Electronic Supplementary Information

A Label-free Fluorescent Adenosine Triphosphate Biosensor via Overhanging Aptamer-triggered Enzyme Protection and Target Recycling Amplification

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1. Experimental section

1.1 Chemicals and materials. Exonuclease III (exo III) was obtained from New England BioLabs (Beijing, China). Adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine triphosphate (GTP), uridine triphosphate (UTP), tris (hydroxymethyl) aminomethane (Tris) and thioflavin T (ThT) were purchased from Sigma-Aldrich and used as received. The ThT solution was freshly prepared before each experiment in order to avoid storage-induced oxidation. Human serum was purchased from Amyjet Scientific Inc., and has been approved by the ethical committee of Nanjing Normal University. All other chemicals used in this work were of analytical grade and directly used without additional purification. All oligonucleotides (HPLC purified, sequences shown in Table S1) were obtained from TaKaRa Inc. (Dalian, China).

Table S1. Sequences of adopted oligonucleotides in this work.

Name	Sequence from 5' to 3'
G-rich MB (GMB)	ACCTTCCTCCCCCAATACTCCCCCAGGT*GGGTTGGGTTGGGTTGGG*TTTTCCCCAACCCAACCCAACCC
Aptamer	ACCTGGGGGAGTATTGGGGGAGGAAGGT
2T-aptamer	**TT**ACCTGGGGGAGTATTGGGGGAGGAAGGT
4T-aptamer	**TTTT**ACCTGGGGGAGTATTGGGGGAGGAAGGT
6T-aptamer	**TTTTTT**ACCTGGGGGAGTATTGGGGGAGGAAGGT
8T-aptamer	**TTTTTTTT**ACCTGGGGGAGTATTGGGGGAGGAAGGT
10T-aptamer	**TTTTTTTTTT**ACCTGGGGGAGTATTGGGGGAGGAAGGT
6T-aptamer-2A	**TTTTTT**ACCTGGGGGAGTATTGGGGGAGGAAGGT**AA**
6T-aptamer-4A	**TTTTTT**ACCTGGGGGAGTATTGGGGGAGGAAGGT**AAAA**
6T-aptamer-6A	**TTTTTT**ACCTGGGGGAGTATTGGGGGAGGAAGGT**AAAAAA**
6T-aptamer-8A	**TTTTTT**ACCTGGGGGAGTATTGGGGGAGGAAGGT**AAAAAAAA**
6T-aptamer-10A	**TTTTTT**ACCTGGGGGAGTATTGGGGGAGGAAGGT**AAAAAAA**

	AAA

The Italic portion represents G-rich sequence that is used to construct G-quadruplex structure. The underlined portions represent ATP aptamer sequences. The bold portions represent overhanging sequences.

1.2 Fabrication of an ATP aptasensor. GMB and overhanging aptamer were firstly mixed equivalently (10 μL 10 μM for each). After that, the mixture was heated up at 90 °C for 5 min, and then cooled to the room temperature slowly in 2 h. Then, different concentration of ATP (4 μL), 4 U exo III (0.5 μL) and 15.5 μL of 10 mM Tris-HCl (pH 8.0) were added to the solution and incubated at 37 °C for 30 min. Then, 5 μL of 1 M KCl was added to the solution and heated up at 90 °C for 5 min. After gradually cooled to room temperature slowly in 2 h, 5 μL of 100 μM ThT was added and kept at room temperature for 30 min.

1.3 Fluorescence measurements. All the measurements were accomplished at room temperature using a FluoroMAX-4C spectrofluorometer (HORIBA Scientific). Setting excitation wavelength at 415 nm, the spectra were recorded in the range from 450 to 600 nm with both excitation and emission slits of 3.0 nm. The fluorescence signals were normalized, and the intensity at the emission wavelength of 485 nm was used for data analysis.

2. Supplementary Results

2.1 Effect of $C_{(G3T3)}/C_{(ThT)}$ ratio on fluorescence intensity of G-quadruplex/ThT complex

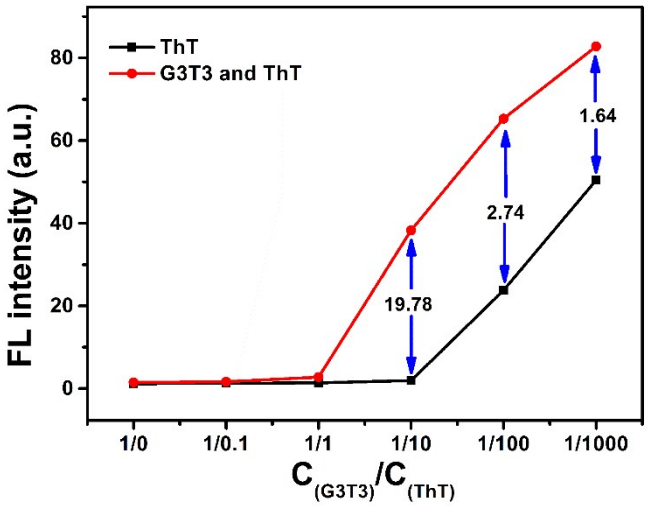

Fig. S1. Fluorescence intensity of different concentrations of ThT with and without G3T3. The concentration of G3T3 was 1 μM.

