Electronic Supplementary Material (ESI) for ChemComm.

Ultra-sensitive MicroRNA-21 detection based on multiple cascaded strand displacement amplification and CRISPR/Cpf1 (MC-SDA/CRISPR/Cpf1)

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Experiment section

Reagents and equipment

The sequence information of all DNA fragments used in this paper can be seen in Table S1, and all of which were purchased from Sangon Biotech (Shanghai, China). T7 Mix, DNase I (2000 U/mL), Cpf1 (LbCas12a), Klenow Fragment (5000 U/mL), Nt.BbvCI (10000 U/mL) and their buffers were offered by New England Biotechnology Co., Ltd (Beijing, China). MiRcute miRNA Isolation Kit (DP501) ordered from TIANGEN Biotechnology Co., Ltd (Beijing, China) was used for the purification of crRNA. Both nucleoside triphosphate (NTPs) and deoxy-ribonucleoside triphosphate (dNTPs) were bought from Sangon Biotech (Shanghai, China). The fluorescence spectrum of the final signal was determined by LS-55 spectrofluorometer (Perkin Elmer, USA). During the measurement, the excitation wavelengths were set at 490 nm in the range of 400 nm to 700 nm.

Synthesis of crRNA

First, 15 μL of system included T7 promoter (10 μM), crRNA-template (10 μM) and appropriate RNase-free water was annealed at 95°C for 5 min. Then, the mixture was cooled to room temperature slowly and left to rest for some time. Afterwards, certain amount of T7 Mix (40 U), 1×RNA polymerase buffer and NTPs (1 μM) was added to the mixture and the final system was adjusted to 30 μL. Then, keep the mixture at 37°C for 16 h to polymerize large number of crRNA. After that, 4 μL of DNase I and 4 μL 10×DNase I buffer were added to the system and the final system was reacted at 37°C for 2 h to degrade the DNA completely. The obtained crRNAs were purified by miRcute miRNA Isolation Kit and stored at -20°C for later use.

Amplification of miR-21 by C-SDA or MC-SDA

First, 5 μL of system contained T-1 (20 nM) or mixture of T-1 (20 nM) and T-2 (20 nM), different concentrations of miR-21, 1 μL 10×NEB buffer 2 and some RNase-free water was annealed at 80°C for 5 min. Then, 0.5 U Klenow Fragment, 1 U Nt.BbvCI, 1 μL 10×CutSmart and 0.5 μL of 25 mM dNTP were added to the system after it had been cooled to room temperature. The final system was adjusted to10 μL using RNase-free water. Then incubate the final system for 80 min at 37°C to obtain activators.

Measurement of the signal

The obtain activators can activate the trans-cleavage activity of CRISPR/Cpf1, disintegrating the ssDNA reporter and generating a fluorescent signal. Specifically, 10 μL CRISPR/Cpf1 system contained 2 μM crRNA, 2 μM Cas12a, 10 μM ssDNA reporters, 2×NEB buffer 2.1 and appropriate RNase-free water was mixed with the previous system. The final reaction system was incubated at 37°C for 1 h. Then, 80 μL ddH_2O was added to the final reaction system and transferred to a fluorescent cuvette. The LS-55 spectrophoto fluorometer was utilized to measure the fluorescent signal.

Gel electrophoresis

The verification of purified crRNA was realized through a 3% agarose electrophoresis. The process was accomplished in 1×TBE buffer at 120 V for 50 min. A bright band which represents crRNA can be seen in Fig. S1. Feasibility of C-SDA and MC-SDA were verified by 15% of polyacrylamide gel electrophoresis (PAGE). It

was completed in 1×TBE buffer at 120 V for 60 min. Gel images were observed using an Azure Biosystems C150 (USA).

Table S1 The DNA sequences used in this work

Name	Information of the DNA sequences (5'-3')
miR-21	UAG CUU AUC AGA CUG AUG UUG A
template 1	AAAAAAAAAAAAAAAAAAAAAAAAGCTGAGGAACCGCTGAGGTCAAC ATCAGTCTGATAAGCTA
template 2	AAAAAAAAAAAAAAAAAAAAAAAAGCTGAGGTCAACATCAGTCTGAT AAGCTGAGGAACCGCTGA
Cleavage reporter	HEX- TATTATT- BHQ1
crRNA template	TTTTTTTTTTTTTTTTTTTTTTATCTACACTTAGTAGAAATTACCCTATA GTGAGTCGTATTAATTTC
T7 promoter	GAAATTAATACGACTCACTATAGGG
miR-155	UUA AUG CUA AUC GUG AUA GGG GU
miR-let7a	UGA GGU AGU AGG UUG UAU AGU U
miR-122	UGG AGU GUG AC A AUG GUG UUU G
miR-16	UAGCAGCACGUAAAUAUUGGCG

Table S2 Comparison of this method with other miR-21 sensors.

