Supplementary information

A CRISPR/Cas13a-based and hybridization chain reaction coupled evanescent wave biosensor for SARS-CoV-2 gene detection

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S1 The preparation of optical-fiber sensing probes

For the preparation of optical fiber probe used in homogeneous detection, the tip of the fiber is etched with hydrofluoric acid until the core diameter is reduced to 220 μm. The length of the etched region is 2.5 cm, and the total length of the optical fiber probe is 5.5 cm.

For the preparation of optical fiber probe used in heterogeneous detection, the etched optical fiber is immersed in piranha solution (H_2SO_4:H_2O_2=3:1) for 30 minutes so that it is hydroxylated. Then, the fiber is ultrasonically cleaned with ultrapure water until the solution becomes neutral. The thoroughly dried fiber is then placed in a 2% ATPES (v/v) toluene solution for 1 hour, followed by rinsing with toluene to introduce amino groups onto the fiber. The fiber is subsequently dried in an oven at 200°C for 1 hour[1].Subsequently, the aminated fiber was immersed in 4.0% (v/v) glutaraldehyde solution for 1 h, washed with ultrapure water, and dried with N_2. After these preparations, the fiber was immersed in 250 mL solution composed of 50 nM cDNA (NH_2-$(CH_2)_6$-GGGGG) in 50 mM Tris-HCl and 100 mM NaCl (pH 7.4) at 4 °C overnight to immobilize cDNA onto the fiber surface. The fiber surface was then dipped in 2 mg/mL BSA solution for 1 h to block the remaining aldehyde sites. The optical-fiber evanescent wave fluorescence biosensor and the prepared optical fiber are shown in Fig. S1. The process of cDNA modified on the optical fiber surface was shown in Fig. S2.

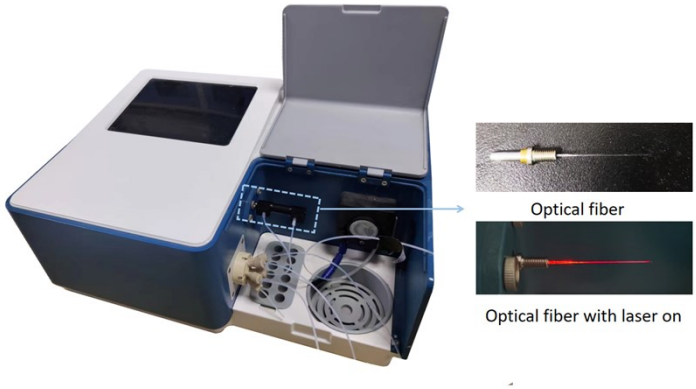
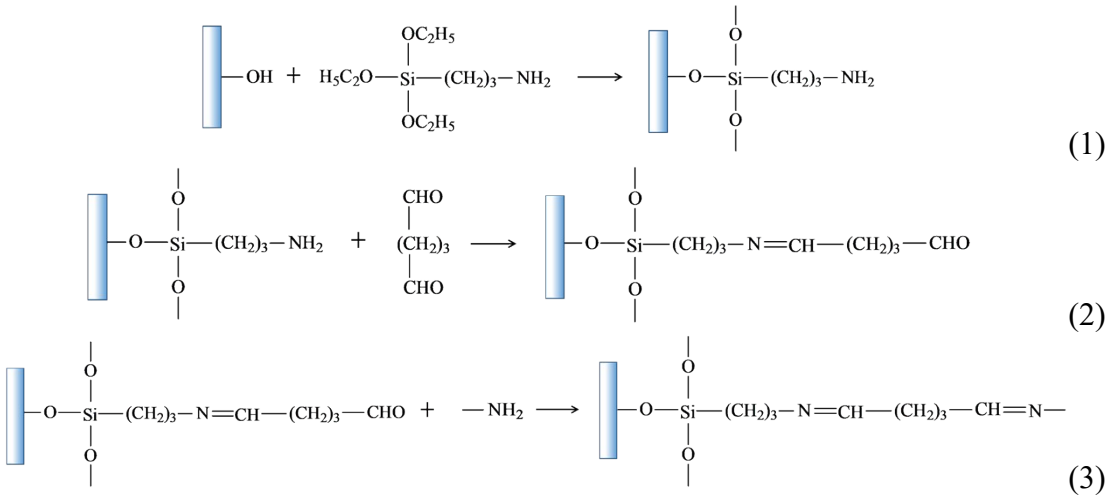


Fig. S1. Photograph of the optical-fiber evanescent wave fluorescence biosensor and the prepared optical fiber.



