Supporting Information

A highly sensitive fluorescence biosensor for detection of *Staphylococcus aureus* based on HCR-mediated three-way DNA junction nicking enzyme assisted signal amplification

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Table S1 DNA sequences in this study

Name	sequence (5' to 3')
T	AAGGTGTAGAGAAATATG GTCCTG (denoted by b* - a*)
MB	FAM-CCAACG GATCATGG TACCTCAGCG TTGG-Dabcyl (denoted by /-f*-d*-/)
H1	CAGGAC CATATTTCTCTACACCTT GAT CGCTGAGGTA a GGAAGG AAGGTGTAGAGAAATATG A (denoted by a-b-c-d-e-b*)
H2	T AAGGTGTAGAGAAATATG GTCCTG CATATTTCTCTACACCTT CCTTCC CCATGATC (denoted by b*- a*-b-e*-f)
H1-1	CAGGAC CATATTTCTCTACACCTT AT CGCTGAGGTA a GGAAGG AAGGTGTAGAGAAATATG (denoted by a-b-c-d-e-b*)
H1-2	CAGGAC CATATTTCTCTACACCTT AT CGCTGAGGTA a GGAAGG AAGGTGTAGAGAAATATG A (denoted by a-b-c-d-e-b*)
H1-3	CAGGAC CATATTTCTCTACACCTT AT CGCTGAGGTA a GGAAGG AAGGTGTAGAGAAATATG TA (denoted by a-b-c-d-e-b*)
H1-4	CAGGAC CATATTTCTCTACACCTT T CGCTGAGGTA a GGAAGG AAGGTGTAGAGAAATATG A (denoted by a-b-c-d-e-b*)
H1-5	CAGGAC CATATTTCTCTACACCTT AGAT CGCTGAGGTA a GGAAGGAAGGTGTAGAGAAATATG A (denoted by a-b-c-d-e-b*)
H1-6	CAGGAC CATATTTCTCTACACCTT AAGAT CGCTGAGGTA a GGAAGGAAGGTGTAGAGAAATATG A (denoted by a-b-c-d-e-b*)
H2-1	AAGGTGTAGAGAAATATG GTCCTG CATATTTCTCTACACCTT CCTTCC CCATGATC (denoted by b*- a*-b-e*-f)
H2-2	TA AAGGTGTAGAGAAATATG GTCCTG CATATTTCTCTACACCTT CCTTCC CCATGATC (denoted by b*- a*-b-e*-f)
1-mismatched strand	AAGGTGTAGAGAAATATGCTCCTG
2-mismatched strand	AAGGTGTAGAGAAATATGCACCTG
3-mismatched strand	AAGGTGTAGAGAAATATCCACCTG
Noncomplementary strand	TGGCATTATCGATCAGTACCAGCC
Forward prime	GCGATTGATGGTGATACGGTT
Reverse prime	TACGCTAAGCCACGTCCATA
nuc amplicon (224 bp)	GCGATTGATGGTGATACGGTTAAATTAATGTACAAAGGTCAACC AATGACATTTAGACTATTATTGGTTGATACACCTGAAACAAAGC ATCCTAAAAAAAGGTGTAGAGAAATATGGTCCTGAAGCAAGTGCA TTTACGAAAAAAATGGTAGAAAATGCAAAGAAAATTGAAGTCG AGTTTGACAAAGGTCAAAGAACTGATAAATATGGACGTGGCTTA GCGTA

Note: The HCR probes were designed according to complementary base pairing through the simulation of NUPACK software (http://www.nupack.org/). The free end of HCR hairpins is marked in blue. The spacer of H1 is marked in red. The mismatched base is marked in green. The primers were designed using Primer-BLAST tool from the NCBI website online, and the specificity of the sequences was verified by the results of searches against NCBI's non-redundant database using the BLASTN algorithm (https://www.ncbi.nlm.nih.gov/pubmed).

