Supporting Information

Target responsive aptamer machine for label-free and sensitive non-enzymatic recycling amplification detection of ATP

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Experimental Section

Materials: ATP, cytidine triphosphate (CTP), uridine triphosphate (UTP) and guanosine triphosphate (GTP) were purchased from Worthington Biochemicals (Lakewood, NJ, USA). All oligonucleotides listed in Table S1 were obtained from Shanghai Sangon Biological Engineering Technology and Services (Shanghai, China). NMM was ordered from J&K Scientific Ltd. (Beijing, China). The obtained NMM was diluted to a stock solution of 5 mM with dimethyl sulfoxide (DMSO), stored at -20 °C and freshly diluted to a suitable concentration (50 μM) with Tris buffer (20 mM Tris-HCl, 10 mM KCl, 15 mM $MgCl_2$, pH 8.0) before usage. Human serums were provided by the 9th People's Hospital of Chongqing (Chongqin, China). Other reagents were purchased from Kelong Chemical Company (Chengdu, China). All the reagents were of analytical grade and used without further purification, and solutions were prepared with ultrapure water (specific resistance of 18.3 MΩ•cm) during the experimental process.

Table S1. The sequences of the oligonucleotides used in the experiments.

Name	Sequence
Aptamer Strand (AS)	5'-GCCCTACCCAATCTGTGAGAGAACCTGGGGGAGTATTG CGGAGGAAGGT-3'
Helper Strand (HS)	5'-CCCAGGTTCTC-3'
Signal Strand (SS)	5'-TCACAGATTGGGTAGGGCGGGTTGGG-3'
Fuel Strand (FS)	5'-AGGTTCTCTCACAGATTGGGTAGGGC-3'

ATP sensing protocol: The three-strand DNA partial duplex probes were first prepared by mixing the AS (500 nM), HS (500 nM) and SS (500 nM) in Tris-HCl buffer, and the mixture was heated to 90 °C for 5 min, followed by slowly cooling down to room temperature. Subsequently, the fuel probes were added to the mixture to make the sensing solution. Next, different concentrations of ATP were added to the sensing solution with a reaction volume of 50 μL and incubated for 80 min at room temperature. This was followed by the introduction of the NMM (2 μM) and further incubation for another 30 min. Finally, the resulting solutions were diluted to 200 μL, and fluorescent measurements were performed.

Fluorescence measurements: Fluorescent measurements were performed on a RF-5301-PC spectrophotometer (Shimadzu, Tokyo, Japan) utilizing excitation and emission slits of 10 nm and 10 nm, respectively. The fluorescent data were collected from 575 nm to 650 nm by exciting the samples at 399 nm with a 150W Xenon lamp (Ushio Inc, Japan) as the excitation source at room temperature. The fluorescent emission at 610 nm was used as the indicator of the performance of the ATP detection approach.

Supplementary Figure:

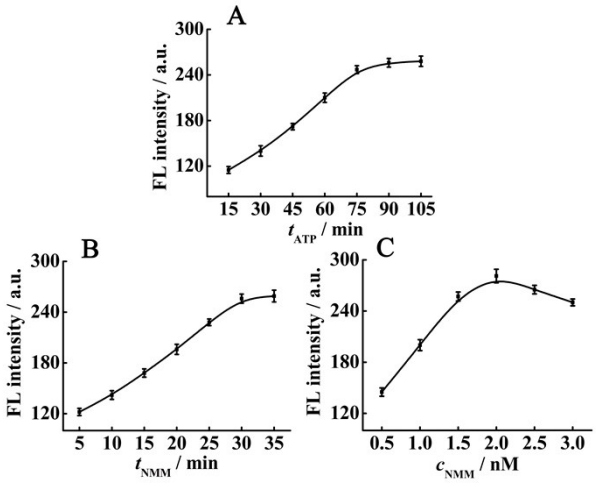

Fig. S1 Effects of (A) ATP interaction time (t_{ATP}), (B) binding time between NMM and G-quadruplexes (t_{NMM}) and (C) the concentration of NMM (c_{NMM}) on the fluorescent responses of the sensing systems. The concentrations of DNA complex probes, fuel probes, ATP were 500 nM, 500 nM, 500 nM, respectively.

In order to achieve optimal performance of our proposed method for ATP detection, the experimental parameters including the ATP interaction time (t_{ATP}), the binding time between NMM and G-quadruplexes (t_{NMM}) and the concentration of NMM (c_{NMM}) were examined. The fluorescent intensity was evaluated at different experimental conditions and treated as the index of method performance. The effect of t_{ATP} on the fluorescence intensity of the sensing solutions with the presence of 500 nM ATP was first investigated from 15 to 105 min with a time interval of 15 min. From Fig. S1A, we can see that evaluated t_{ATP} leads to increasing fluorescent response up to 80 min and then levels off after 80 min (with the concentration of NMM at 1.5 μM and the NMM/G-quadruplex binding time of 30 min). Therefore, 80 min of t_{ATP} was selected for our assay protocol. According to Fig. S1B, the fluorescence intensity increases with the prolonged t_{NMM} in the range of 5 to 30 min and remains almost unchanged thereafter (with the concentration

of NMM at 1.5 μM and interaction time of 80 min). Thus, an optimal t_{NMM} of 30 min was used in subsequent experiments. As can be seen from Fig. S1C, the fluorescence intensity presents a peak-shape dependence upon the concentration of NMM (with interaction time of 80 min and the NMM/ G-quadruplex binding time of 30 min) in the range from 0.5 to 3.0 μM and reaches the maximum value at the concentration of 2.0 μM, indicating that 2.0 M of NMM concentration is suitable for the following experiments.

Table S2. Different signal amplification approaches for ATP detection.

Signal amplification	Detection method	Linear range	Limit of detection	Ref
Exo III-assisted target recycling	Fluorescence	0.25-500 μM	0.25 μM	1
Endonuclease-assisted target recycling	Fluorescence	0.02-100 μM	20 nM	2
Gold nanoparticles (AuNPs)	Electrochemi luminescence	8-2000 nM	7.6 nM	3
DNAzyme wrapped magnetic nanoparticles	Fluorescence	10-80 nM	10 nM	4
Nicking enzyme-mediated DNA recycling	Quartz crystal microbalance assay	2-10 nM	1.3 nM	5
Silver nanoparticles	Fluorescence	200-200 μM	48 nM	6
DNA-fueled aptamer machine	Fluorescence	50 -500 nM	25 nM	This work

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