(1)

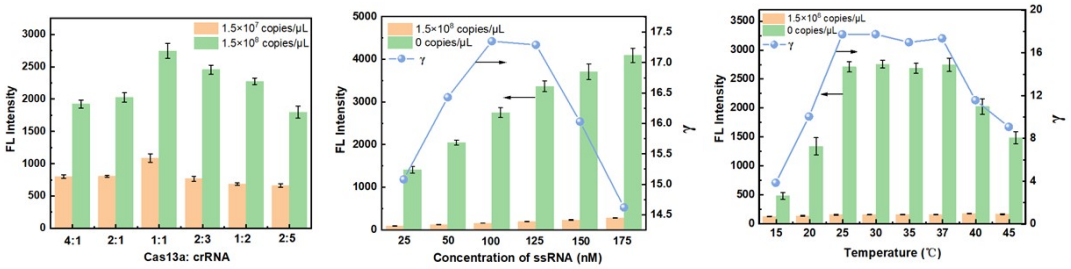
(2)

(3)

Fig. S2. Surface modification schematic of optical-fiber with cDNA.

S2 Investigation of optimal reaction conditions of CRISPR/Cas13a

The results in Fig. S3(a) indicate that when the ratio of Cas13a to crRNA is 1:1, the maximum collateral cleavage activity is achieved, resulting from the maximal formation of RNP at this point. The increase in ssRNA concentration is accompanied by an increase in fluorescence intensity, as shown in Fig. S3(b). Defining γ as the signal ratio between the presence and absence of targets, its maximum value of 17.35 occurs at an ssRNA concentration of 100 nM. The influence of reaction temperature on CRISPR/Cas13a is shown in Fig. S3(c). It can be seen that the reaction performs well within the temperature range of 25°C to 37°C.



(a) (b) (c)

Fig. S3. Investigation of optimal ratio for Cas13a, crRNA, and ssRNA. (a) CRISPR/Cas13a system with 50 nM Cas13a, the varying amount of crRNA1, 100 nM ssRNA reporter, 10 μL pseudovirus RNA extract, total volume of 20 μL, incubated for 60 min at 37 ℃ and (b) with 50 nM Cas13a, 50 nM crRNA1, varying the amount of ssRNA, 10 μL pseudovirus RNA extract, total volume of 20 μL, incubated for 60 min at 37 ℃. (c) CRISPR/Cas13a reaction at various temperatures, with 50 nM Cas13a, 50 nM crRNA1, 10 μL pseudovirus RNA extract, 100 nM ssRNA, total volume of 20 μL incubated for 60 min.

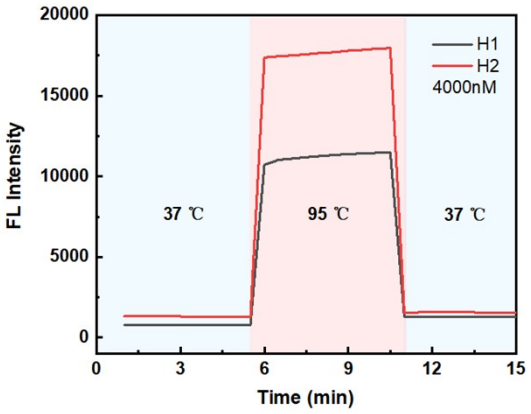


Fig. S4. The fluorescence intensity of hairpins H1 and H2 at different temperatures.