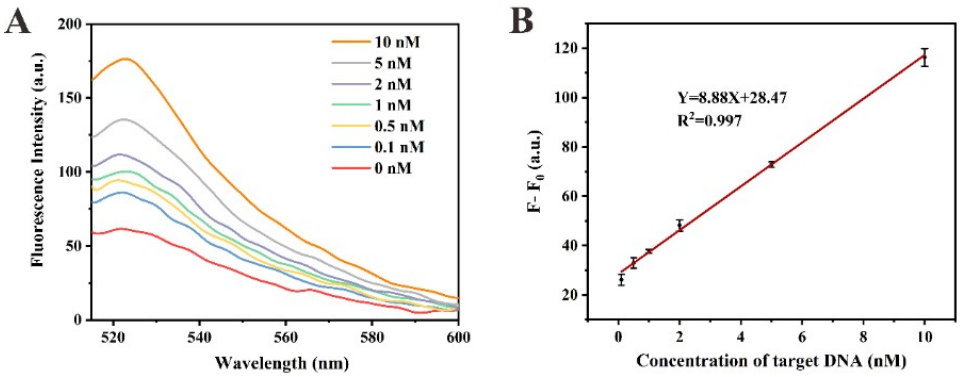

Fig. S1 Sensitivity of the pure HCR strategy for target DNA detection. (A)Fluorescent signal response with different concentrations of target DNA (B)Calibration curves of target DNA concentration with corresponding relative fluorescence intensities. Error bars: the standard deviations of triplicate analyses.

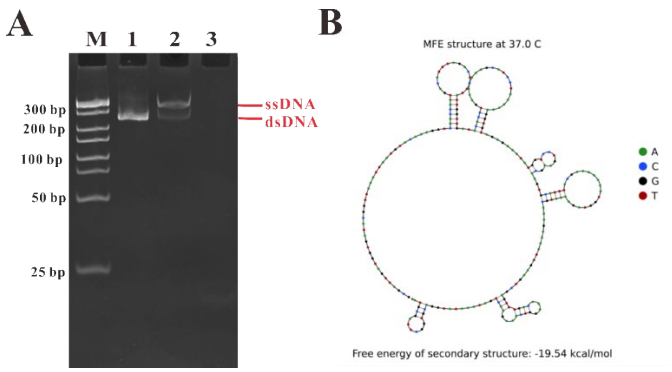

Fig. S2 Various experimental results of aPCR. (A) PAGE analysis. Lane M: DNA marker; Lane 1: traditional PCR of *S. aureus*; Lane 2: aPCR of *S. aureus*; Lane 3: negative control. (B) Simulation studies on the secondary structures of the ssDNA product from aPCR of *S. aureus.*

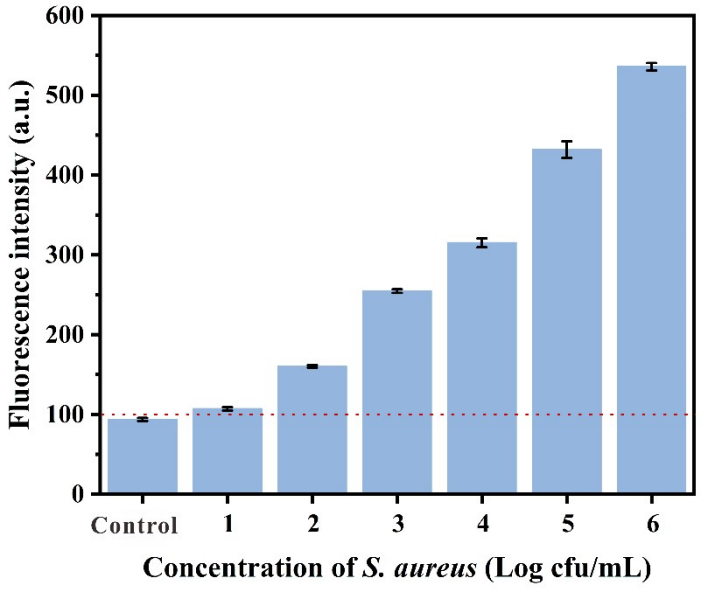

Fig. S3 Fluorescence response from the proposed strategy to different concentration of *S. aureus* in water (1.2×10^1 - 1.2×10^6 cfu/mL). The dotted lines represented cut-off value. Error bars: the standard deviations of triplicate analyses.

