Supporting Information

Entropy-driven amplification strategy-assisted lateral flow assay biosensor for ultrasensitive and convenient detection of nucleic acids

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Contents

Figures:

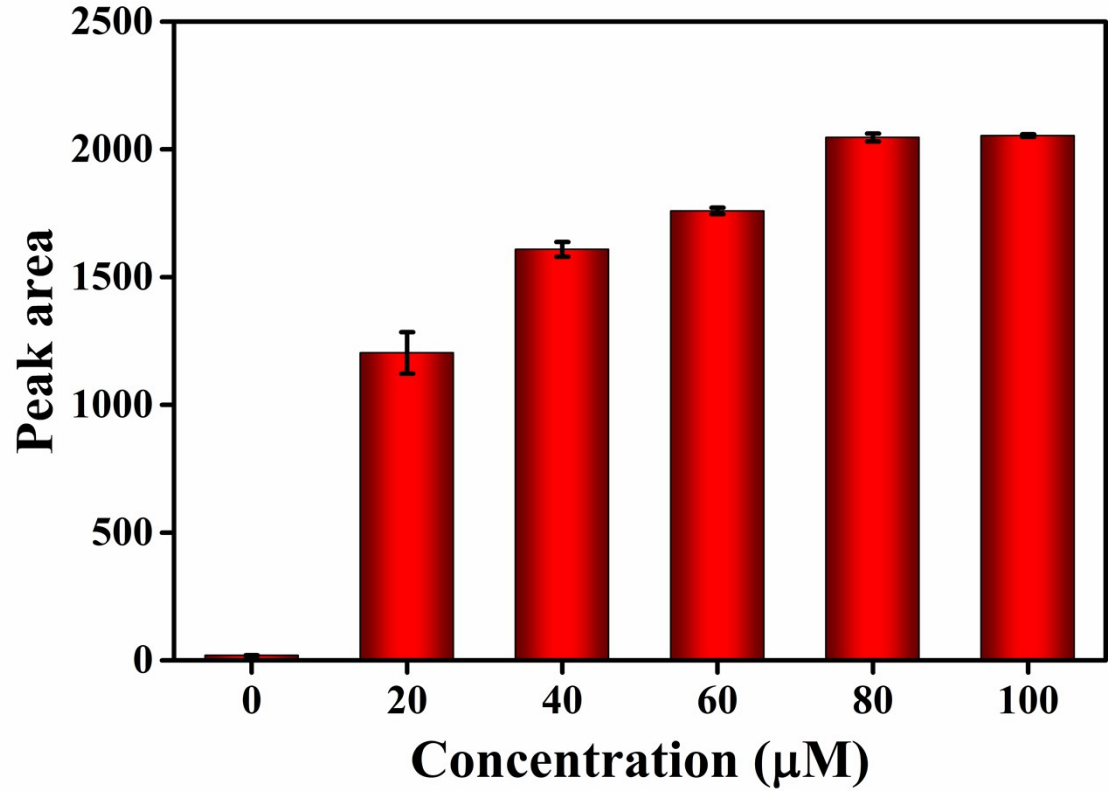

Figure S1. The effect of capture-DNA concentration on the response of biosensor for target detection.