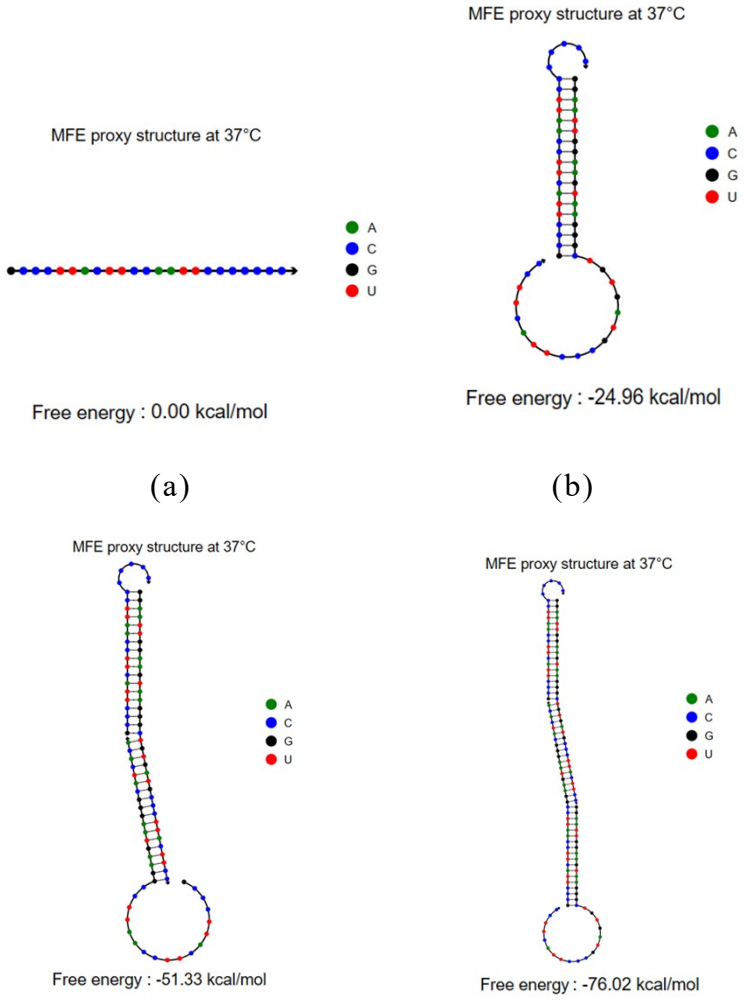


Fig. S5. The HCR reaction triggered by I_0. (a) The sequence of RNA initiator I_1. (b) The structure of I_1+H1. (c) The structure of I_1+H1+H2. (d) The structure of I_1+H1+H2+H1.

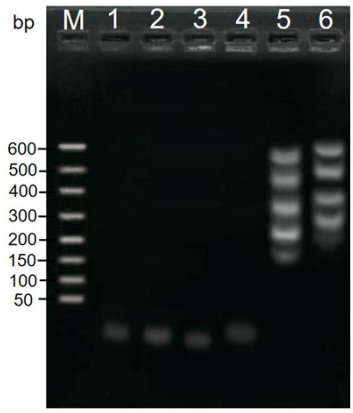
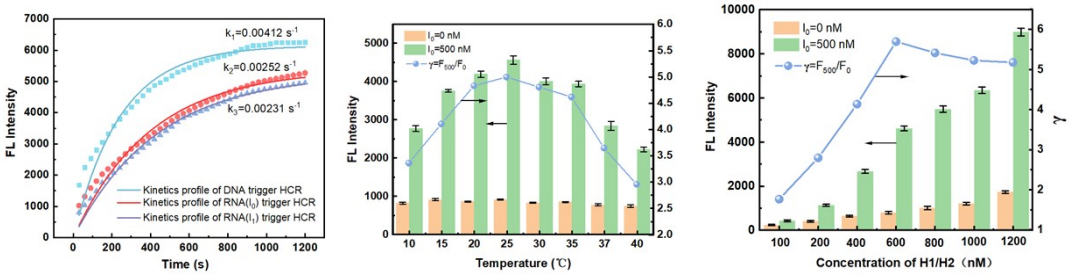


Fig. S6. The agarose gel electrophoresis result of the I_1 triggered HCR. Lane M: markers; lane 1: the RNA initiator I_1 (2000 nM); lane 2: The

hairpin H1 (600 nM); lane 3: The hairpin H2 (600 nM); lane 4: H1 (600 nM)+ H2 (600 nM); lane 5: I_1 (50 nM) + H1 (600 nM) +H2 (600 nM); lane 6: I_1 (10 nM) + H1 (600 nM) +H2 (600 nM)

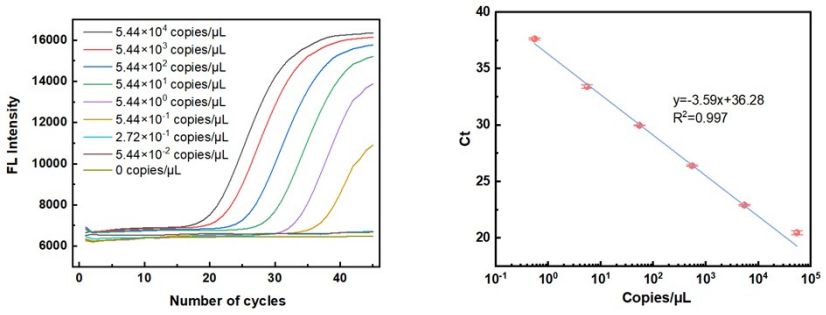


(a) (b) (c)

Fig. S7. Exploration of reaction conditions for the HCR reaction. (a) Kinetic fitting of the first-order HCR reaction with DNA, RNA (I_0 or I_1) as the initiator, with initiator at 500 nM, H1 and H2 at 400 nM, reaction at 37°C. (b) The influence of temperature on the HCR reaction, with H1 and H2 at 400 nM. (c) The influence of the concentrations of H1 and H2 on the HCR reaction, reaction at 37°C.

