Electronic Supplementary Material (ESI) for ChemComm.

Trans-Cleavage of CRISPR-Cas12a-Aptamer System for One-Step Antigen Detection

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Supporting Information

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1. Experiments Procedures

1.1 Regents

All DNA and RNA oligonucleotides used in this research were synthesized by Sangon Biotechnology Inc. (Shanghai, China). The sequence was shown in Table S1. The diethylpyrocarbonate (DEPC)-treated water, RNase inhibitor, prostate-specific antigen (PSA) ELISA Kit were purchase from Sangon Biotechnology Inc. (Shanghai, China). The Cas12a and buffer were purchase from New England Biolabs (Beijing) Ltd. (Beijing, China). Clinical samples were provided by Renji Hospital (Shanghai, China). The regents were all chromatography grade or analytical grade and used as received. The solutions were prepared using the deionized water purified through a Millipore system (18.2 MΩ cm).

1.2 Fluorescence measurements for cleavage kinetics study

For the trans cleavage kinetics study, the aptamer (50 nM) with protein target as experimental group or the buffer only as the control group was added to the well plate coated with capture antibody and incubated with 1.0 h. The Cas 12a-crRNA complexes were assembly by incubated with the 250 nM Cas12a and 250 nM crRNA for 30 min at 37 °C. The fluorescence intensity (510 nm) was measured at excitation wavelength of 480 nm using a FluoroMax-4 spectrofluorometer (Horiba Scientific) for every 5 min.

1.3 Fluorescence measurements for establishment of titration curves

For the establishment of titration curves, the protein targets with concentration from 0.16 ng/mL to 10 ng/mL were added to the to the well plate coated with capture antibody and incubated with 1.5 h. The follow steps were same as 1.2. And we defined signal change as:

$$Signal\ Change = Fluorescent\ (Blank) - Fluorescent\ (target)$$

1.4 Fluorescence measurements for clinical samples

The clinical samples1, 2 and 4 were diluted 3-fold with PB buffer to be used. The follow steps were same as 1.2 and 1.3.

1.5 ELISA assay for protein detection

The workflow of ELISA detection assay was provided by Sangon Biotechnology Inc. (Shanghai, China).

Websites:

https://cdn1.sinobiological.com/reagent/KIT002.pdf

2. Supplementary Figures

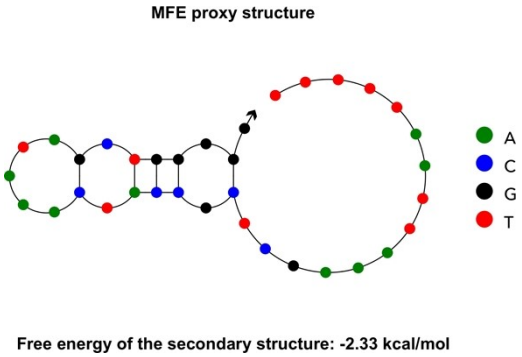

Fig. S1. The minimum free energy (MFE) proxy structure of PSA aptamer provided by NUPACK.

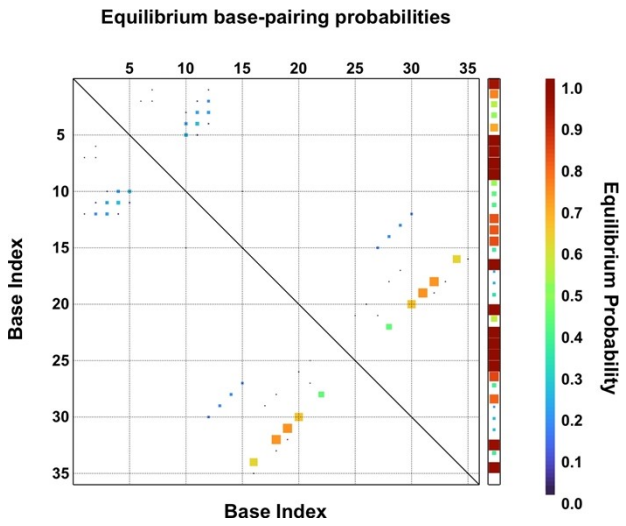

Fig. S2. The equilibrium base-pairing probabilities of PSA aptamer provided by NUPACK.

