Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Chemiluminescence resonance energy transfer as simple and sensitive readout mode for CRISPR/Cas12a-based biosensing platform

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Procedure of fluorescence detection of target DNA with CRISPR/Cas12a-based biosensing

Firstly, LbCas12a protein (100 nM) was incubated with the crRNA (100 nM) in 1×NEBuffer 2.1 (10 mM Tris-HCl, 50 mM NaCl, 10 mM Mg^{2+}, 100 μg/mL BSA, pH 7.9) at 37 ℃ for 15 min. Secondly, 20 μL Rnase-free water, 5 μL 10×NEBuffer 2.1, 5 μL Mn^{2+} (6 mM) and 5 μL Taqman probe (5 μM) were added successively into the Cas12a−gRNA mixture. And then target DNA with certain concentration was added into the mixture solution. The above solution was incubated at 37℃ for 60 min. The reaction solution was added into 70 μL Tris–HCl buffer (pH 7.9) for fluorescence test. The fluorescence emission spectrum of the resultant reaction solution was collected by F-4700 fluorescence spectrophotometer (Hitachi, Japan). The fluorescence spectra were recorded in the 490–580 nm range with an excitation wavelength of 480 nm. The fluorescence intensity in the 530 nm was used to quantify the concentration of target DNA. Both excitation and emission slit widths were set at 10 nm, and the PMT of fluorescence spectrophotometer was set at 700 V.

Table S1. Sequences of oligonucleotides used in this work.

Name	Sequence (5′–3′)
HPV-16	CAAATATGTCATTATGTGCTGCCATATCTACTTCAGAAACTACA TATAA
[a]SNP HPV-16	AATATGTCATTATGTGCTGCCAAATCTACTTCAGAAACT
HPV-18	CCAGGTACAGGAGACTGTGTAGAAGCACATATTGTTAAATTGG TACTGGG
PB-19	CATTATTAAGTCCACTATTGTGGAAGCTGCAAAAGCTATT
Scrambled	TCAAAGACTTCATCTATACCGTCGTGTATTACTGTATAA
[b]crRNA (HPV-16)	UAAUUUCUACUCUUGUAGAUUGAAGUAGAUAUGGCAGCAC
Taqman probe	FAM-TTATT-BHQ

[a] The bases in red is the mismatched base.

[b] The blue letters are the recognizing sequence of target DNA (HPV-16).

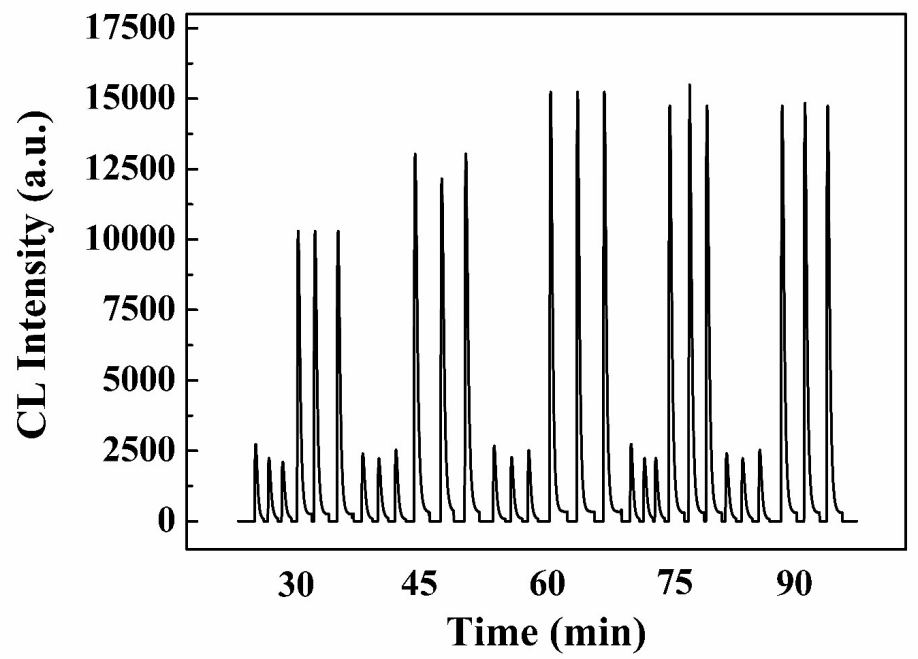

Figure S1. Effect of the incubation time of CRISPR/Cas12a and Taqman probe on CL response.

