Supporting Information

CRISPR-empowered hybridization chain reaction amplification for attomolar electrochemical sensor

Ling Wan[†, 1], Jianfeng Ma[†, 1], Jiasheng Yi[1], Yan Dong[1], Renjie Niu[1], Yan Su[2], Qian Li[3], Dan Zhu[1], Jie Chao[1], Shao Su*[, 1], Chunhai Fan[3], Lianhui Wang[1], Ying Wan*[, 2]

[1]State Key Laboratory of Organic Electronics and Information Displays & Jiangsu Key Laboratory for Biosensors, Institute of Advanced Materials (IAM), Nanjing University of Posts and Telecommunications, 9 Wenyuan Road, Nanjing 210023, China.

[2]School of Mechanical Engineering, Nanjing University of Science and Technology, Nanjing 210094, China.

[3]School of Chemistry and Chemical Engineering, Frontiers Science Center for Transformative Molecules and National Center for Translational Medicine, Shanghai Jiao Tong University, Shanghai 200240, China.

[†]These authors contributed equally to this work.

E-mail: iamssu@njupt.edu.cn, wanying@njust.edu.cn

Experimental section

Reagents and materials. SPCE were purchased from Ningbo Yuangan Biotechnology Co., Ltd. Chloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$, ≥99%), polyacrylic acid (PAA), polyethyleneimine (PEI), ethylenediaminetetraacetic acid (EDTA), boric acid and mercaptohexanol (MCH) were purchased from Sigma-Aldrich (USA). The TMB-H_2O_2 mixed solution was purchased from Shanghai Niujin Biotechnology Co., Ltd. Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$, ≥99.0%), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$, ≥99.0%), magnesium chloride ($MgCl_2$), sodium chloride (NaCl) and trishydroxymethyl aminomethane (Tris) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The DNA and RNA sequences required in the experiment were synthesized by Takara Biotechnology Co., Ltd. (Shanghai, China), which was listed in Table S1. LwaCas13a was purchased from Guangzhou Bio-Lifesci Co., Ltd., which was recombinantly expressed in E. coli. H7N9-crRNA-LwaCas13a ternary complex consisted of 50 nM LwaCas13a, 50 nM crRNA, and varying amounts of target H7N9 in reaction buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3).

Apparatus. The morphology of SPCE-HFGNs was characterized by scanning electron microscope (SEM). Cyclic voltammetry (CV), chronoamperometry (i-t) and differential pulse voltammetry (DPV) were performed on the CHI660D electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd.). The working electrode (WE), reference electrode (RE) and auxiliary electrode (CE) of a typical SPCE was carbon, Ag/AgCl and carbon, respectively. CV and i-t were performed at room temperature in TMB-H_2O_2 solution. CV measurement was performed at a potential window ranging from -0.2 to 0.6 V with a scan rate of 100 mV s^{-1}. I-t measurement was performed at a fixed potential of -50 mV and recorded within 100 s. DPV was performed with the potential window ranging from -0.45 to 0.1 V in 1× PBS buffer.

Assemble of HRP on HP2 and HP3. HP2 and HP3 were purchased from Takara Biotechnology Co., Ltd. (Beijing, China). HRP enzymes labelled with hairpin DNA strands (HP2 and HP3) were prepared according to a protocol described previously[1-3]. Briefly, 20 μM HRP enzyme solution was added into the solution of 50-fold excess bis (sulfosuccinimidyl) suberate (BS^3) and reacted for 2 h in phosphate buffer solution (10 mM, pH=7.4), allowing one n-hydroxysuccinimide (NHS) ester group of the BS^3 to react with the lysine residues on the enzyme surface. In the meantime, the amino-modified hairpin DNA strands were annealed from 95°C to room temperature in two hours

to form hairpin structure. Subsequently, the BS^3 modified enzyme solution was mixed with 10-fold excess amino-modified hairpin DNA strands. In this regard, the other NHS ester groups of the BS^3 will react with the amino of hairpin DNA strands. After purification, HPR-labelled HP2 and HPR-labelled HP3 were obtained.

