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Electronic Supplementary Information

Proximity-Induced exponential amplification reaction triggered by proteins and small molecules

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1. Experimental section.

1.1. Materials and reagents

All HPLC-purified DNA oligonucleotides (Table S1) were obtained from Sangon Biotech. Co. Ltd. (Shanghai, China). Nicking endonuclease Nb.BbvCI, Klenow fragment polymerase (KFP) and NEB buffer 2 were obtained from New England Biolabs (Beijing, China). Adenosine triphosphate (ATP), cytidine triphosphate (CTP), uridine triphosphate (UTP), guanosine triphosphate (GTP) and deoxyribonucleoside 5'-triphosphate mixture (dNTPs) were obtained from Sangon Biotech. Co. Ltd. (Shanghai, China). Thioflavin T (ThT) was obtained from Sigma. Streptavidin (SA) was obtained from Solarbio. Super GelRed was obtained from US Everbright INC. All chemical reagents were of analytical grade and used without further purification. Ultrapure water obtained from a Milli-Q filtration system of Sigma-Aldrich was used in all experiments.

Table S1. The oligonucleotides used in this work.

Oligonucleotides	Sequence (5′-3′)
6bp	TCCCTATCCCTATCCCATTACTCCTCAGCCATCACAACATTTTTTTTTTTTAGTTGTG
6bp-a	TCCCTATCCCTATCCCATTACTCCTCAGCCATCACAACATTT
6bp-b	TTTTTTTTTAGTTGTG
5bp	TCCCTATCCCTATCCCATTACTCCTCAGCCATCACAACATTTTTTTTTTTTATTTGTG
5bp-a	TCCCTATCCCTATCCCATTACTCCTCAGCCATCACAACATTT
5bp-b	TTTTTTTTTATTTGTG
4bp	TCCCTATCCCTATCCCATTACTCCTCAGCCATCACAACATTTTTTTTTTTTATATGTG
4bp-a	TCCCTATCCCTATCCCATTACTCCTCAGCCATCACAACATTT
4bp-b	TTTTTTTTTATATGTG
3bp	TCCCTATCCCTATCCCATTACTCCTCAGCCATCACTTTTTTTTTTTTTTTTTTTTGTG
3bp-a	TCCCTATCCCTATCCCATTACTCCTCAGCCATCACTTTTTTT
3bp-b	TTTTTTTTTTTTTGTG
2bp	TCCCTATCCCTATCCCATTACTCCTCAGCCATCATTTTTTTTTTTTTTTTTTTTTTTG
2bp-a	TCCCTATCCCTATCCCATTACTCCTCAGCCATCATTTTTTTT
2bp-b	TTTTTTTTTTTTTTTG
Pri-bio	Biotin-TTTTTTGAAG
Tem-bio	ATCCCTATCCCTATCCCTATCCCTACCTCAGCAACATTACTCCTCAGCAACATTACTCCTTCTTTT-Biotin
ATP-pri	TGCGGAGGAAGGTTTTGAAG
ATP-tem	ATCCCTATCCCTATCCCTATCCCTACCTCAGCAACATTTGACCTCAGCAACATTTGACCTTCTTTTACCTGGGGGAGTAT

The recognition sequences for Nb.BbvCI are underlined.

1.2. Screening of suitable complementary base number

1 μM Nbp-a and Nbp-b (N=2, 3, 4, 5, 6) were mixed with 0.6 mM dNTPs, 1 U KFP and 3.5 U Nb.BbvCI in 1 × NEB buffer 2 (50 mM NaCl, 10 mM Tris-HCl (pH=7.9), 1 mM DL-Dithiothreitol, 10 mM $MgCl_2$). The mixture was incubated at 37 °C for 2 h. 1 μM Nbp (N=2, 3, 4, 5, 6) was mixed with 0.6 mM dNTPs, 1 U KFP and 3.5 U Nb.BbvCI in 1 × NEB buffer 2 (50 mM NaCl, 10 mM Tris-HCl (pH=7.9), 1 mM DL-Dithiothreitol, 10 mM $MgCl_2$). The mixture was incubated at 37 °C for 2 h. Then their fluorescence intensity at 604 nm was used for recorded under the excitation at 302 nm.