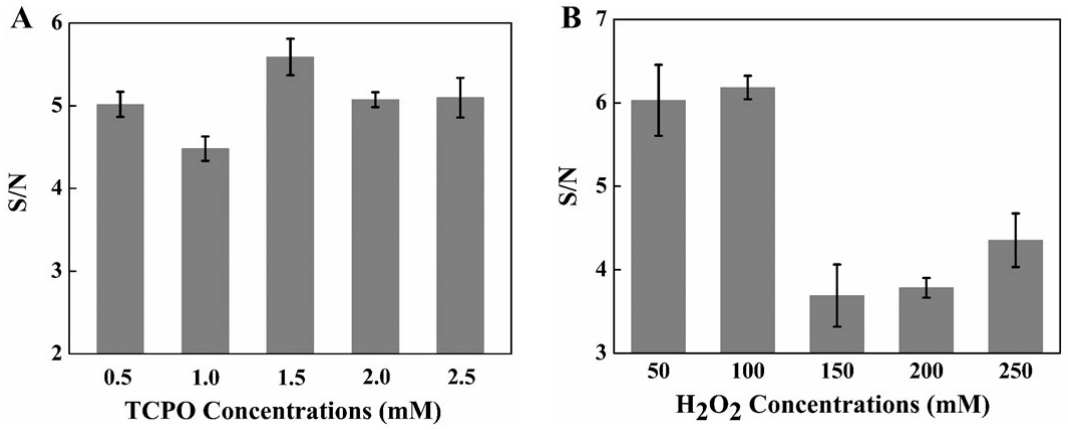

Figure S2. (A) Effect of TCPO concentration on the CL response of this biosensing for DNA detection, and (B) effect of H_2O_2 concentration on the CL response of this biosensing for DNA detection.

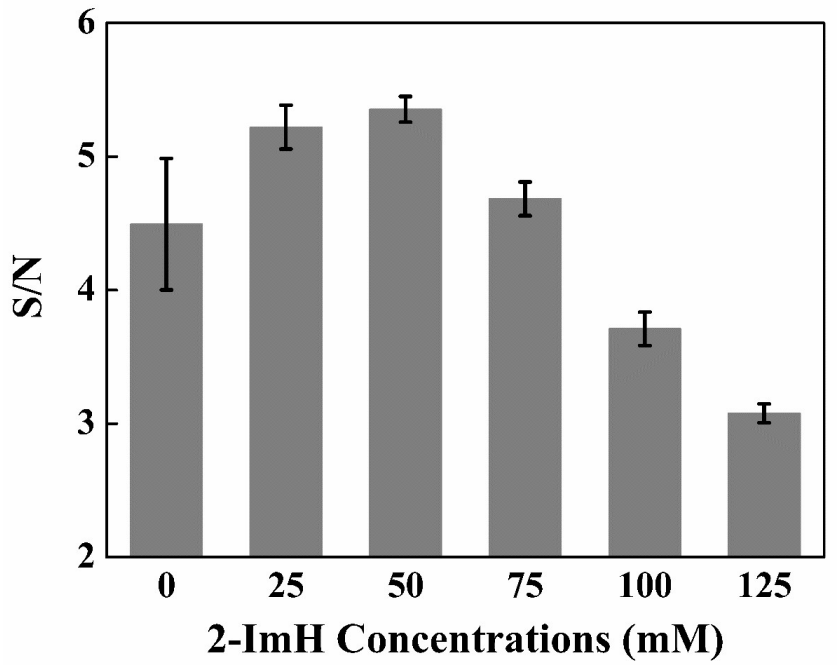

Figure S3. Effect of 2-ImH concentration on the CL response of this biosensing for DNA detection.