S3 Detection of SARS-CoV-2 gene using qPCR

To begin the experiment, carefully combine the extracted pseudovirus (10 μL), RT-PCR reaction mixture (12.5 μL), enzyme mixture (1.3 μL), and primer-probe mixture (1.2 μL) in a PCR tube. Gently mix the components to ensure thorough blending. Once the mixture is prepared, transfer it to the qPCR machine. Set the amplification program as follows: initiate with a reverse transcription step at 50 °C for 5 min, followed by a pre-denaturation step at 95 °C for 1 min. Then, proceed with 45 cycles, each consisting of a denaturation phase at 95 °C for 5 s and an annealing and extension phase (with fluorescence detection) at 60 °C for 10 s. The real-time fluorescence curves obtained from qPCR for targets of varying concentrations are shown in Fig S8(a). The fitting curve between gene concentrations of different levels and Ct values is depicted in Fig S8(b). It is evident that there is a linear relationship between the logarithm of gene concentration and Ct values, with an R^2 of 0.997.



(a) (b)

Fig. S8. (a) The PCR amplification curve and (b) the corresponding standard curve

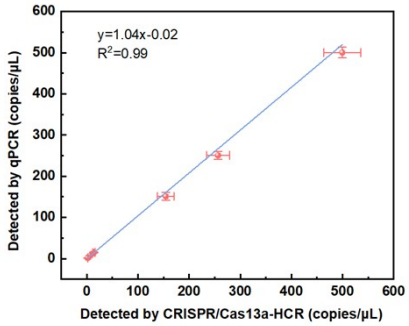


Fig. S9. Correlation analysis of detection concentrations between heterogeneous CRISPR/Cas13a-HCR and qPCR. The detection concentrations were 1.5, 7.5, 15, 150, 200, and 500 copies/μL, respectively.

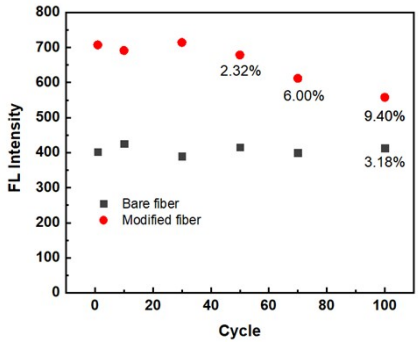


Fig. S10. The regeneration cycles for bare fiber and modified fiber

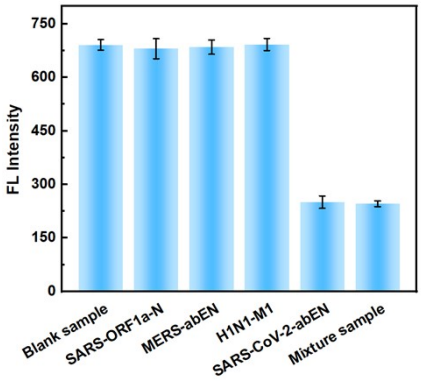


Fig. S11. Specificity recognition capability verification of the detection, the spiked concentrations were all 150 copies/μL