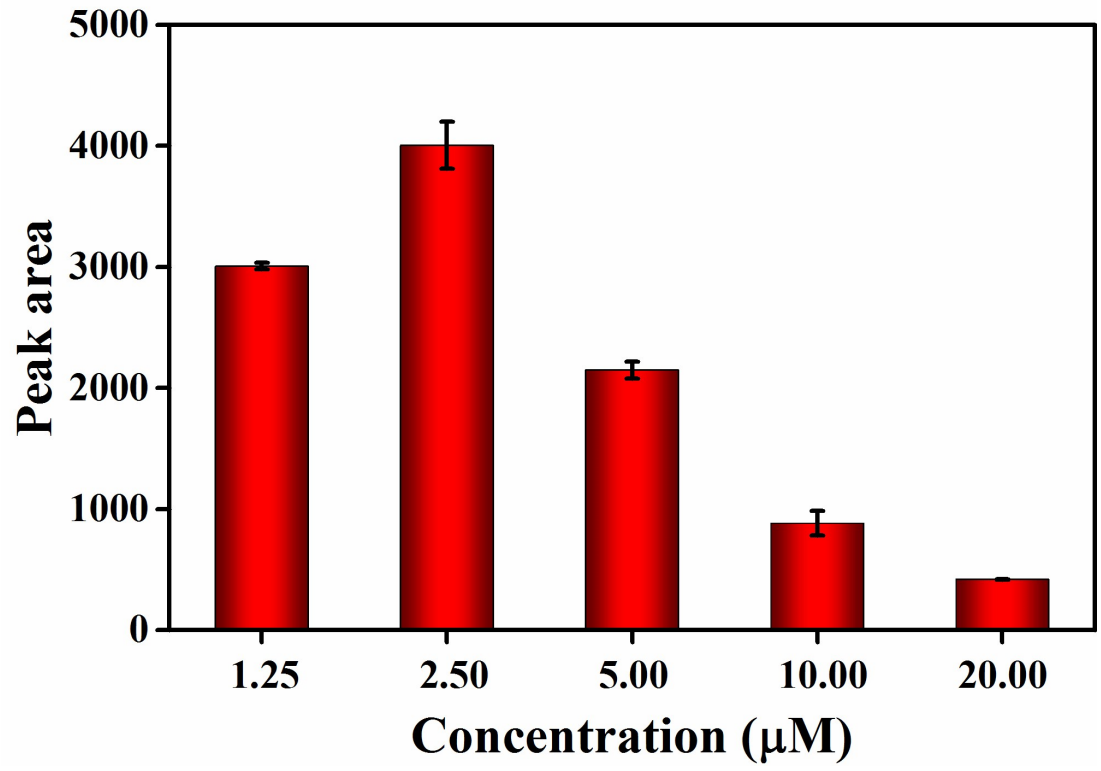

Figure S2. The effect of reporter-DNA concentration on the response of biosensor for target detection.

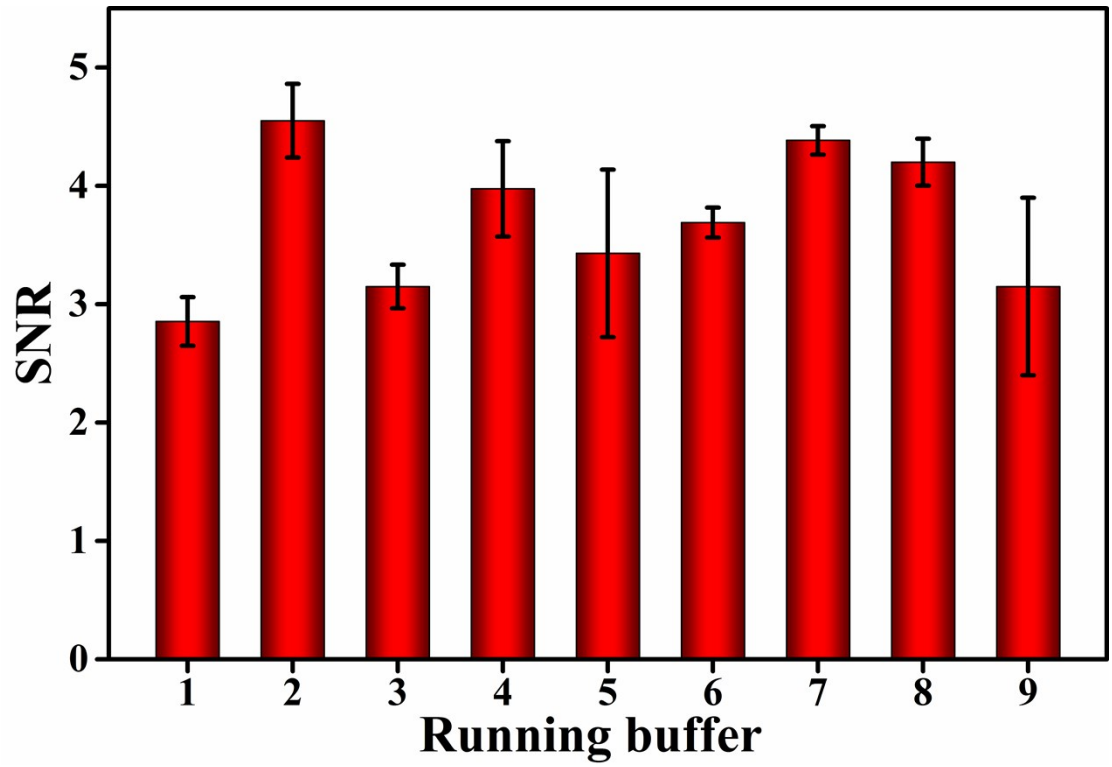

Figure S3. The effect of running buffer on the target detection: (1) 0.5% Tween, (2)1.0% Tween, (3) 4.0% Tween, (4) 1.0% Tween+0.5% BSA, (5)1.0% Tween+1.0% BSA, (6)1.0% Tween+4.0% BSA, (7) 1.0% Tween+0.5% sucrose, (8) 1.0% Tween+1.0% sucrose, and (9) 1.0% Tween+4.0% sucrose.

