

Supplementary Materials

CRISPR-Cas12a Trans-Cleaves DNA G-Quadruplex

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Materials and Methods

Nucleic acid preparation

G-quadruplex (G4) DNA and crRNA sequences were obtained from Sangon Biotech company (Shanghai, China). The SARS-CoV-2 N-gene and SARS N-gene were amplified via polymerase chain reaction (PCR) from pUC57- SARS-CoV-2-N vector and pUC57-SARS-N vector, respectively. PCR products were purified by gel extraction kit (Axygen company, New York, American). The primers and vectors used were provided by Tsingke company (Beijing, China). For cleavage assays, G4 oligonucleotides were heated at 95°C for 10 min and cooled down before use. Relevant DNA or RNA sequences were designed by referring previous report¹ and listed below.

Table S1 Nucleotide sequences used in this study.

Name	sequence
SARS-CoV-2 N-gene crRNA	5'-UAAUUUCUACUAAGUGUAGA <u>UCCCCCAGCGCUU</u> <u>CAGCGUUC</u> -3'
SARS-CoV-2 N-gene-FWD	5'-CCAAATTGGCTACTACCGAAGAGCTAC-3'
SARS-CoV-2 N-gene-REV	5'-CACAGTTTGCTGTTTCTTCTGTCTCTGCGG-3'
SARS N-gene-FWD	5'-CCAAATTGGCTACTACCGAAGAGCTAC-3'
SARS N-gene-REV	5'-CACAGTGGGCTGCTTCTTTTGTCTCTGCGG-3'
Fluo Telomere G4	5'-FAM-TTAGGGTTAGGGTTAGGGTTAGGG-TAMRA-3'
Telomere G4	5'-TTAGGGTTAGGGTTAGGGTTAGGG-3'
Fluo TBA	5'-FAM-GGTTGGTGTGGTTGG-TAMRA-3'
FQ ssDNA	5'-FAM-TTATT-BHQ1-3'

Note: the underline indicated the sequence targeting SARS-CoV-2 N-gene.

> cDNA of SARS-CoV-2 N-gene

CCAAATTGGCTACTACCGAAGAGCTACCAGACGAATTCGTGGTGGTGACGGT
AAAATGAAAGATCTCAGTCCAAGATGGTATTTCTACTACCTAGGAACTGGGC
CAGAAGCTGGACTTCCCTATGGTGCTAACAAAGACGGCATCATATGGGTTGC
AACTGAGGGAGCCTTGAATACACCAAAGATCACATTGGCACCCGCAATCCT
GCTAACAATGCTGCAATCGTGCTACAACCTCCTCAAGGAACAACATTGCCAA
AAGGCTTCTACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTC
CTCATCACGTAGTCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAGG
GGAACCTTCTCCTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTT
GCTGCTGCTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTGGTAAAGGC
CAACAACAACAAGGCCAAACTGTCACTAAGAAATCTGCTGCTGAGGCTTCTA
AGAAGCCTCGGCAAAAACGTACTGCCACTAAAGCATAACAATGTAACACAAG
CTTTCGGCAGACGTGGTCCAGAACAAACCCAAGGAAATTTTGGGGACCAGGA
ACTAATCAGACAAGGAACTGATTACAAACATTGGCCGCAAATTGCACAATTT
GCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGCATTGGCATGGAAGTCA
CACCTTCGGGAACGTGGTTGACCTACACAGGTGCCATCAAATTGGATGACAA
AGATCCAAATTTCAAAGATCAAGTCATTTTGCTGAATAAGCATATTGACGCAT
ACAAAACATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAAGAAGGCTG
ATGAAACTCAAGCCTTACCGCAGAGACAGAAGAAACAGCAAACCTGTG.