1.3. Polyacrylamide gel electrophoresis (PAGE) analysis

25 μL of reaction mixture containing 1 μM DNA, 0.6 mM dNTPs, 1 U KFP, 3.5 U Nb.BbvCI, 1 × NEB buffer 2 (50 mM NaCl, 10 mM Tris-HCl (pH=7.9), 1 mM DL-Dithiothreitol, 10 mM $MgCl_2$) was prepared and incubated at 37 °C for 2 h. Then, 10 μL of reaction solution was sufficiently mixed with 2 μL 6 × loading buffer. The mixture was analyzed by 10 % PAGE in 1 × TAE buffer at a 110 V constant voltage for 40 min. The gel was photographed by a gel documentation system (Huifuxingye, Beijing, China) after staining by Super GelRed solution for 40 min.

1.4. Streptavidin detection

400 nM Pri-bio and 200 nM Tem-bio were mixed with 0.2 mM dNTPs, 1 U KFP, 15 μM ThT and 2.5 U Nb.BbvCI in 1 × NEB buffer 2 (50 mM NaCl, 10 mM Tris-HCl (pH=7.9), 1 mM DL-Dithiothreitol, 10 mM $MgCl_2$). The mixture added with different concentrations of Streptavidin was incubated at 37 °C for 2 h, and then its fluorescence spectrum was recorded in the range of 460 ~ 550 nm under the excitation at 425 nm. The fluorescence intensity at 485 nm was used for Streptavidin detection.

1.5. ATP detection

400 nM ATP-pri and 200 nM ATP-tem were mixed with 0.2 mM dNTPs, 1 U KFP, 15 μM ThT and 3.5 U Nb.BbvCI in 1 × NEB buffer 2 (50 mM NaCl, 10 mM Tris-HCl (pH=7.9), 1 mM DL-Dithiothreitol, 10 mM $MgCl_2$). The mixture added with different concentrations of ATP was incubated at 37 °C for 70 min, and then its fluorescence spectrum was recorded in the range of 460 ~ 550 nm under the excitation at 425 nm. The fluorescence intensity at 485 nm was used for ATP detection.

2. Simulation of DNA folding

We simulated the hybridization of DNA duplex on this website: http://www.unafold.org/. The temperature parameter was set to 37 °C, since the reaction was conducted at 25 °C. The other parameters were set according to the reaction condition with $[Na^+]$ = 50 mM, $[Mg^{2+}]$ = 10 mM.