2.2 Optimal concentration and incubation time of exo III for the aptasensor

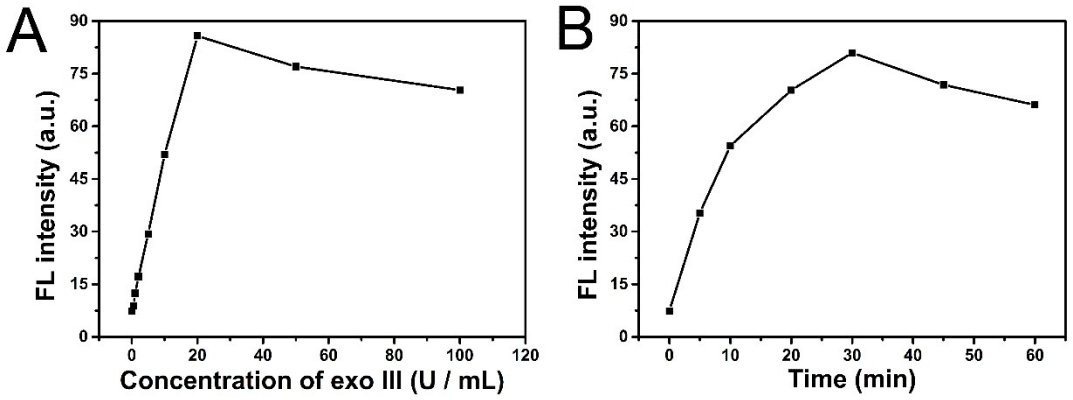

Fig. S2. Fluorescence intensity of GMB catalyzed by exo III under (A) different concentration and (B) incubation time.

2.3 Effect of $C_{(GMB)}/C_{(aptamer)}$ ratio on fluorescent noise of the aptasensor.

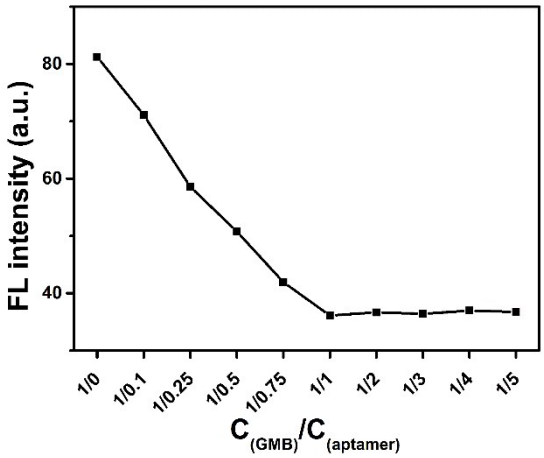

Fig. S3. Fluorescence intensity of different concentrations of 6T-aptamer. The concentration of GMB was 1 μM.

2.4 Comparison of the performance of several ATP aptasensors

Table S2. Comparison of the performance of several ATP aptasensors.

Method	Detection limit (M)	Linear range (M)	Real sample detection	Ref.
Electrochemiluminescence	6.0×10^{-9}	1.8×10^{-8}-9.1×10^{-5}	NR[1]	S1
Photoelectrochemistry	3.2×10^{-9}	1.0×10^{-9}-1.0×10^{-6}	R[2]	S2
Surface-Enhanced Raman Scattering	1.24×10^{-11}	1.24×10^{-11}-2.0×10^{-9}	NR	S3
Fluorescence	2.0×10^{-11}	3.0×10^{-11}-1.0×10^{-7}	R	S4
Chemiluminescence	1.4×10^{-9}	2.0×10^{-9}-8.0×10^{-8}	R	S5
Electrochemistry	3.4×10^{-11}	1.0×10^{-10}-2.0×10^{-8}	NR	S6
Fluorescence	2.8×10^{-10}	5.0×10^{-10}-5.0×10^{-7}	R	this work

[1]NR represents Not Reported. [2]R represents Reported.

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