Preparation of SPCE-HFGNs. SPCE-HFGNs were prepared according to our previous work.[4] Briefly, the SPCE was washed with ethanol and ultrapure water three times, respectively. After drying, the SPCE was alternately immersed 6 times into 20% PAA and 20% PEI solution to form a polyelectrolyte multilayer and to obtain shape-controlled gold nanostructures. Using $HAuCl_4$ (0.4 mM) as the precursor solution, well-defined hierarchical flower-like gold nanostructures-decorated SPCE was obtained at -0.3 V for 30 min.

Construction of electrochemical biosensors. HP1 contained LwaCas13a cleavage site (AUAUAU) was assembled on SPCE-HFGNs surface via Au-S bond, named as HP1/SPCE-HFGNs.[5] Subsequently, MCH was used to reduce non-specific adsorption of HP1/SPCE-HFGNs. After the addition of H7N9, the specific binding of crRNA, H7N9, and LwaCas13a increased the cleavage activity of LwaCas13a,[6, 7] changing the structure of HP1 from hairpin to double-strand. As a result, the cleaved probe DNA exposed a special sequence, which initiated an HCR reaction in the presence of HPR-labelled HP2 and HPR-labelled HP3. Expectedly, ultralong dsDNA structures loaded with large amounts of HRP were formed on the surface of SPCE-HFGNs to efficiently amplify the electrochemical signal in the TMB-H_2O_2 mixture solution.

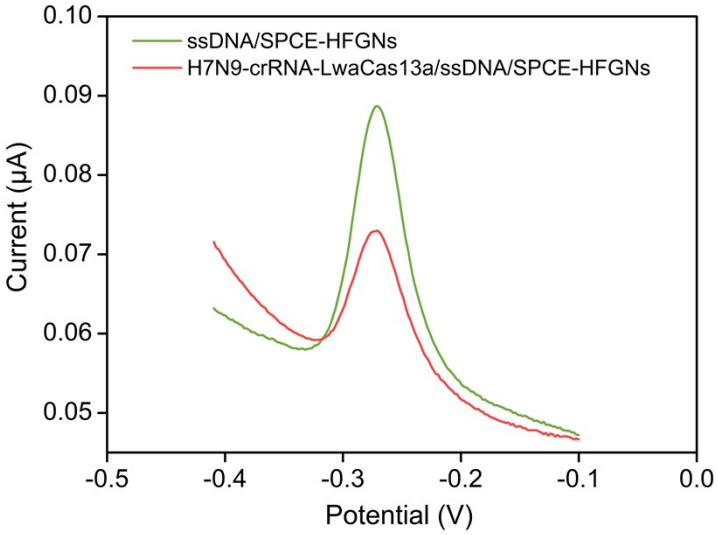

Figure S1. The DPV curves of sensing platform with or without the incubation of H7N9-crRNA-LwaCas13a ternary complex.

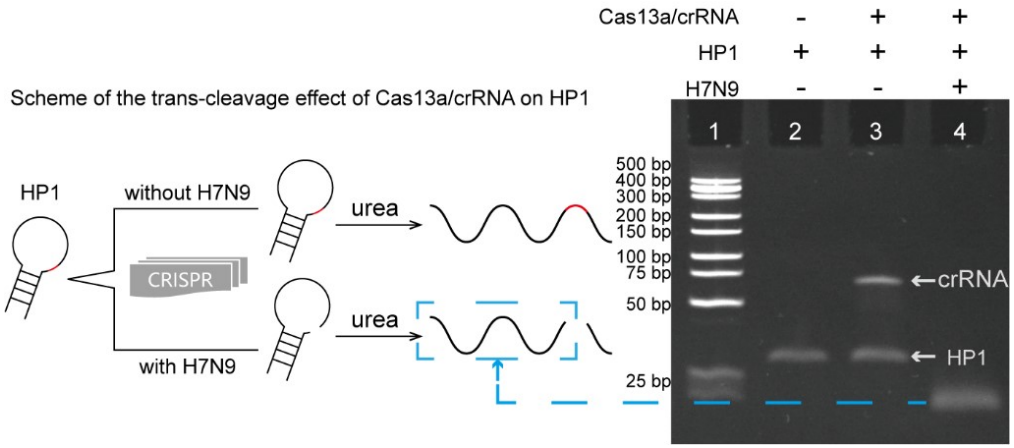

Figure S2. Schematic illustration and gel image of the cleavage effect of Cas13a on HP1.