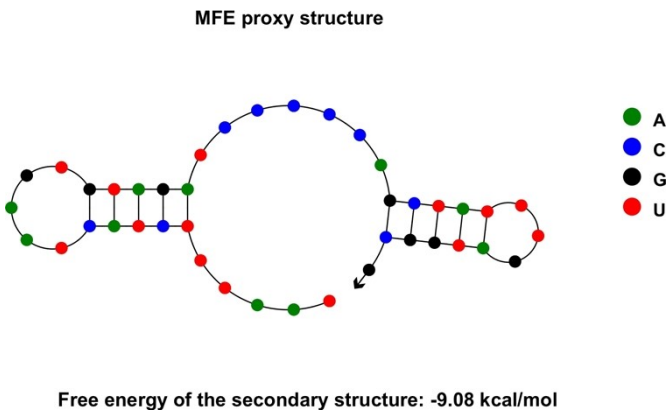

Fig. S3. The minimum free energy proxy structure of crRNA provided by NUPACK.

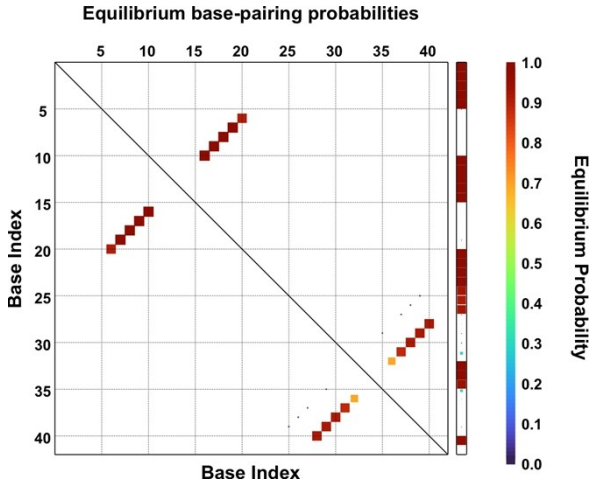

Fig. S4. The equilibrium base-pairing probabilities of crRNA provided by NUPACK.

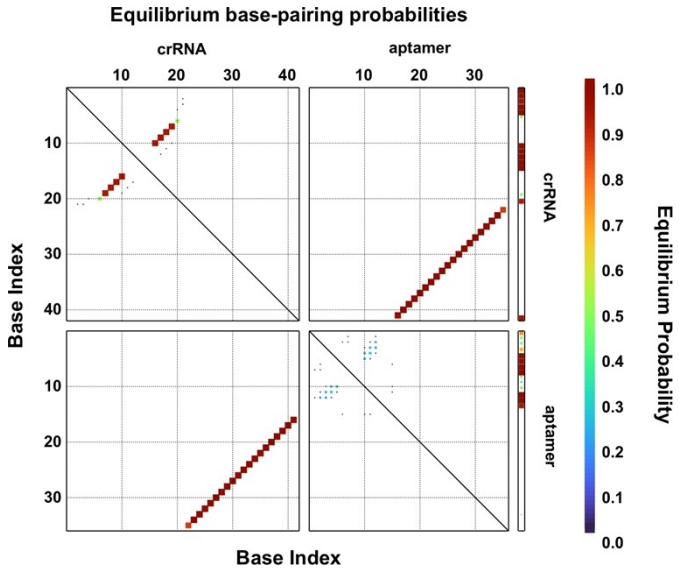

Fig. S5. The equilibrium base-pairing probabilities between crRNA and PSA aptamer provided by NUPACK.