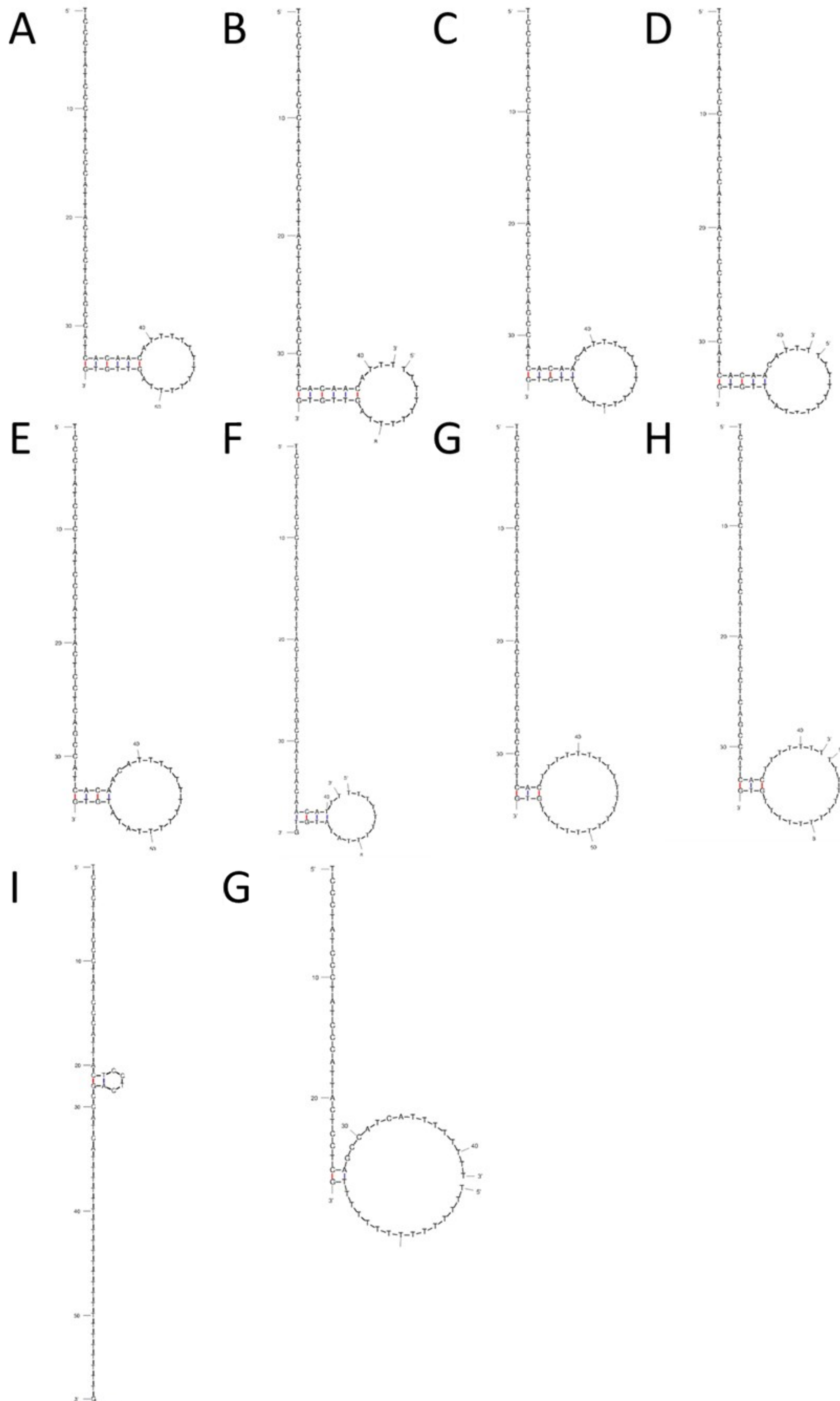

Figure S1. DNA folding form of 6bp, 6bp-a&6bp-b, 5bp, 5bp-a&5bp-b, 4bp, 4bp-a&4bp-b, 3bp, 3bp-a&3bp-b, 2bp and 2bp-a&2bp-b.

Table S2. The simulation results of DNA folding indicated that

	ΔG	ΔH	ΔS	Tm	Fold
6bp	-3.2	-48.5	-146.2	58.6°C	Right
6bp-a&6bp-b	-6.1	-55.1	-158.0	19.6°C	Right
5bp	-0.9	-38.5	-121.2	44.6°C	Right
5bp-a&5bp-b	-3.5	-35.1	-102.0	-7.7°C	Right
4bp	-0.2	-32.6	-104.4	39.3°C	Right
4bp-a&4bp-b	-3.2	-24.9	-70.1	-24.8°C	False
3bp	1.0	-26.2	-87.7	25.5°C	Right
3bp-a&3bp-b	-2.3	-30.8	-92.0	-21.2°C	False
2bp	1.1	-14.8	-51.1	16.5°C	False
2bp-a&2bp-b	0.1	-10.3	-33.5	-111.6°C	False

We can find the Tm of hairpin DNA is much higher than that of separate primer and template, which is consistent with the experimental results. What's more, 4bp-a&4bp-b, 3bp-a&3bp-b, 2bp and 2bp-a&2bp-b can't fold as expected and can't initiate reaction.