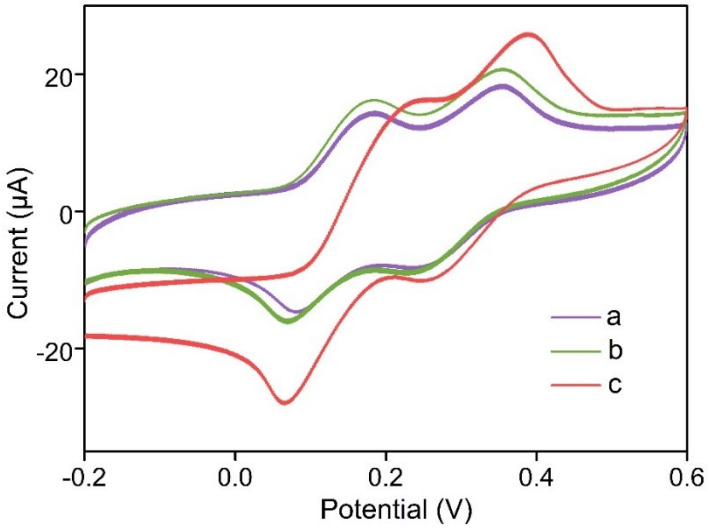

Figure S3. CVs of (a) SPCE-HFGNs, (b) HP3/HP2/crRNA-LwaCas13a/HP1/SPCE-HFGNs and (c) HP3/HP2/H7N9-crRNA-LwaCas13a/HP1/SPCE-HFGNs.

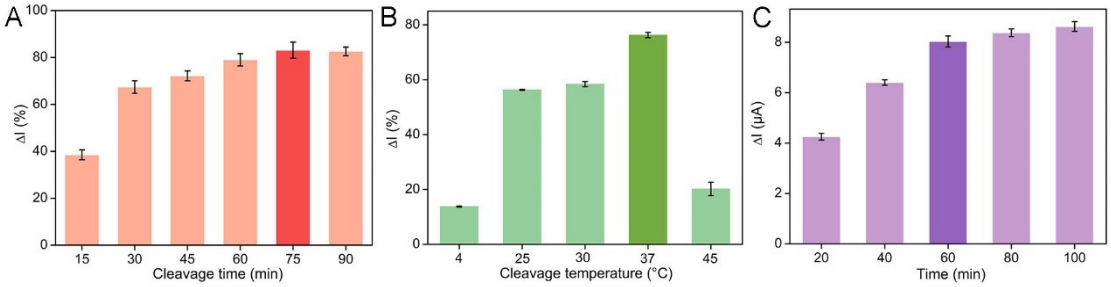

Figure S4. The effects of (A) cleavage time, (B) cleavage temperature, and (C) HCR reaction time on electrochemical responses for 1 fM H7N9 detection.

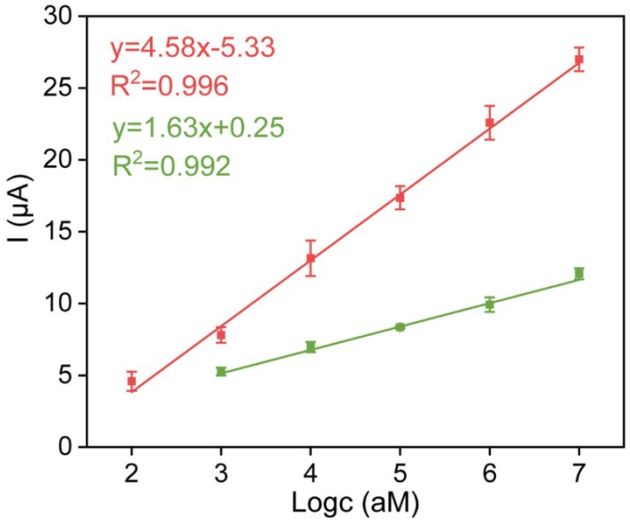

Figure S5. Comparison of detection performance with (red curve) and without HCR (green curve).