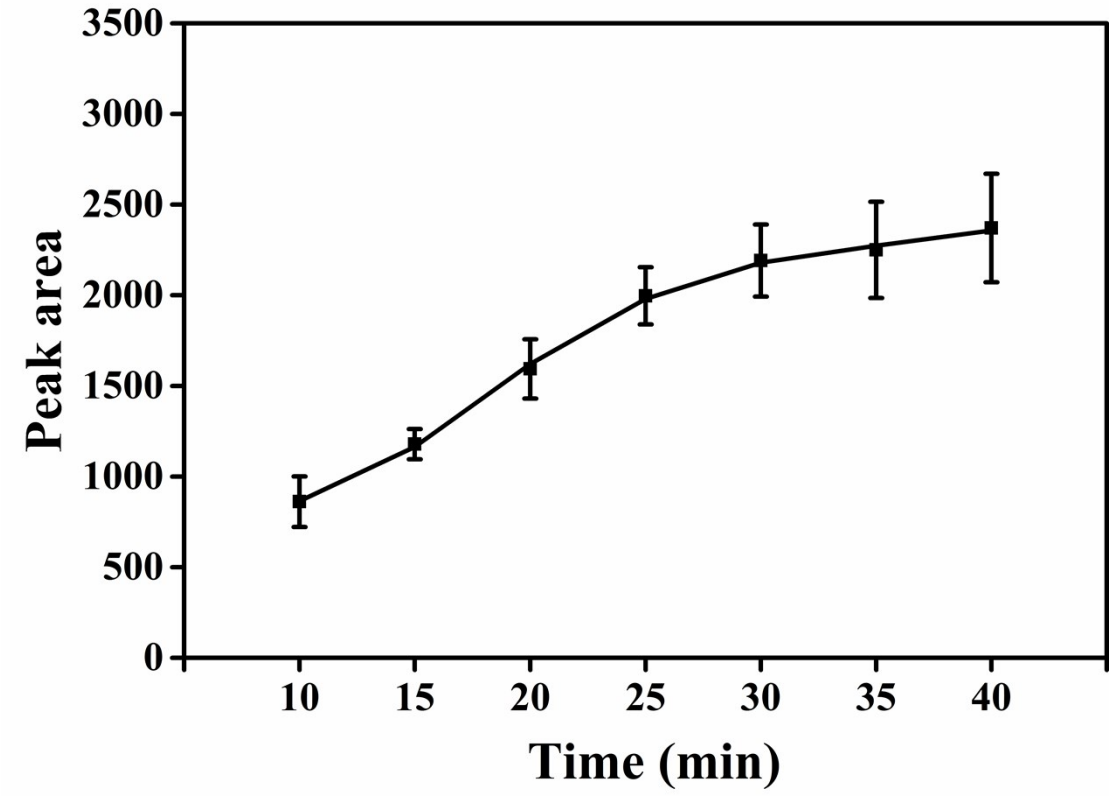

Figure S4. The effect of running time on the response of biosensor for target detection.