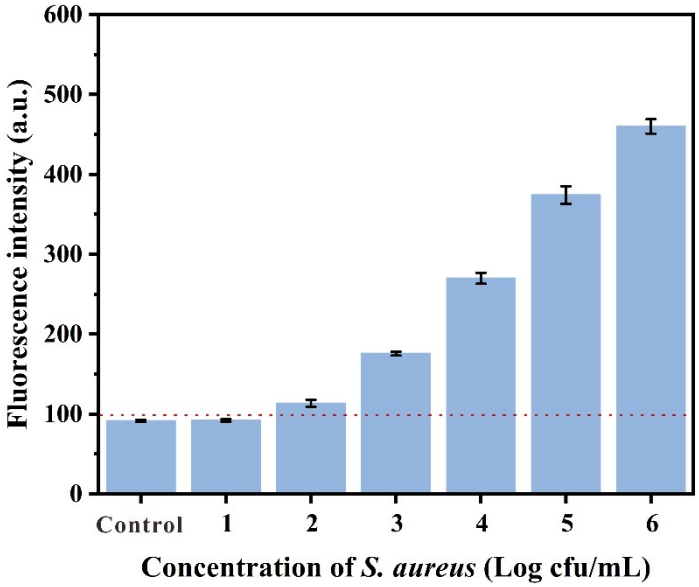

Fig. S4 Fluorescence response from the proposed strategy to different concentration of *S. aureus* in milk (1.3×10^1 - 1.3×10^6 cfu/mL). The dotted lines represented cut-off value. Error bars: the standard deviations of triplicate analyses.

Table S2 Comparison of the proposed assay and other 3WJ-NEASA or HCR-based biosensors for DNA detection

Method	Reaction time	Operation procedure	Linear range	LOD	Ref.
Fluorescence detection based on 3WJ-NEASA and triplex DNA	30 min	one-step	100 pM -200 nM	65 pM	[1]
Amperometric detection based on 3WJ-NEANA and flower-like carbon nanotubes-polyaniline nanohybrid	2.5 h	multi-step	1 fM -10 nM	0.33 fM	[2]
Fluorescence detection based on HCR and magnetic particles	2h	multi-step	0.01- 100 nM	10 pM	[3]
Fluorescence detection based on four-way branched migration HCR	1.5 h	one-step	0.2- 60 nM	67 pM	[4]
Chemiluminescence detection of DNAzyme and nonlinear HCR	1.5 h	multi-step	0.5- 10 pM	7 pM	[5]
Fluorescence detection based on silver nanoclusters/graphene oxide and HCR	4.5 h	multi-step	10-100 nM	1.18 nM	[6]
Fluorescence detection based on HCR-mediated 3WJ-NEASA	30 min	one-step	10 pM -10 nM	6.7 pM	This work

Table S3 Comparison of previously reported methods for *S. aureus* detection

Method	Materials	LOD (cfu/mL)	Ref.
Multiplex PCR	DNA	1.0×10^5 (milk)	7
Multiplex real-time PCR coupled with SDS and PMA	DNA	1.0×10^2 (milk)	8
Immunomagnetic separation via cell binding domain of lysin	antibody	4.0×10^3 (milk)	9
Competitive ELISA	antibody	1.0×10^4 (culture)	10
Conductometric biosensor based on magnetic analyte separation	aptamer	4.0×10^3 (tap water)	11
Microfluidic chip coupled with fluorescent silica nanoparticles label	aptamer	2.7×10^2 (water)	12
Fluorescence biosensor based on HCR-mediated 3WJ-NEASA	DNA	1.2×10^1 (water) 1.3×10^2 (milk)	This work

Note: SDS, sodium dodecyl sulphate; PMA, propidium monoazide; ELISA, enzyme linked immunosorbent assay.

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