3. PAGE analysis of Streptavidin and ATP sensing system

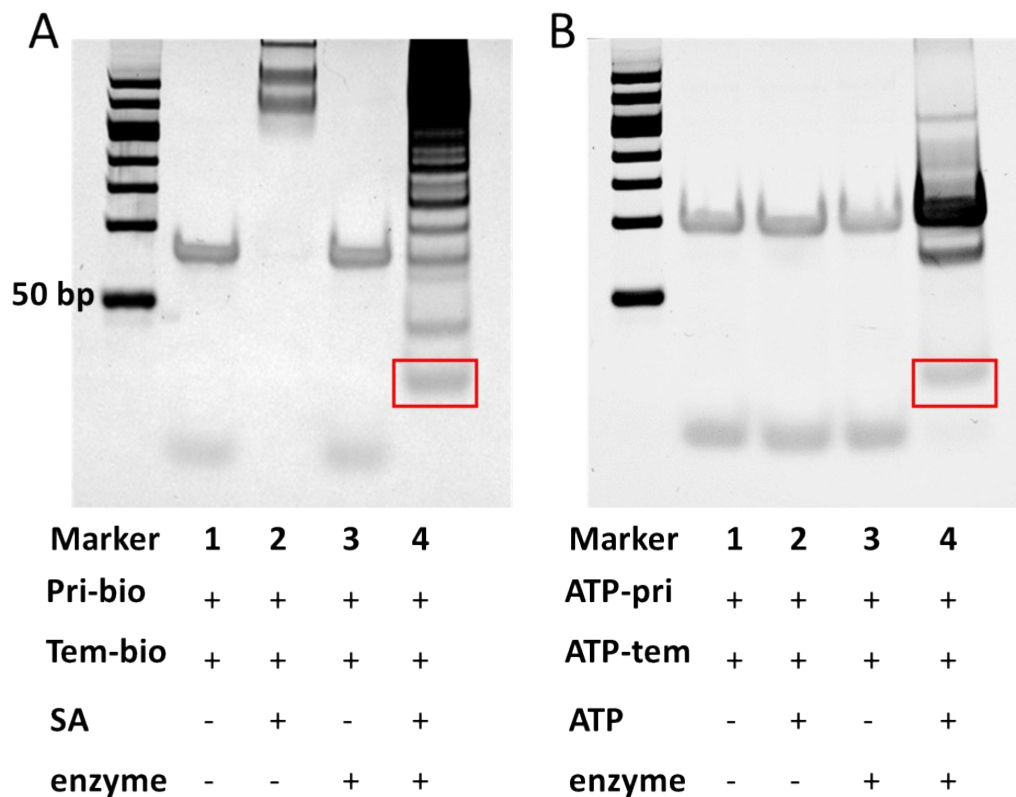

Figure S2. PAGE analysis of Streptavidin and ATP sensing system.

The experimental results suggested that the EXPAR process would be initiated only in the presence of streptavidin or ATP. The G-rich product (30bp, in red boxes) were observed, thus demonstrating the feasibility of the EXPAR reaction triggered by streptavidin and ATP.