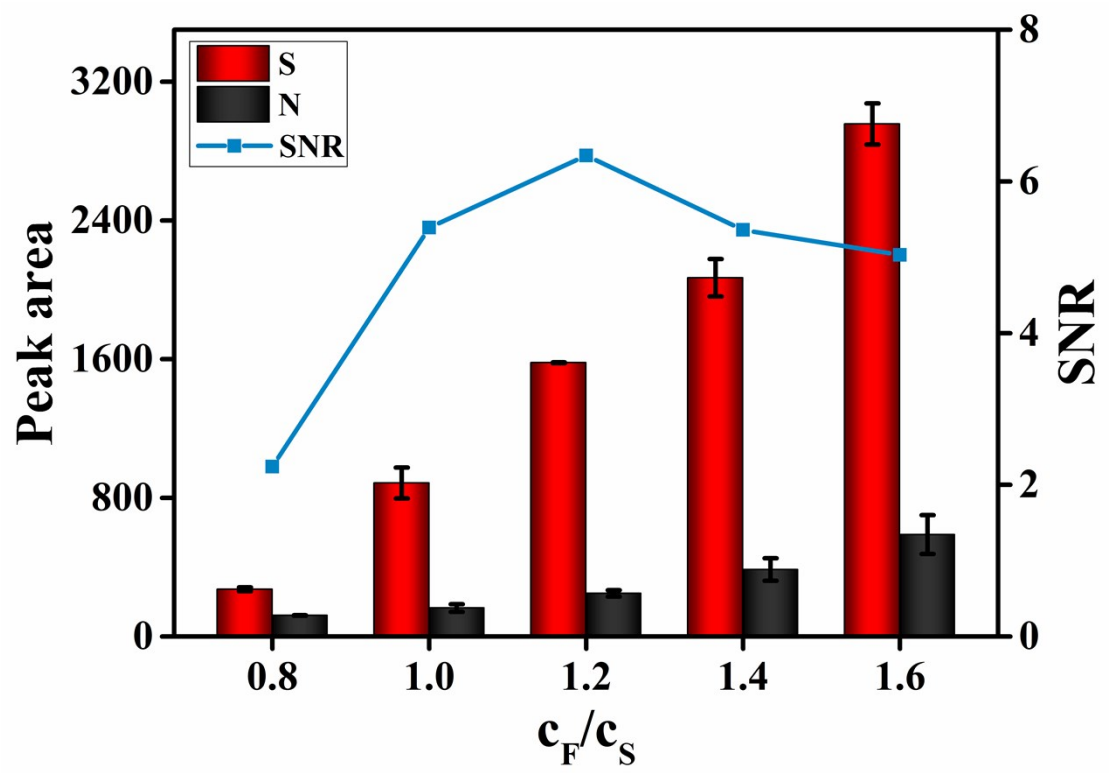

Figure S5. The effect of the concentration ratio of fuel and S on the performance of biosensor.

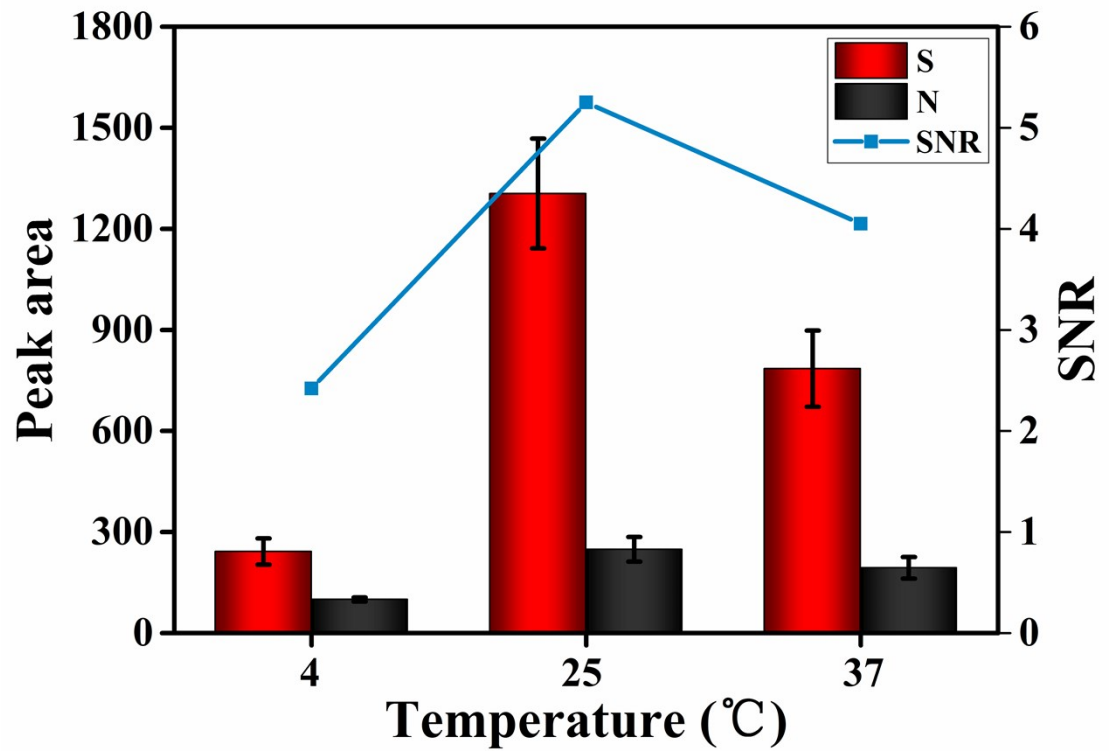

Figure S6. The effect of reaction temperature on the performance of biosensor.

Tables：

Table S1. Sequences used in the experiment.