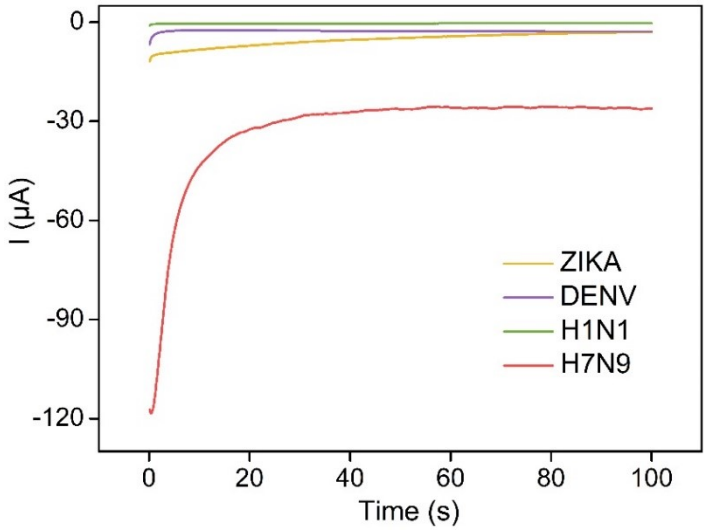

Figure S6. Chronoamperometry curves of this sensor for ZIKA, DENV, H1N1 and H7N9 detection at the same concentration (10 pM).

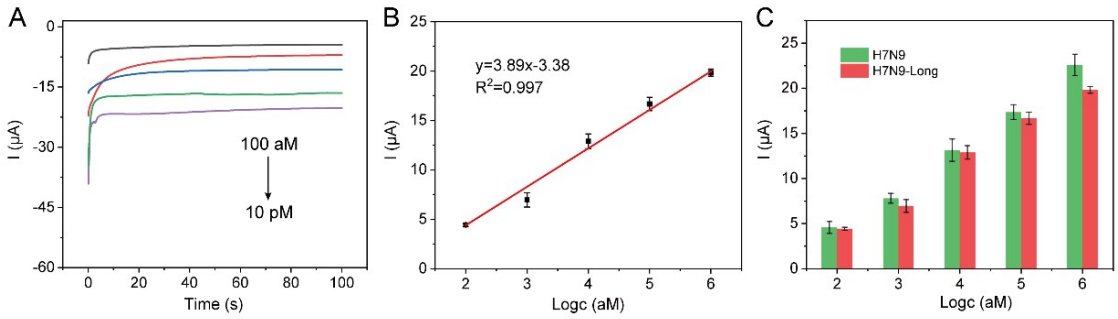

Figure S7. (A) i-t curves of this sensor for different concentration of H7N9-Long detection in the range of 100 aM-10 pM. (B) The linear range of this sensor for H7N9-Long detection. (C) Comparison of the performance of this sensor for H7N9 and H7N9-Long detection.

Table S1. DNA and RNA sequences required in the experiment (5' to 3' ends).

Name	Sequence
HP1	SH-TTTTTAATTCAGCACAATCTAT*AUAUAU*GCTG
crRNA	*GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGAUUGAC CCAGUCAAACUAAGCAGCGGC*
H7N9	*GCCGCUGCUUAGUUUGACUGGGUCAAUCU*
H7N9-Long	*CAACCUCCAGCCGCUGCUUAGUUUGACUGGGUCAAUCUAUG GUCGUGAG*
ssDNA	SH-TTTTTT*AUAUAU*-MB
HP2	HRP-ACAATCTATTCTGAAACATAGATTGTGCTGAATT
HP3	GTTTCAGAATAGATTGTAATGTCTCACAATCTAT-HRP
ZIKA	*AGCAUAUUGACGUGGGAAAGAC*
DENV	*UGGUGCUGUUGAAUCAACAGGUUCU*
H1N1	*GGAAAGAAAUGCUGGAUCUGGUA*

Table S2. Comparison of the analytical performance of other biosensors.