>cDNA of SARS N-gene

CCAAATTGGCTACTACCGAAGAGCTACCCGACGAGTTCGTGGTGGTGACGGC
AAAATGAAAGAGCTCAGCCCCAGATGGTACTTCTATTACCTAGGAACTGGCC
CAGAAGCTTCACTTCCCTACGGCGCTAACAAAGAAGGCATCGTATGGGTTGC
AACTGAGGGAGCCTTGAATACACCCAAAGACCACATTGGCACCCGCAATCCT
AATAACAATGCTGCCACCGTGCTACAACCTCCTCAAGGAACAACATTGCCAA
AAGGCTTCTACGCAGAGGGAAGCAGAGGCGGCAGTCAAGCCTCTTCTCGCTC
CTCATCACGTAGTCGCGGTAATTCAAGAAATTCAACTCCTGGCAGCAGTAGG
GGAAATTCTCCTGCTCGAATGGCTAGCGGAGGTGGTGAAACTGCCCTCGCGC

TATTGCTGCTAGACAGATTGAACCAGCTTGAGAGCAAAGTTTCTGGTAAAGG
CCAACAACAACAAGGCCAAACTGTCTACTAAGAAATCTGCTGCTGAGGCATCT
AAAAAGCCTCGCCAAAAACGTACTGCCACAAAACAGTACAACGTCCTCAA
GCATTTGGGAGACGTGGTCCAGAACAAACCCAAGGAAATTTTCGGGGACCAA
GACCTAATCAGACAAGGAACTGATTACAAACATTGGCCGCAAATTGCACAAT
TTGCTCCAAGTGCCTCTGCATTCTTTGGAATGTCACGCATTGGCATGGAAGTC
ACACCTTCGGGAACATGGCTGACTTATCATGGAGCCATTAAATTGGATGACA
AAGATCCACAATTCAAAGACAACGTCATACTGCTGAACAAGCACATTGACGC
ATACAAAACATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAAAAAGAC
TGATGAAGCTCAGCCTTTGCCGCAGAGACAAAAGAAGCAGCCCCTGTG.

***In vitro* cleavage assay**

Lachnospiraceae bacterium ND2006 Cas12a (LbCas12a) used in this study was purchased from Meige company (Guangzhou, China). Unless mentioned otherwise, Cas12a-mediated cleavage assays were carried out in cleavage buffer consisting of 10 mM Tris (pH 7.9), 50 mM NaCl, 10 mM MgCl₂. First, LbCas12a (1 µL, 2 µM), crRNA (2 µL, 1 µM) and 18 µL cleavage buffer were mixed and preincubated at 37 °C for 10 min. After the formation of LbCas12a/crRNA complex, target DNA (SARS-CoV-2 N-gene; 5 µL, 50 ng/µL, 100 nM) or non-target DNA (SARS N-gene; 5 µL, 50 ng/µL, 100 nM) or cleavage buffer (5 µL) and G4 DNA sequence (25 µL, 500 nM) were added to form a 50-µL reaction solution. The cleavage reaction was performed at 37 °C for 15 min.²⁻³

It is to be noted that we modified the concentration of G4 in the following experiments in consideration of the detection sensitivity of fluorescence spectrometer, CD spectrometer and NMR spectrometer. Accordingly, we also adapted the concentrations of other reagents during the experiments to obtain sufficient signal for the analysis.

Measurement of fluorescence spectroscopy

To carry out FRET assay, the 5'-FAM and 3'-TAMRA labelled G4 sequences were diluted to a final concentration of 25 nM. Fluorescence measurements were performed on a FluoroMax-4 spectrofluorometer (Horiba, Japan) at room temperature (~25 °C). The excitation and emission slits were both 5 nm. Excitation was set at 488 nm, and emission was collected from 500 to 750 nm. In the experiment testing the effect of target concentration on cleavage efficiency, the final target concentration varied from 0 to 10 nM.

For the time-course cleavage assay, the holder for loading the quartz cuvette was pre-heated and kept at 37 °C through a water circulation. LbCas12a (1.5 µL, 2 µM) was preincubated with crRNA (3 µL, 1 µM) at 37 °C for 10 min in 20 µL cleavage buffer that has 50mM Na⁺, 100mM Na⁺, 150mM Na⁺, 200mM Na⁺ or 70mM K⁺ respectively. Then G4 sequences (50 µL, 5 µM) and 425 µL relevant buffer were mixed with the 20-µL reaction solution. This mixture was added into the cuvette and incubated in the holder for another 10 min. After that, the measurement was initiated immediately after the target (5 µL, 100 nM) was added into the cuvette. The interval was set as 1-5 min based on the cleavage kinetics under various conditions.

