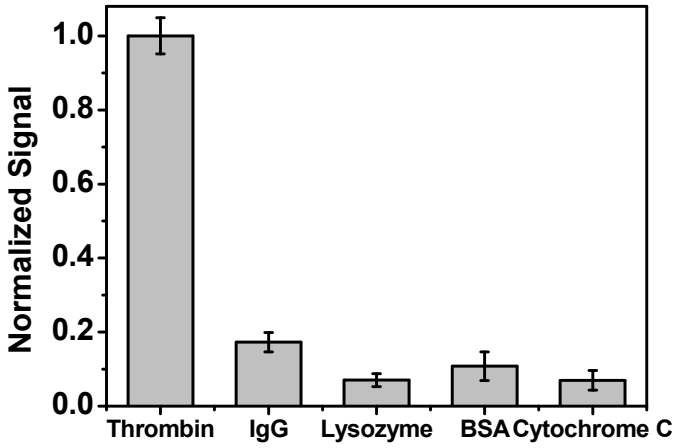

Figure S4. The selectivity of thrombin using IgG, lysozyme, BSA and cytochrome C at the concentration of 200 nM. The fluorescence signal decreases were normalized to the thrombin sample.

Mutant thrombin-aptamers

Three mutant aptamers DNA4-6 were tested. Fig. S5 showed a relative high selectivity of T9 aptamer for thrombin against the three mutant aptamers.

Table S3. Three mutant aptamers binding with thrombin

No	Sequence
T9	5-GGTTGGTGTGGTTGG TTTTTTTTTT CAACC-3
DNA4	5′-AATTAATGTAATTAA TTTTTTTTTT TAATT-3′
DNA5	5′-AGTTAGTGTAGTTAG TTTTTTTTTT TAACT-3′’
DNA6	5′-GATTAGTGTGATTAG TTTTTTTTTT TAATC-3′’

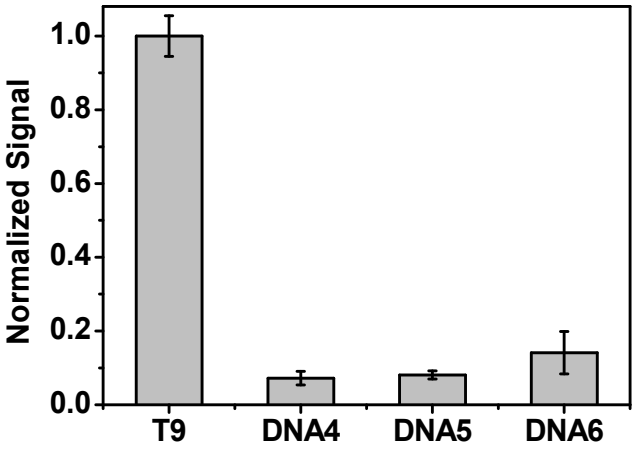

Figure S5. The selectivity of aptamer-thrombin system using three mutant aptamers. The fluorescence signal decreases were normalized to T9 sequence.