Amplification strategy	**Linear range**	**Detection limit**	**References**
LRET	10 pM-10 nM	7 pM	8
DNA tetrahedral	120 fM-1.2 nM	100 fM	9
EXPAR and HCR	5 pM-100 pM	9.4 fM	10
optical trapping and bead-based fluorescence	10 pM-1 nM	1 pM	11
HCR and DNA triplex assembly	0.2 nM-100 nM	0.14 nM	12
exonuclease III-assisted cycling amplification	1 fM-100 pM	31 aM	13
CHA and imaging array	0.5 nM-25 nM	141 pM	14
modular DNA circuits	0.1 pM-100 nM	24 pM	15
MoS_2-Au@Pt and CHA	10 pM-50 nM	2.8 pM	16
CRISPR, HCR and enzyme	100 aM-10 pM	58 aM	This work

catalytic reaction

Abbreviations：

[LRET] luminescence resonance energy transfer, [EXPAR] isothermal exponential amplification,

[HCR] hybridization chain reaction, [CHA] catalytic hairpin assembly.

References:

1 J. Fu, M. Liu, Y. Liu, N. W. Woodbury and H. Yan, *J. Am. Chem. Soc.*, 2012, **134**, 5516-5519.

2 S. Lu, T. Hu, S. Wang, J. Sun and X. Yang, *ACS Appl. Mater. Interfaces*, 2017, **9**, 167-175.

3 Y. Chen, G. Ke, Y. Ma, Z. Zhu, M. Liu, Y. Liu, H. Yan and C. J. Yang, *J. Am. Chem. Soc.*, 2018, **140**, 8990-8996.

4 S. Su, Y. Wu, D. Zhu, J. Chao, X. Liu, Y. Wan, Y. Su, X. Zuo, C. Fan and L. Wang, *Small*, 2016, **12**, 3794-3801.

5 Patrick D. Hsu, Eric S. Lander and F. Zhang, *Cell*, 2014, **157**, 1262-1278.

6 L. Cong, F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, X. Wu, W. Jiang, L. A. Marraffini and F. Zhang, *Science*, 2013, **339**, 819-823.

7 O. O. Abudayyeh, J. S. Gootenberg, P. Essletzbichler, S. Han, J. Joung, J. J. Belanto, V. Verdine, D. B. T. Cox, M. J. Kellner, A. Regev, E. S. Lander, D. F. Voytas, A. Y. Ting and F. Zhang, *Nature*, 2017, **550**, 280-284.

8 W. W. Ye, M.-K. Tsang, X. Liu, M. Yang and J. Hao, *Small*, 2014, **10**, 2390-2397.

9 S. Dong, R. Zhao, J. Zhu, X. Lu, Y. Li, S. Qiu, L. Jia, X. Jiao, S. Song, C. Fan, R. Hao and H. Song, *ACS Appl. Mater. Interfaces*, 2015, **7**, 8834-8842.

10 Y. Yu, Z. Chen, W. Jian, D. Sun, B. Zhang, X. Li and M. Yao, *Biosens. Bioelectron.*, 2015, **64**, 566-571.

11 D. Cao, C.-Y. Li, Y.-F. Kang, Y. Lin, R. Cui, D.-W. Pang and H.-W. Tang, *Biosens. Bioelectron.*, 2016, **86**, 1031-1037.

12 L. Zou, T. Li, R. Shen, S. Ren and L. Ling, *Talanta*, 2018, **189**, 137-142.

13 C. Song, Y. Liu, X. Jiang, J. Zhang, C. Dong, J. Li and L. Wang, *Talanta*, 2019, **205**, 120137.

14 D. Jiang, Y. Tian, Y. Zhang, X. Lu, D. Xiao and C. Zhou, *Anal. Chim. Acta*, 2021, **1171**, 338645.

15 D. Zhu, Z. Ma, Z. Wang, Q. Wei, X. Li, J. Wang, S. Su, X. Zuo, C. Fan, J. Chao and L. Wang, *Anal. Chem.*, 2021, **93**, 13861-13869.

16 Y. Dong, L. Wan, S. Lv, D. Zhu, S. Su, J. Chao and L. Wang, *Langmuir*, 2022, **38**, 1791-1796.