To test the influence of ion conditions over the cleavage efficiency of LbCas12a, we used the FQ-labelled non-target ssDNA (5'-FAM-TTATT-BHQ1-3') as the reporter.² LbCas12a (1 µL, 2 µM), crRNA (2 µL, 1 µM) and 18 µL cleavage buffer (containing 50mM Na⁺, 100mM Na⁺, 150mM Na⁺, 200mM Na⁺ or 70mM K⁺ respectively) were mixed and preincubated at 37 °C for 10 min. After the formation of LbCas12a/crRNA complex, target DNA (SARS-CoV-2 N-gene; 5 µL, 50 ng/µL) and non-target ssDNA reporter (25 µL, 500 nM) were added to form a 50-µL reaction solution. The cleavage reaction was performed at 37 °C for 5 or 15 min. The reaction system was incubated at 65°C for 15 min to inactivate the LbCas12a. To carry out fluorescence measurement, the ssDNA reporter was diluted to a final concentration of 25 nM with relevant buffer. The excitation and emission slits were both 5 nm. Excitation was set at 488 nm, and emission was collected from 500 to 600 nm.

Circular dichroism (CD) experiments

CD experiments were carried out on Chirascan (AppliedPhotophysics, UK). The data were collected in a 0.1 cm path length cuvette by taking the average of three scans recorded from 220 to 320 nm at a scanning rate of 100 nm/min at room temperature (~25 °C). LbCas12a (1.5 µL, 2 µM) was preincubated with crRNA (3 µL, 1 µM) in a total of 20 µL buffer that contains 50 mM Na⁺, 100 mM Na⁺, 150 mM Na⁺ or 70 mM K⁺ respectively for 10 min at 37 °C. Then G4 oligonucleotide (25 µL, 25 µM) and target (5 µL, 60 ng/µL) were added into the reaction solution and the mixtures were incubated at 37 °C for 2 h. FRET assay was performed before the CD measurement to ensure that the G4 was fully cleaved. The mixture was diluted to a total of 250 µL for CD detection. All spectra were corrected with matched buffer blanks. For the time-course CD measurement, the cuvette holder was pre-heated and kept at 37 °C and the temperature of the testing solution was monitored with a sensor. LbCas12a (1.5 µL, 20 µM) was preincubated with crRNA (3 µL, 10 µM) in a total of 20 µL cleavage buffer (100 mM Na⁺) for 10 min at 37 °C. Then telomere G4 sequence (220 µl, 10 µM) and the target (10 µl, 120 ng/µl) were mixed with the 20-µL reaction solution, following the CD measurement immediately with an interval of 10 minutes.

Analysis of Cas12a-digested products by agarose gel electrophoresis and denaturing PAGE

LbCas12a (1.5 µL, 2 µM) was preincubated with crRNA (3 µL, 1 µM) in a total of 20-µL buffer (50 mM Na⁺, 100 mM Na⁺ 150 mM Na⁺ or 70 mM K⁺, respectively) for 10 min at 37 °C. Then G4 oligonucleotide (25 µL, 10 µM) and target (5 µL, 60 ng/µL) were added into the reaction solution and the mixtures were incubated at 37 °C for relevant time. The reaction system was incubated at 65°C for 15 min to inactivate the LbCas12a. The cleaved products were separated with agarose gel (3.5%, w/v) pre-stained with Gelred (Biotium) at 150 V/180 mA for 1 h. We exposed the DNA agarose gel to the UV light device (BioRad Company, Shanghai, China) to take the gel graph.

To analyze the cleaved products more closely, we also conducted denaturing polyacrylamide gel electrophoresis (PAGE). FAM-labelled DNA was first digested by Cas12a at 37°C for different time and then heated at 65 °C for 15 min to stop the reactions.

Then the solution was mixed with the loading buffer and loaded into 20 % denaturing PAGE containing 8 M urea. Electrophoresis was performed at 120V (about 40 V/cm) for about 90 min (Mini-PROTEAN Tetra Cell system, Bio-Rad) in 1×TBE buffer. The gel was scanned using Bio-rad ChemiDoc MP (170-8280) (BioRad Company, Shanghai, China) in the mode of “fluorescein”.

