Oxidative Cleavage-Based HCR-CRISPR/Cas12a Biosensor for Highly Sensitive Detection of Hypochlorous Acid

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1.Experimental section

1.1. Reagents and chemicals

All oligonucleotide strands used in this study were purchased from Shangya Biotechnology Co., Ltd. (Fuzhou, China). Detailed sequences of the oligonucleotide strands are provided in (Table S2). The EnGen® LbaCas12a (Cpf1) enzyme was obtained from New England Biolabs (Beijing, China).GelRed Nucleic Acid Gel Stain was obtained from Shanghai Liji Biotechnology Company. HClO, $MgCl_2$, and NaCl were purchased from Aladdin Reagent (Shanghai, China). All chemical reagents were of analytical grade and used without further purification. All buffers and solutions were prepared with deionized water (18.2 MΩ cm) from a Milli-Q Plus system.

1.2. Instruments.

Fluorescence spectra were recorded from 500 to 600 nm at 490 nm excitation and 520 nm emission wavelengths using a Cary Eclipse fluorescence spectrometer from Agilent. The gel electrophoresis system and gel imaging system used are all provided by Bio Rad (America). UV spectrophotometry was recorded by a GENESYS180 spectrometer from Thermo Fisher, USA. The morphology of the HCR reaction was characterized using a high-resolution Bruker MultiMode 8 atomic force microscope (AFM). The samples were centrifuged using a HT150R benchtop high-speed cryo-centrifuge from Xiangyi Instruments (Hunan, China).

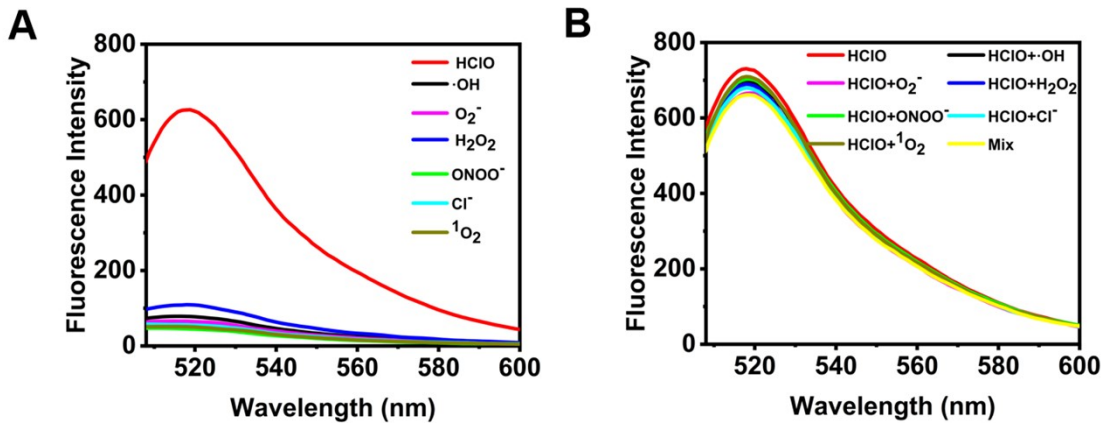

Figure S1. Specificity of HCR-Cas12a for HClO detection.(A) Specificity analysis of the HCR-Cas12a system to different competitors and (B) their mixtures with HClO.

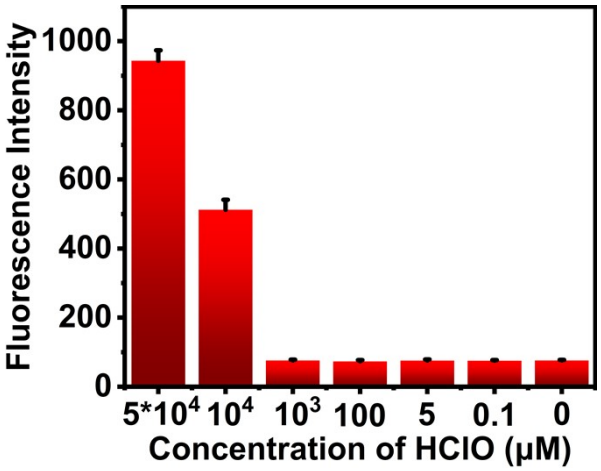

Figure S2. Fluorescence emission spectra of the effects of different concentrations of hypochlorous acid on DNA