4. Optimization of Streptavidin sensing system

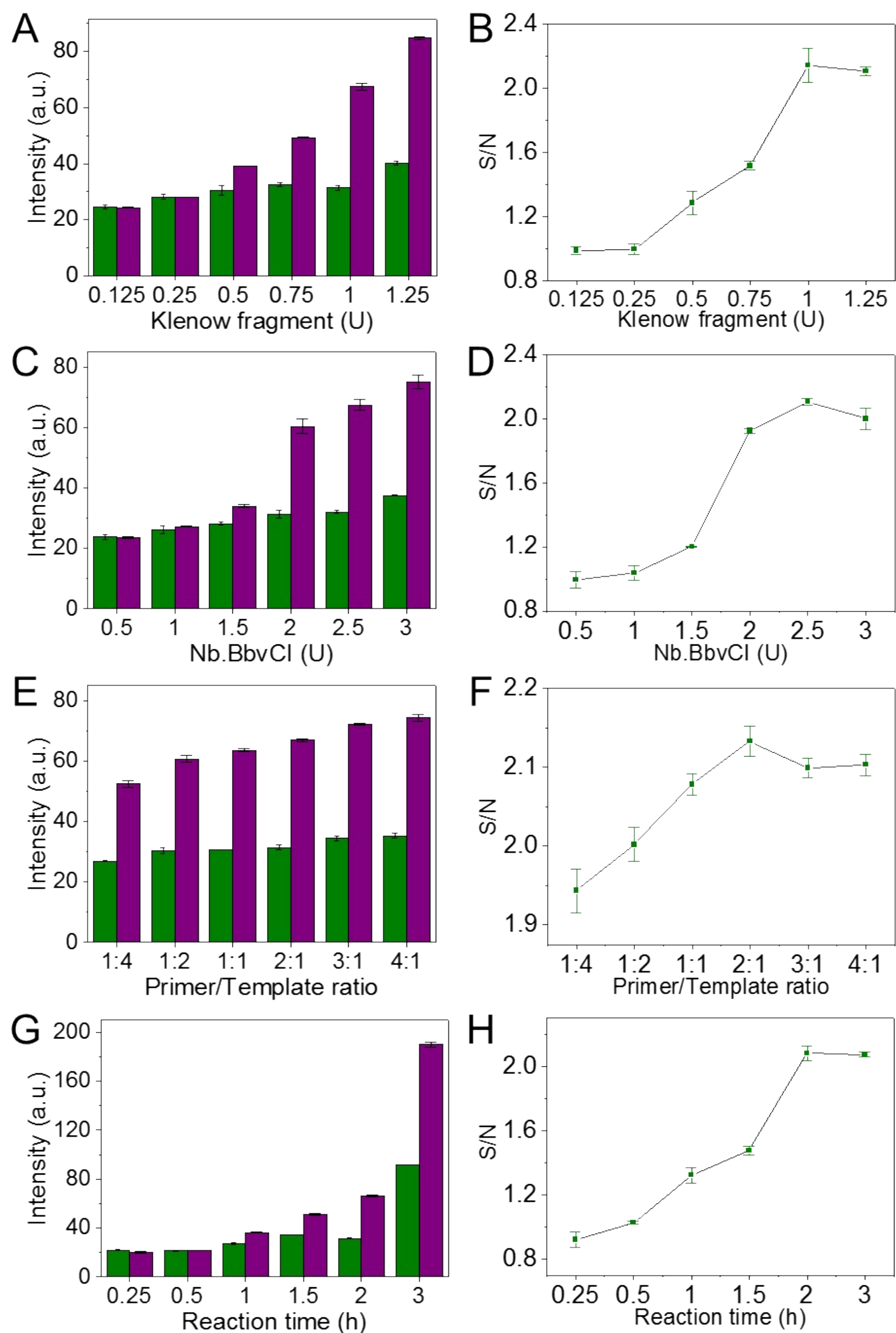

Figure S3. Fluorescence intensity change of the sensing system with (A) Klenow fragment concentration, (C) Nb.BbvCI concentration, (E) Primer/Template ratio and (G) reaction time. Signal-to-noise ratio (F/F0) change of the sensing system with (B) Klenow fragment concentration, (D) Nb.BbvCI concentration, (F) Primer/Template ratio and (H) reaction time. The concentration of SA was 0.01 nM.

5. Baseline fluorescence spectra of ATP sensing system

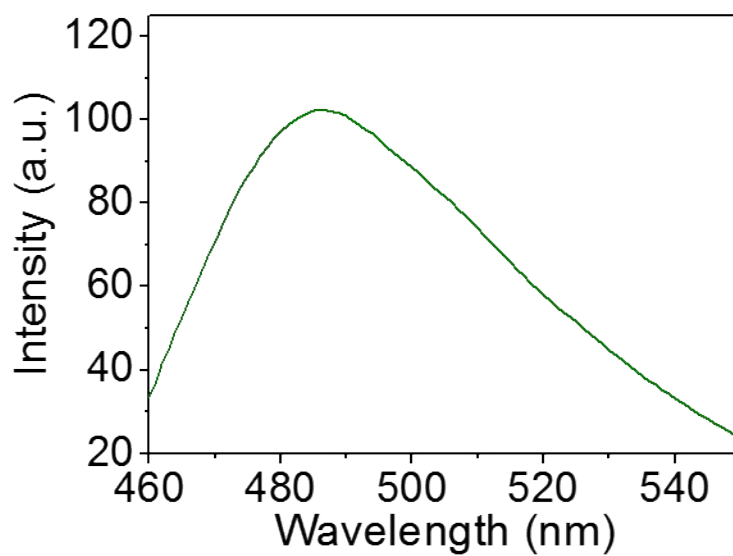

Figure S4. Fluorescence spectra of the sensing systems containing 400 nM ATP-pri, 200 nM ATP-tem and 15 μM ThT, without any ATP and enzyme.