NMR experiments

¹H NMR was measured on a Bruker AVANCE 700 MHz spectrometer. Spectra were recorded at 10 °C. We dissolved the telomere G4 sample (0.3 mM, without labelling fluorescent probes), LbCas12a (0.5 μM), and crRNA (0.5 μM) in 400 μL of 90% H₂O/10% D₂O, 10 mM Tris (pH 7.9), 100 mM NaCl, 10 mM MgCl₂ buffer. NMR experiment was first performed on the non-cleaved sample. Then target DNA (15 nM) was added to the sample and incubated at 37 °C for overnight cleavage. CD spectroscopy was tested for the cleaved sample before conducting NMR measurement to make sure that the G4 was fully degraded. Then ¹H NMR measurement was performed on the cleaved sample with the same settings as the non-cleaved sample.

References

1. Broughton, J. P.; Deng, X.; Yu, G.; Fasching, C. L.; Servellita, V.; Singh, J.; Miao, X.; Streithorst, J. A.; Granados, A.; Sotomayor-Gonzalez, A.; Zorn, K.; Gopez, A.; Hsu, E.; Gu, W.; Miller, S.; Pan, C. Y.; Guevara, H.; Wadford, D. A.; Chen, J. S.; Chiu, C. Y., CRISPR-Cas12-based detection of SARS-CoV-2. *Nat Biotechnol* **2020**, 38, 870–874.
2. Chen, J. S.; Ma, E.; Harrington, L. B.; Da Costa, M.; Tian, X.; Palefsky, J. M.; Doudna, J. A., CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science* **2018**, 360 (6387), 436-439.
3. Li, S.-Y.; Cheng, Q.-X.; Liu, J.-K.; Nie, X.-Q.; Zhao, G.-P.; Wang, J., CRISPR-Cas12a has both cis- and trans-cleavage activities on single-stranded DNA. *Cell Research* **2018**, 28 (4), 491-493.
4. Krainer, G.; Hartmann, A.; Schlierf, M., farFRET: Extending the Range in Single-Molecule FRET Experiments beyond 10 nm. *Nano Lett* **2015**, 15 (9), 5826-9.

Supporting Figures

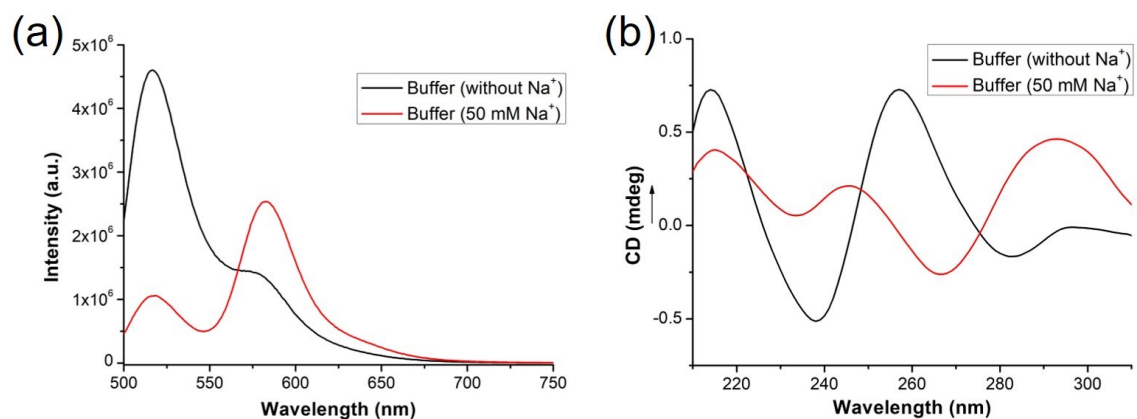


Figure S1 Fluorescence spectra (a) and CD spectra (b) of the telomere sequence (5'-FAM-(TTAGGG)₄-TAMRA-3') in Tris-HCl buffer (pH7.9) with or without 50 mM Na⁺.