Method	**Linear range**	**LOD**	**Ref.**
Au@Si nanocomposite labeled lateral flow assay	10 pM-1 nM	1 pM	S1
DSN and visible-light-induced oxidase mimic activity	50 fM-3 pM	44.76 fM	S2
phosphorescent resonance energy transfer of Mn-ZnS QDs	8-80 nM	1.6 nM	S3
sulfonamide-bound antisense hybridization	10 fM-10 nM	20 fM	S4
MoS_2 and fluorescent dye-labeled DNA probe	0-40 nM	500 pM	S5
Thionine and Gold Nanoparticles Co-Functionalized MoS2 Nanosheet	1 pM-10 nM	0.26 pM	S6
CHA and DNA-templated silver nanoclusters (DNA/AgNCs)	200 pM-20 nM	200 pM	S7
DSN-assisted signal amplification strategy and PRET	0.25-40 nM	0.16 nM	S8
RCA and polygonal-plate fluorescent-hydrogel	0.5-50 nM	5 pM	S9
MC-SDA and CRISPR/Cpf1	10 fM-10 pM; 10 pM-100 nM	10 fM	**This work**

Table S3 Detection of miR-21 in normal human serum by the proposed MC-SDA/CRISPR/Cpf1.

Target added (%)	Detected results (fM)	Recovery (%)
0	--	--
100 fM	99.49	99.49
1 pM	1147.47	114.75
10 pM	9896.15	98.96

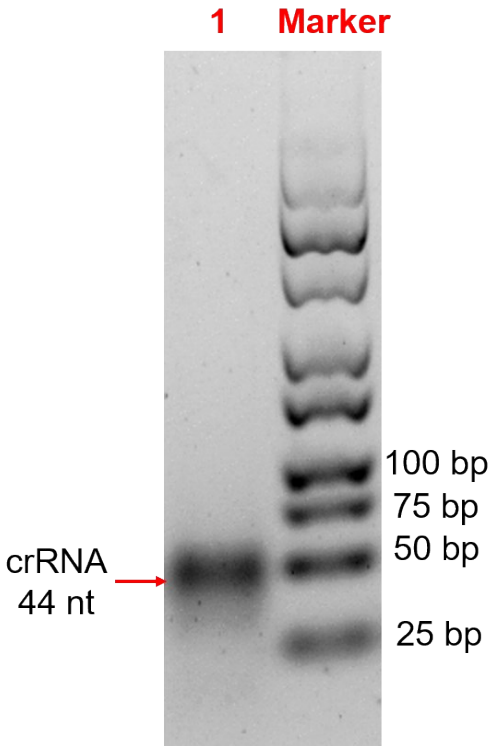

Figure S1 Gel electrophoresis analysis of crRNA.

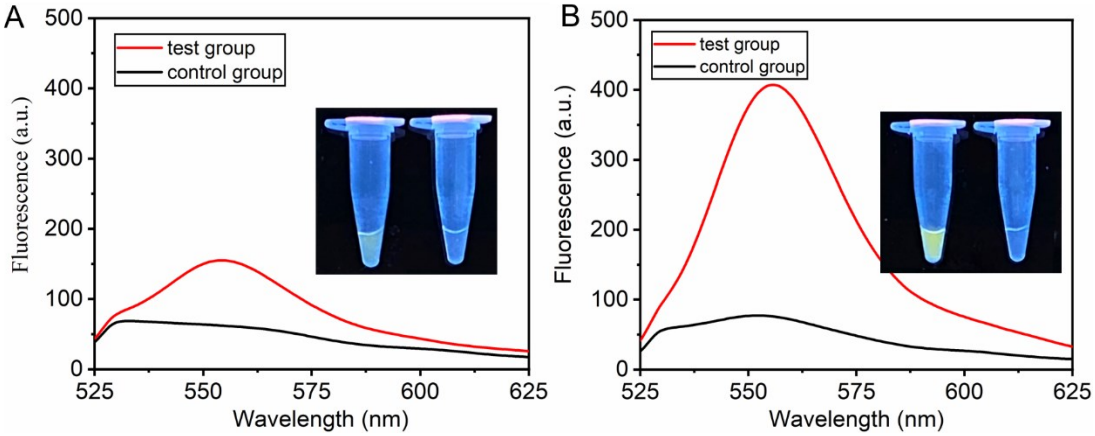

Figure S2 Feasibility of the miR-21 detection based on C-SDA/CRISPR/Cpf1 (A) and MC-SDA/CRISPR/Cpf1 (B).