6. Optimization of ATP sensing system

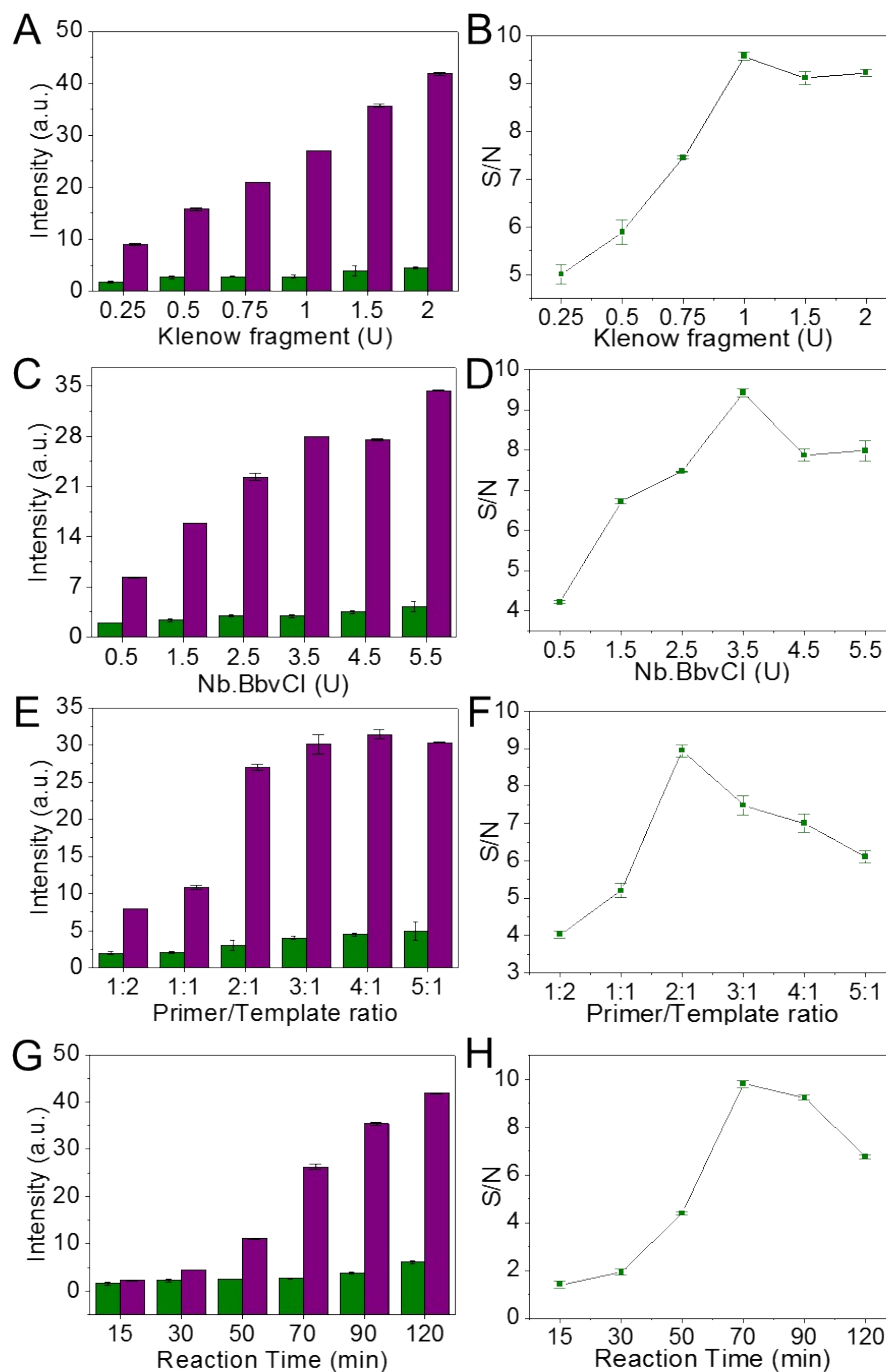

Figure S5. Fluorescence intensity change of the sensing system with (A) Klenow fragment concentration, (C) Nb.BbvCI concentration, (E) Primer/Template ratio and (G) reaction time. Signal-to-noise ratio (F/F0) change of the sensing system with (B) Klenow fragment concentration, (D) Nb.BbvCI concentration, (F) Primer/Template ratio and (H) reaction time. The concentration of ATP was 0.03 nM.

7. Response of detection system to target in low concentration range

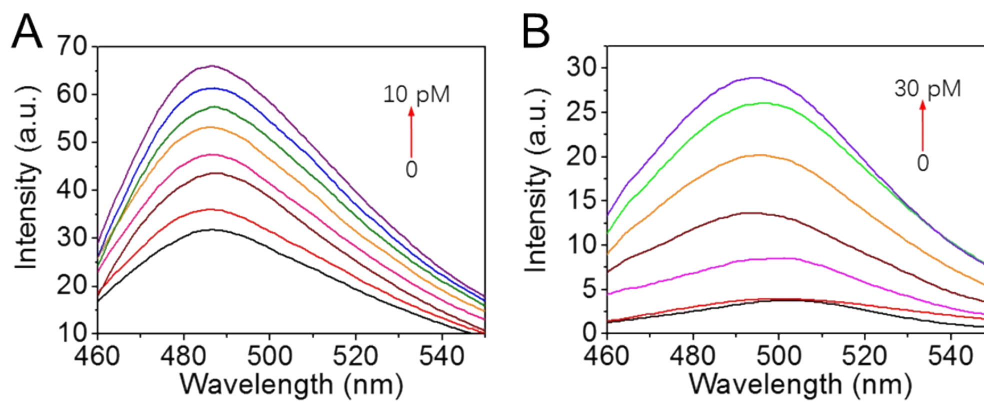

Figure S6. (A) Fluorescence spectra of the sensing systems containing different amounts of streptavidin (0-10 pM) (B) Fluorescence spectra of the sensing systems containing different amounts of ATP (0-30p M)

8. Comparison of our Streptavidin and ATP detection method with other reported ones

Table S3. Comparison of several Streptavidin detection methods

Method	Operation and procedures	Detection limit	Reference
Optical biosensor	Relied on the change of the optical response generated by variations in the dielectric constant of the voids due to a bio-recognition probe-target event.	54 nM	1
Fluorescence	Triggering strand displacement by Binding of the two affinity ligands to the same target	0.1 nM	2