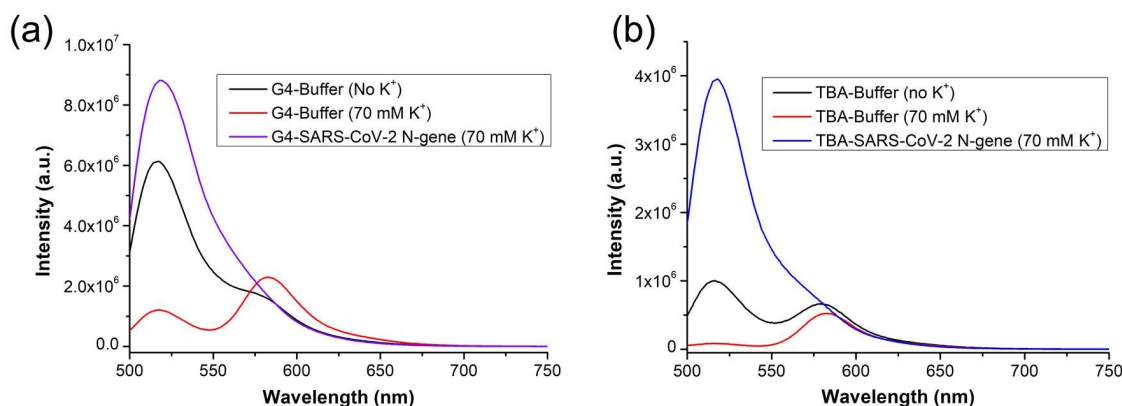


Figure S2 (a) Fluorescence spectra of telomere sequence (5'-FAM-(TTAGGG)₄-TAMRA-3'). In G4-Buffer (no K⁺), the sequence is a linear structure and the FRET efficiency could be neglected. In G4-Buffer (70 mM K⁺), the sequence formed a G4 structure that generated a high FRET efficiency. In G4- SARS-CoV-2 N-gene (70 mM K⁺), the cleavage resulted in the separation of the two fluorophores, which caused an even lower FRET efficiency than that of G4-Buffer (no K⁺). (b) Fluorescence spectra of TBA (5'-FAM-GGTTGGTGTGGTTGG-TAMRA-3'). In TBA-Buffer (no K⁺), the linear sequence still generated a moderate FRET efficiency since the oligonucleotide only has 15 nucleotides (<10 nm in length) and the two fluorophores can generate FRET signals.⁴ In TBA-Buffer (70 mM K⁺), TBA formed a G4 structure that generated higher FRET efficiency than that of TBA-Buffer (no K⁺). In TBA- SARS-CoV-2 N-gene (70 mM K⁺), the cleavage caused dramatic decrease of the FRET efficiency.