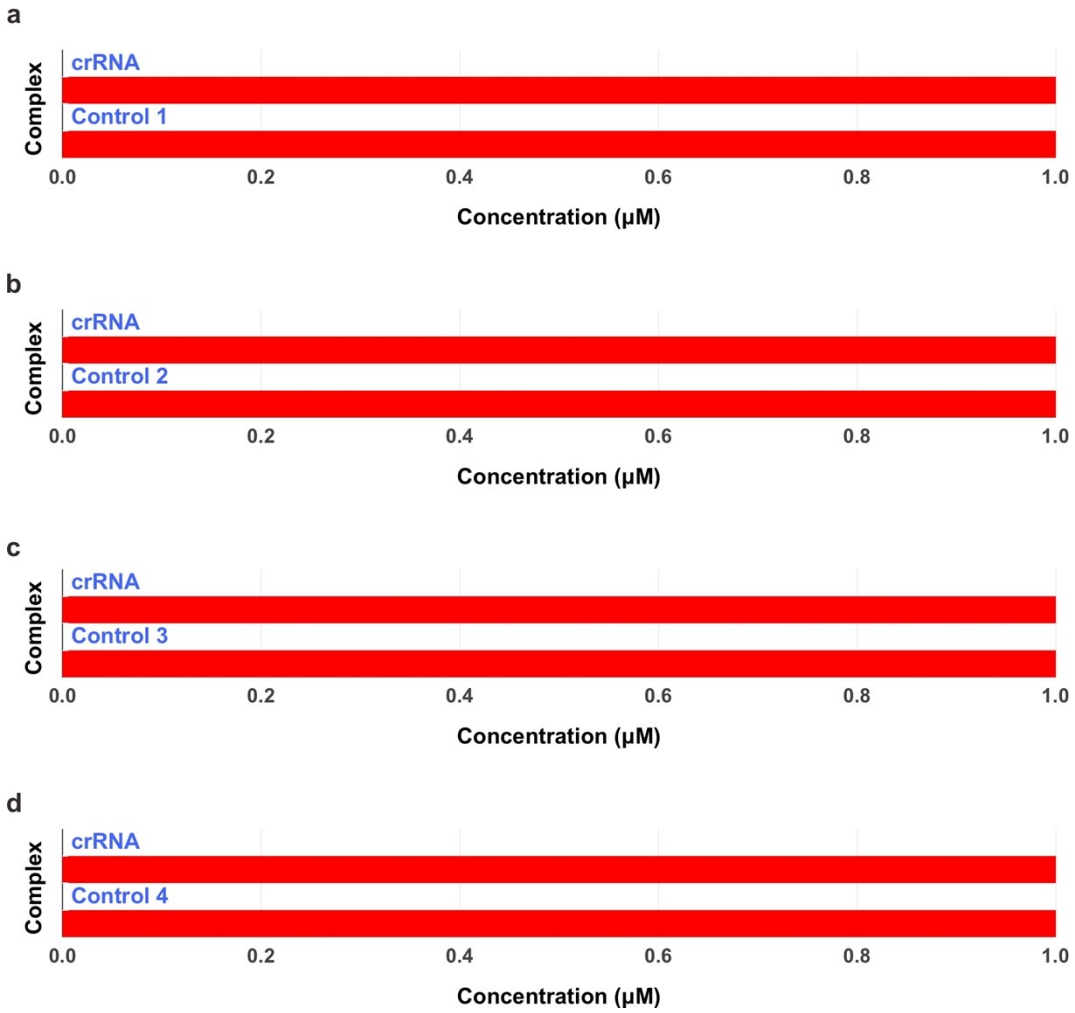

Fig. S6. The equilibrium complex concentration of mixture of crRNA and control 1, control 2, control 3 and control 4 provided by NUPACK.

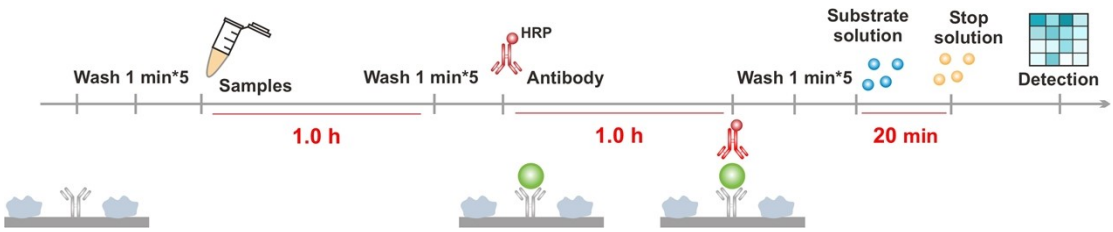

Fig. S7. The workflow of ELISA detection assay.