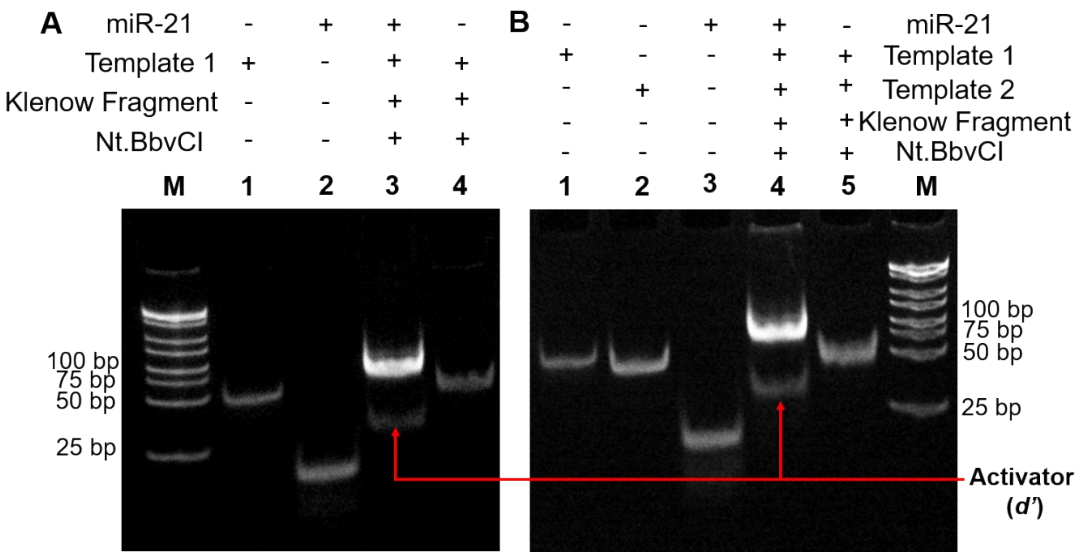

Figure S3 (A) 15% polyacrylamide gel electrophoresis of C-SDA (M: Marker; Lane 1: Template 1; Lane 2: miR-21; Lane 3: experimental group; Lane 4: control group), (B) 15% polyacrylamide gel electrophoresis of MC-SDA (Lane 1: Template 1; Lane 2: Template 2; Lane 3: miR-21; Lane 4: experimental group; Lane 5: control group; M: marker).

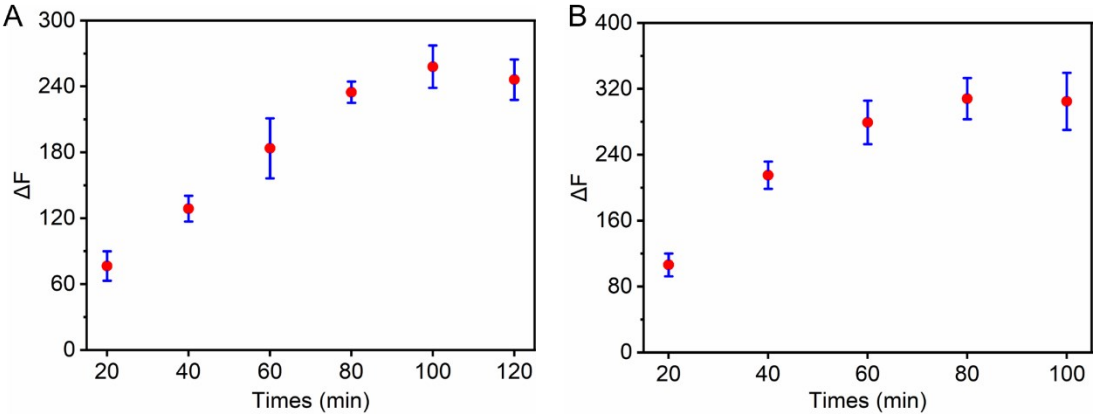

Figure S4 Optimizations of the reaction time of MC-SDA (A) and the shearing time of CRISPR/Cpf1 (B).

S1 T. Dong, R. Yin, Q. Yu, W. Qiu, K. Li, L. Qian, H. Li, B. Shen and G. Liu, Anal Chim Acta, 2021, **1147**, 56-63.
S2 Y. Xia, T. Chen, L. Zhang, X. Zhang, W. Shi, G. Chen, W. Chen, J. Lan, C. Li, W. Sun and J. Chen, Biosens Bioelectron, 2021, **173**, 112834.
S3 J. Lv, Y. Miao and G. Yan, RSC Advances, 2017, **7**(65), 41063-41069.
S4 D.A. Smith, L.J. Newbury, G. Drago, T. Bowen and J.E. Redman, Sens Actuators B Chem, 2017, **253**, 335-341.
S5 B. Cai, S. Guo and Y. Li, Analytical Methods, 2018, **10**(2), 230-236.
S6 D. Zhu, W. Liu, D. Zhao, Q. Hao, J. Li, J. Huang, J. Shi, J. Chao, S. Su and L. Wang, ACS Appl Mater Interfaces, 2017, **9**(41), 35597-35603.
S7 J. Gu, Z. Qiao, X. He, Y. Yu, Y. Lei, J. Tang, H. Shi, D. He and K. Wang, Analyst, 2020, **145**(15), 5194-5199.
S8 J.J. Yang, Z.F. Zhang and G.Q. Yan, Anal Biochem, 2017, **539**, 127-133.
S9 H. Song, Y. Zhang, S. Wang, K. Huang, Y. Luo, W. Zhang and W. Xu, Sensors and Actuators B: Chemical, 2020, **306**, 127554.