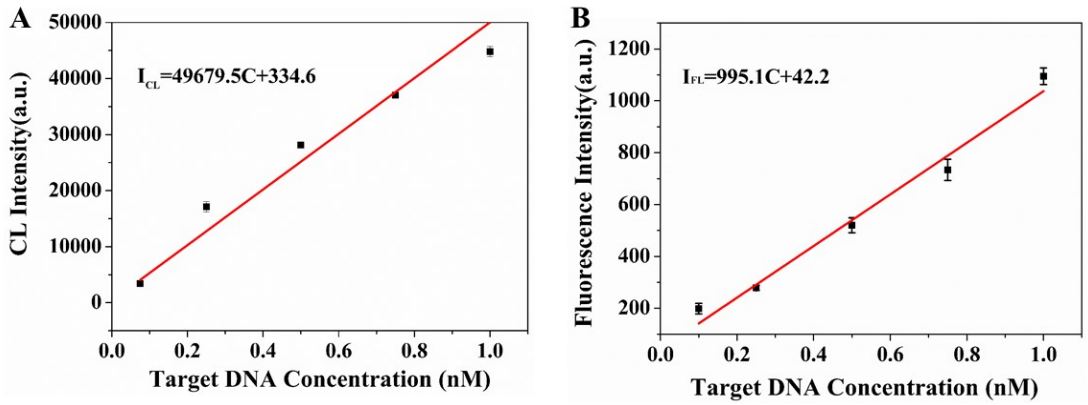

Figure S4. (A) The linear relationship between the CL intensity and target DNA concentration, and (B) the linear relationship between the fluorescence intensity and target DNA concentration. Experimental conditions: 2.5 μL crRNA (100 nM), 2.5 μL Cas12a (100 nM), 5 μL Mn^{2+} (6 mM) 5 μL Taqman probe (5 μM), and 10 μL different concentrations of target DNA.

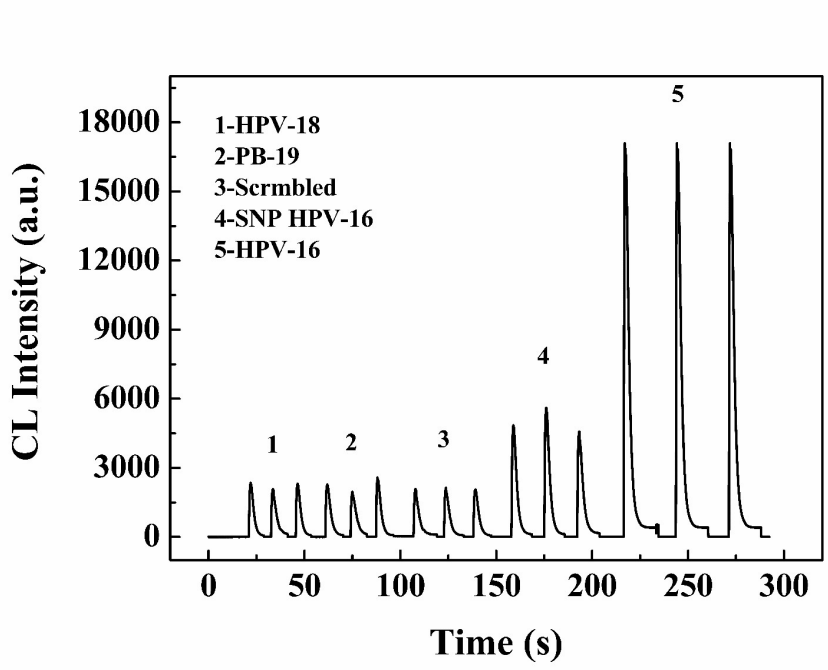

Figure S5. Specificity of this proposed method for target DNA (HPV-16) against other similar DNAs. The concentration of each DNA was 0.25 nM.

Table S2. Recovery experiment for the detection of different concentrations of HPV-16 in diluted serum sample (1%).

Sample	Added (nM)	Found (nM)	Recovery (%)	RSD (%) (n=3)
No. 1	0.2	0.20	100	1
No. 2	0.5	0.49	98	1
No. 3	1.0	0.98	98	4.2