Flow cytometry	Using flow cytometry to analyze biomarker-bearing particles.	0.75 nM	3
Chemiluminiscence	based on a multipedal DNA walker along a three-dimensional DNA functional magnet particles track	6.5 pM	4
Real-time PCR	based on binding-induced DNA assembly	0.1 pM	5
This work	based on proximity-induced exponential amplification reaction	2.9 fM	

Table S4. Comparison of several ATP detection methods

Method	Operation and procedures	Detection limit	Reference
Electrochemistry	Based on target-induced hairpin-mediated sensing interface	5.04 pM	6
Fluorescence	responding to ATP with ZIF-8 decomposition and fluorescence off–on switch via a competitive coordination interaction	35 μM	7
Fluorescence	a four-stage signal amplification based on an allosteric probe-conjugated strand displacement amplification (SDA) integrated with CRISPR/Cpf1 system (ASD-Cpf1)	1.8 pM	8
Surface-enhanced Raman spectroscopy	aptasensor based on ratiometric SERS strategy	20 pM	9
Single molecule fluorescent colocalization	based on dual-color fluorescent colocalization of spilt aptamers	100 fM	10
Electrochemistry	sandwich electrochemical sensor based on a new conception of bivalent split aptamer signal probe	41 nM	11
This work	based on proximity-induced exponential amplification reaction	31.3 fM	

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