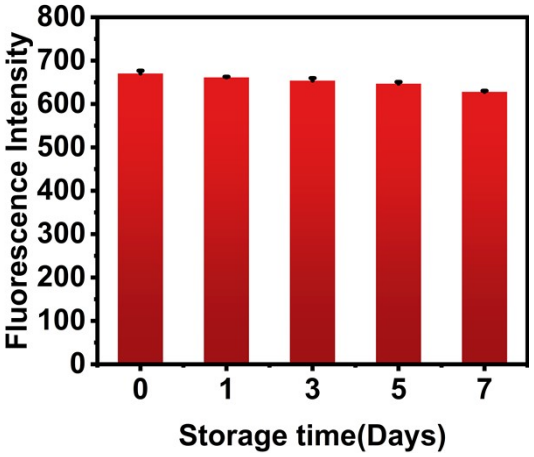

Figure S3. Measurement of the stability of the HCR-Cas12a system.

Table S1. Comparison of the present method with reported methods for the determination of HClO.

Method	Detection technique	Linear rage	LOD	Ref.
Iridium (III) Complex	chemiluminescence	1~80 μM	0.14 μM	[1]
Heterobimetallic Ruthenium(II) − Gadolinium(III) Complex	Resin magnetic resonance	0~100 μM	0.64 μM	[2]
Selenomorpholine-caged cyanine dye	fluorescence	0~11 μM	31.5 nM	[3]
Phenothiazine triphenylamine	fluorescence	0.2~10 μM	55 nM	[4]
CRISPR/Cas12a biosensors	fluorescence	1~5 μM	0.33 μM	[5]
HCR-Cas12a system	fluorescence	0.01~1 μM	9.14 nM	This work

Table S2. Oligonucleotide sequences used in this work.

*indicates phosphorothioate-modified site.

NAME	Nucleotide Sequence （5'→3'）
H0	AGTCTAGGAAACTGCGTGGGTTAAT*CAACATCAGTCTGATAAGCTA TTAACCCA
H1	TTAACCAACGCAGTTTCCTAGACTCAGTGTAGTCTAGGAAACTGCGTG
H2	AGTCTAGGAAACTGCGTTGGTTAATACGCAGTTTCCTAGACTACACTG
crRNA	UAAUACGACUCACUAUAGGGUAAUUUCUACUAAGUGUAGAUC UAGACUACACUGAGUCUAG
FQ-reporter	FAM-TTATT-BHQ1
L-crRNA-15nt	CTTAGTA* GAAATTA
L-crRNA-19nt	ACA*CTTAGTA*GAAATTA
L-crRNA-23nt	TCTACA* CTTAGTA* GAAATTA
L-crRNA-25nt	AGATCTACA* CTTAGTA* GAAATTA

References

1. D. Yongcheng, Z. Kexin, Y. Xiaohan, X. Xiaobo, Z. Zixuan and L. Yi, *Analytical chemistry*, 2023, 95.
2. S. Wenbo, S. Bo, L. Zhiwei, Z. Wenzhu, T. Mingqian, S. Fengling and Y. Jingli, *Analytical chemistry*, 2020, 92, 11145-11154.
3. S. Zhang, L. Ning, Z. Song, X. Zhao, F. Guan, X.-F. Yang and J. Zhang, *Analytical Chemistry*, 2022, 94, 5805-5813.
4. P. Wu, Y. Zhu, L. Chen, Y. Tian and H. Xiong, *Analytical Chemistry*, 2021, 93, 13014-13021.
5. J. Y. Ma, B. Liu, S. Raza, H. X. Jiang, A. N. Tang and D. M. Kong, *SENSORS AND ACTUATORS B-CHEMICAL*, 2023, 376.