Name	Sequence (5' to 3')
Report	Amino-CCTACGTCTCCAACTAACTTACGG
Linker	Biotin-TGGAGACGTAGGGTATTGAATGAGGGCCGTAAGTTAGTTGGAGACGTAGG
By product	TTTTTTCCCTCATTCAATACCCTACG
Fuel	CCTACGTCTCCAACTAACTTACGGCCCTCATTCAATACCCTACG
target	CATTCAATACCCTACGTCTCCA
Capture	Biotin-TTTTTTTTTTCCGTAAGTTAGT
m-T	CATTCATTACCCTACGTCTCCA
m-C	CATTCACTACCCTACGTCTCCA
m-G	CATTCAGTACCCTACGTCTCCA
i-T	CATTCATATACCCTACGTCTCCA
i-A	CATTCAAATACCCTACGTCTCCA
i-C	CATTCACATACCCTACGTCTCCA
i-G	CATTCAGATACCCTACGTCTCCA
d-A	CATTCATACCCTACGTCTCCA
Linker-H1N1	Biotin-TTGTGACTGGGTGTATATTCTGAGGGCCGTAAGTTAGTTGGAGACGTAGG
By product-H1N1	TTTTTTCCCTCAGAATATACACCCAG
Fuel-	CCTACGTCTCCAACTAACTTACGGCCCTCAGAATATACACCCA

H1N1	G
H1N1-DNA	CAGAATATACACCCAGTCACAA
H1N1-M1	CAGAATATACAGCCAGTCACAA
H1N1-M2	CAGAATCTACAGCCAGTCACAA
H1N1-M3	CAGAATCTACAGCCAGACACAA
H3N2	CTTCAAAATACGAAGTGGGAAA
H5N1	TTCAGATCATCCCCAAAAGTTC
H9N2	ATTCTTTCAGGAGAGAGCCACG
H7N9	TGTCACCTCTGACTAAGGGGAT

Table S2. Comparison of the proposed method with previous reported probes for nucleic acids detection.

Materials	**Amplification strategy**	**LOD**	**Linear range**	**Ref.**
AuNPs	/	60 pM	0.075-10 nM	1
AuNPs	/	68 pM	0.1-10 nM	2
AuNPs	/	1 nM	1-100 nM	3
AuNPs	aPCR	1 pg mL^{-1}	1 pg mL^{-1}-1 ng mL^{-1}	4
AuNPs	UP-APCR	0.1 pg mL^{-1}	0.1-100 pg mL^{-1}	5
AuNPs	NASBA	0.5 pg mL^{-1}	0.5-500 pg mL^{-1}	6
AuNPs	RCA	40 pM	0.02-200 nM	7
AuNPs	HCR	5 pM	/	8
AuNPs	HCR	6.8 pM	0.02-10 nM	9
Fluorescent nanospheres	EDA	1.43 pM (9.44 fg mL^{-1})	3-150 pM	**This work**

aPCR: asymmetric PCR

UP-APCR: universal primer-mediated asymmetric PCR

NASBA: nuclear acid sequence-based amplification

RCA: rolling circle amplification

HCR: hybridization chain reaction

EDA: Entropy-driven amplification

Table S3. The application of LFA biosensor in real human serum samples.

Samples	Spiked (pM)	Found (pM)	Recovery (%)	RSD (%)
1	10.00	9.58	95.80	2.3
2	30.00	30.70	102.3	2.7
3	100.0	98.31	98.31	3.7

Table S4. Comparison of the proposed detection method for H1N1 RNA with previous reported probes.

Detection Method	Amplification strategy	LOD	Linear range	Ref.
Impedimetric	/	577 pM	/	10
Fluorescence	/	0.13 mM	0.2-10 mM	11
Colorimetric	/	10 pM	10 pM-100 nM	12
Fluorescence polarization	/	3.45 nM	10 nM-100 nM	13

Current	PCR	20-30 fg mL^{-1}	/	14
Colorimetric	RT-PCR	14.1 pg mL^{-1}	/	15
Fluorescence	EDA	2.02 pM (13.4 fg mL^{-1})	3-150 pM	**This work**

PCR: polymerase chain reaction

RT-PCR: reverse transcription-polymerase chain reaction

Table S5. The application of LFA biosensor in real human serum samples.

Samples	**Spiked (pM)**	**Found (pM)**	**Recovery (%)**	**RSD (%)**
1	15.00	14.41	96.1	1.9
2	75.00	76.31	101	4.6
3	150.0	143.1	95.4	1.4

Reference

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