Table S1 The sequences used in this work

Name	Sequence (5'-3')
I_0	GCCCUUACUUCCAAUUCC
I_1	GCCCUUACUUCCAAUUCCCCCCC
H1	GGAATTGGAAG (FAM) TAAGGGCTGTGATGCCCTTA-BHQ1-CTTCC
H2	GCCCT (FAM) TACTTCCAATTCCGGAAGTA-BHQ1-AGGGCATCACA
H1 (evanescent wave detection)	GGAATTGGAAGT (Cy3) AAGGGCTGTGATGCCCTTA-BHQ2-CTTCC
H2 (evanescent wave detection)	GCCCT (Cy3) TACTTCCAATTCCGGAAGTA-BHQ2-AGGGCATCACA
cDNA	NH_2-$(CH_2)_6$-GGGGG
crRNA1	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCGACAUUCCGAAGAACGCUGAAGCGCUG
crRNA2	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUUUGCGGCCAAUGUUUGUAAUCAGUUCC
SARS-CoV-2 gene N	AAUGUCUGAUAAUGGACCCCAAAAUCAGCGAAAUGCACCCCGCAUUACGUUUGGUGGACCCUCAGAUUCAACUGGCAGUAACCAGAAUGGAGAACGCAGUGGGGCGCGAUCAAAACAACGUCGGCCCCAAGGUUUACCCAAUAAUACUGCGUCUUGGUUCACCGCUCUCACUCAACAUGGCAAGGAAGACCUUAAAUUCCCUCGAGGACAAGGCGUUCCAAUUAACACCAAUAGCAGUCCAGAUGACCAAAUUGGCUACUACCGAAGAGCUACCAGACGAAUUCGUGGUGGUGACGGUAAAAUGAAAGAUCUCAGUCCAAGAUGGUAUUUCUACUACCUAGGAACUGGGCCAGAAGCUGGACUUCCCUAUGGUGCUAACAAAGACGGCAUCAUAUGGGUUGCAACUGAGGGAGCCUUGAAUACACCAAAAGAUCACAUUGGCACCCGCAAUCCUGCUAACAAUGCUGCAAUCGUGCUACAACUUCCUCAAGGAACAACAUUGCCAAAAGGCUUCUACGCAGAAGGGAGCAGAGGCGGCAGUCAAGCCUCUUCUCGUUCCUCAUCACGUAGUCGCAACAGUUCAAGAAAUUCAACUCCAGGCAGCAGUAGGGGAACUUCUCCUGCUAGAAUGGCUGGCAAUGGCGGUGAUG

	CUGCUCUUGCUUUGCUGCUGCUUGACAGAUUGAACCAGCUUGA GAGCAAAAUGUCUGGUAAAGGCCAACAACAACAAGGCCAAACU GUCACUAAGAAAUCUGCUGCUGAGGCUUCUAAGAAGCCUCGGC AAAAACGUACUGCCACUAAAGCAUACAAUGUAACACAAGCUUU CGGCAGACGUGGUCCAGAACAAACCCAAGGAAAUUUUGGGGAC CAGGAACUAAUCAGACAAGGAACUGAUUACAAACAUUGGCCGC AAAUUGCACAAUUUGCCCCCAGCGCUUCAGCGUUCUUCGGAAUG UCGCGCAUUGGCAUGGAAGUCACACCUUCGGGAACGUGGUUGA CCUACACAGGUGCCAUCAAAUUGGAUGACAAAGAUCCAAAUUU CAAAGAUCAAGUCAUUUUGCUGAAUAAGCAUAUUGACGCAUAC AAAACAUUCCCACCAACAGAGCCUAAAAAGGACAAAAAGAAGA AGGCUGAUGAAACUCAAGCCUUACCGCAGAGACAGAAGAAACA GCAAACUGUGACUCUUCUUCCUGCUGCAGAUUUGGAUGAUUUC UCCAAACAAUUGCAACAAUCCAUGAGCAGUGCUGACUCAACUCA GGCCUAA

Table S2 Comparison of sensing performance of amplification-free CRISPR-based SARS-CoV-2 detections

Method	LOD	Time	Reference
CRISPR-HCR	10 aM (6 copies/μL)	60 min	[2]
CRISPR/Cas13a-CHA	18.6 copies/μL	50 min	[3]
CRISPR-Cas-Driven Single Micromotor	5.2 aM (3 copies/μL)	60 min	[4]
CRISPR-Cas13a mobile phone microscopy	0.16 fM (96 copies/μL)	30 min	[5]
CRISPR-Cas13a gFETs	1 aM (0.6 copies/μL)	30 min	[6]
Ultralocalized Cas13a Assay	6 copies/μL	63 min	[7]
SERS-based CRISPR/Cas12a platform	1 fM (602 copies/μL)	40 min	[8]
Cellphone-Based	50 copies/μL	71 min	[9]

CRISPR/Cas			
CRISPR-HCR	0.47 copies/μL	33 min	This work

Table S3 Recovery of SARS-CoV-2 pseudovirus spiked saliva samples by CRISPR/Cas13a-HCR biosensor

Samples	**Spiked (copies/μL)**	**Measured (copies/μL)**	**Recovery (%)**	**RSD (%, n=3)**
Saliva 1	1.5	1.5	100.00	10.41
	15	14	93.33	14.29
	150	161	107.33	8.63
Saliva 2	1.5	1.7	108.44	19.29
	15	17	113.33	12.01
	150	178	118.67	9.75
Saliva 3	1.5	1.3	86.67	16.43
	15	16	106.67	9.35
	150	177	118.00	17.54

References

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