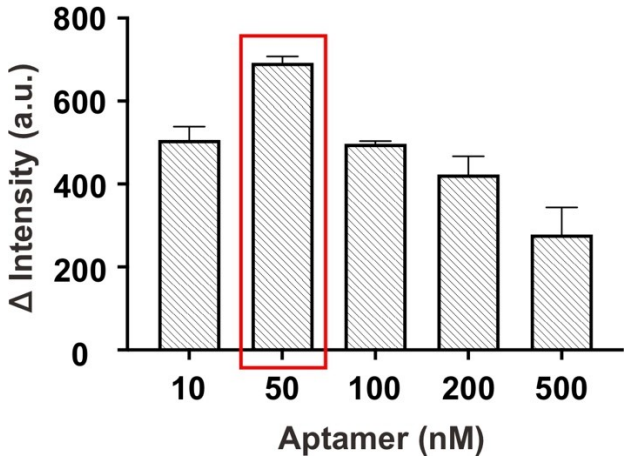

Fig. S8. Effect of aptamer concentration on signal response. When the aptamer concentration was 50 nM, the signal change was maximum. The PSA concentration was constantly 10 ng/mL. Error bars, mean ± s.d. (n = 3).

3. Supplementary Tables

Table S1 DNA sequence used in the experiment

Sequence name	Sequence (5'-3')
crRNA-PSA	UAAUUUCUACUAAGUGUAGAUCCCCCAGCUAUUUGAUGGCG
PSA-aptamer	TTTTTAATTAAAGCTCGCCATCAAATAGCTGGGGG
Signal-probe	BHQ1-CCACCACCACCA-FAM
Control-1	GTGGCAGGTCAGTCATGTATACTGCACTAATTC
Control-2	TAGTGCAGTATACATGACTGACCTGCCACGTCA
Control-3	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGT
Control-4	TCAACTGCCTGGTGATAAAACGACACTACGTGG

Table S2 Comparison of different methods for the detection of PSA[1-6].

Method	Time-cost	Total steps	Pretreatment	Complex Equipment
This work	1.5 h	1 step	No	No
Radioimmunoassay	10.75 h	3 steps	Yes	Yes
ELISA	1.75 h	7 steps	No	No
SPR	0.5 h	4 steps	Yes	Yes
ECL	1 h	4 steps	Yes	No
SERS	1 h	2 steps	Yes	Yes
Electrochemistry	4 h	4 steps	Yes	No

*ELISA: enzyme-linked immunosorbent assay

*SPR: surface plasmon resonance

*ECL: electrochemiluminescence

*SERS: surface-enhanced Raman scattering

Table S3 Comparison of the performance of our method with ELISA method.

Method	Time-cost	Total steps	LOD	Work range
This work	1.5 h	1 step	0.16 ng/mL	0.31-5 ng/mL
ELISA	1.75 h	7 steps	0.19 ng/mL	0.31-20 ng/mL

*The ELISA kit and data was provided by Sangon Biotechnology Inc.

(https://store.sangon.com/productImage/DOC/D711048/D711048_ZH_P.pdf).

Table S4 The source data of clinical samples detection.

ID	Parallel 1	Parallel 2	Parallel 3	AVER.	Δ	Log C	C
1	996	903	1002	967	620	1.68	3.20
2	1109	989	1106	1068	1109	0.91	1.88
3	1089	997	917	1001	586	1.42	2.68
4	1072	998	894	988	1072	1.52	2.86
5	987	896	991	958	629	1.75	3.36
6	1579	1439	1449	1489	98	-2.29	0.21
7	1406	1369	1415	1397	190	-1.59	0.33
8	1379	1398	1375	1384	203	-1.49	0.36
9	1367	1329	1408	1368	219	-1.37	0.39
10	1140	1089	1083	1104	483	0.64	1.56
Control	1607	1573	1581	1587	/	/	/

*AVER.: The average results of three parallel experiments.

*Δ: $= Fluorescence\ (Control) - Fluorescence\ (AVER.)$

*Log C: $\log concentration$

*C: The concentration of PSA on the clinical samples.

*Unit:

Parallel 1 /Parallel 2/Parallel 3/AVER.: a.u.

Log C: log ng/mL

C: ng/mL

4. Reference

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