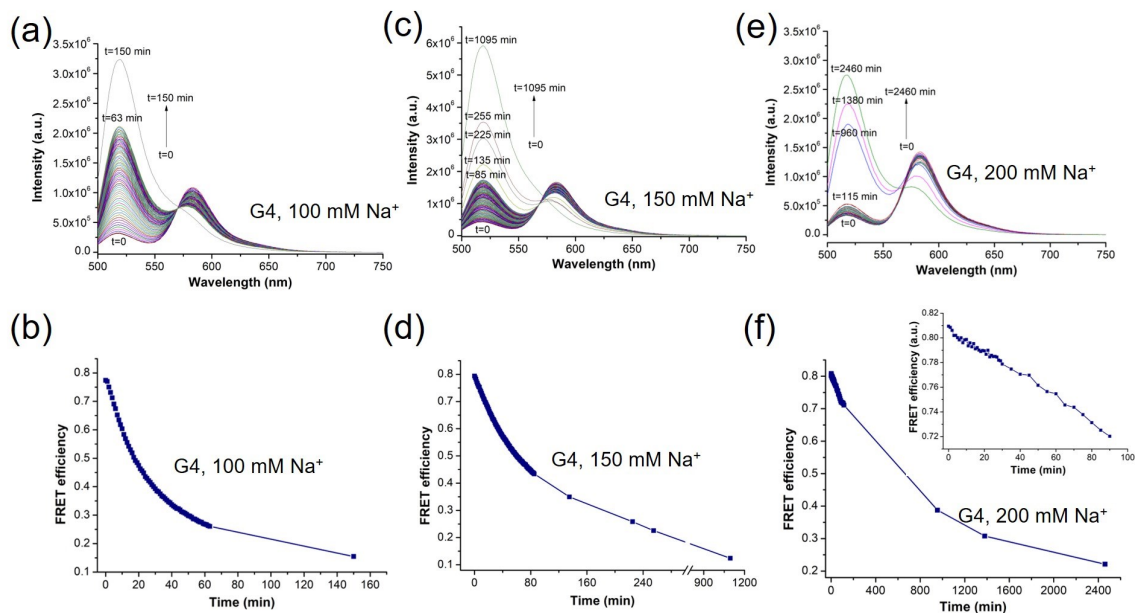


Figure S3 Time lapse trans-cleavage activity on various concentrations of Na^+ -induced G4 examined by fluorescence spectrometer. (a, c, e) Fluorescence spectra to show the on-going cleavage progress of 100 mM (a), 150 mM (c) and 200 mM (e) Na^+ -induced G4. In (a), the interval was 1 min from $t=0$ to $t=63$ min. In (c), the interval was 1 min from $t=0$ to $t=85$ min. In (e), the interval was 1 min from 0-30 min, and 5 min from 30-90 min, respectively. At $t=63$ min for (a), $t=255$ min for (c), and $t=2460$ min for (e), the peak at 583 nm (emission peak of TAMRA) didn't disappear completely, indicating the cleavage was not sufficient and it required a relatively longer time to fully cut the G4. (b, d, f) Plot of the 100 mM Na^+ (b), 150 mM (d) and 200 mM (f) Na^+ induced G4 FRET efficiency against time. The inset in (f) indicated the plot from 0-90 min.

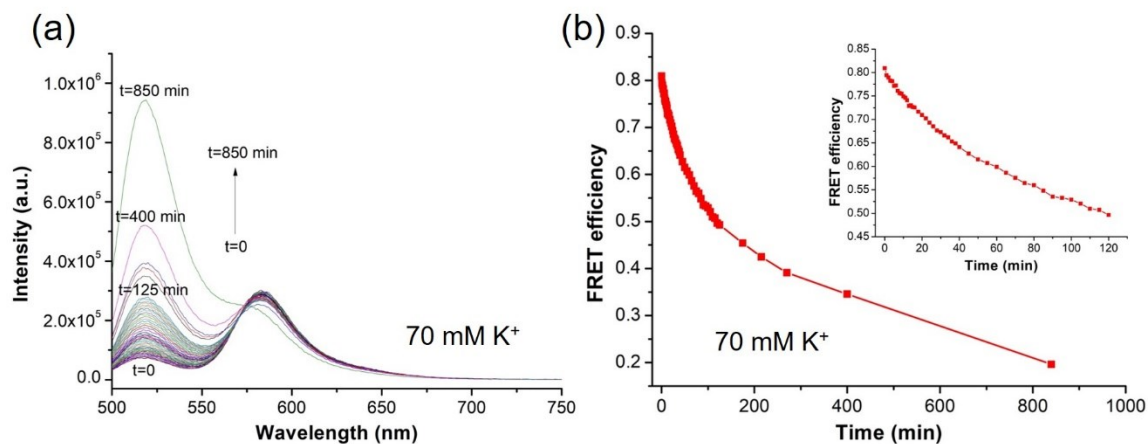


Figure S4 Time lapse trans-cleavage activity on 70 mM K⁺-induced G4 examined by fluorescence spectrometer. (a) Fluorescence spectra to show the on-going cleavage progress. The interval was 1 min from 0-16 min, 2 min from 16-40 min, 5 min from 40-125 min, respectively. At t=850 min, the peak at 583 nm (emission peak of TAMRA) was still there, indicating that it required a longer time to fully cut the G4. (b) Plot of the G4 FRET efficiency against time. The inset indicated the plot from 0-120 min.

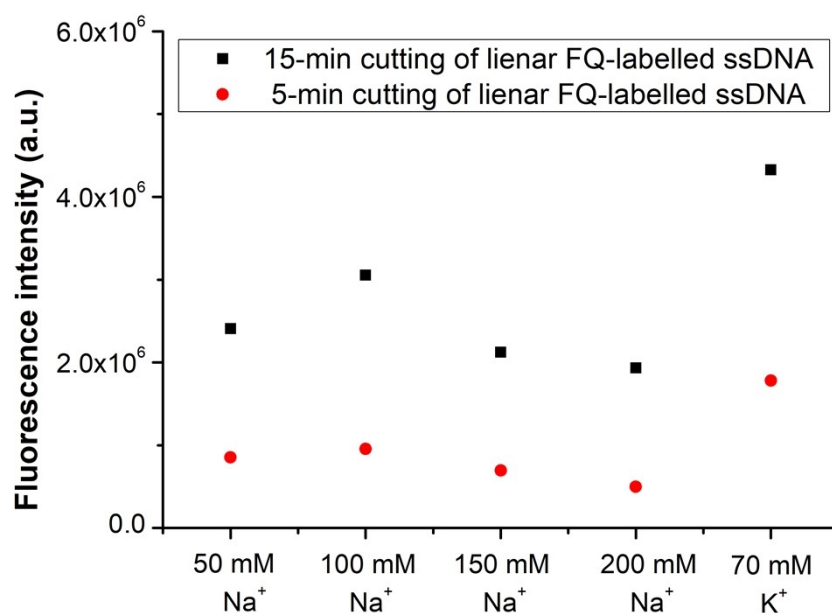


Figure S5 Testing of the effect of ion conditions on the cleavage activity of Cas12a by using a FQ-labelled ssDNA (5'-FAM-TTATT-BHQ1-3') as the reporter. The results demonstrated that sodium concentration did not influence the enzyme activity significantly. However, the enzyme seems to be more effective in potassium than in sodium to cleave the ssDNA.

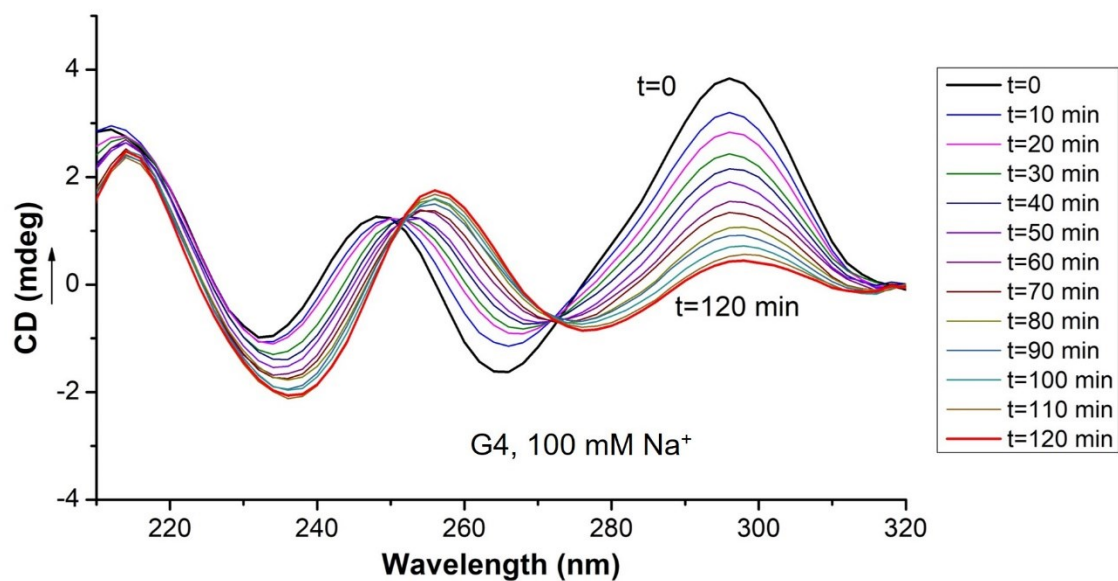


Figure S6 CD spectra showing the time course of LbCas12a trans cleavage on 100 mM-induced G4. The typical peaks of antiparallel G4 (positive peak at 295 nm, negative peak at 265 nm) changed with the on-going cleavage. At the end point, the 295 nm peak decreased apparently and 265 nm peak shifted to around 280 nm.