Supporting Information

The photonic crystal sensing array based on tandem CRISPR/Cas13a system for ultra-sensitive and high-throughput detection of CVA6 virus

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1. Experimental section

1.1. Reagents and materials

The target RNA and guide RNA sequences used in the experiment were listed in Table S2, and they were synthesized by Zixi Biotechnology Co., Ltd., a subsidiary of Qingke Biotechnology Co., Ltd., and all the products were purified by high-performance liquid chromatography (HPLC). The effector protein of the Class II Type VI CRISPR system (LbuCas13a Nuclease, C2c2), the dedicated buffer for the LbuCas13a protein trans-cleavage reaction (5×LbuCas13a Cleavage Buffer), and the RNA probes (ssRNA reporter) were provided and synthesized by Guangzhou Edigen Technology Co., Ltd., with the probes purified by HPLC. RNase inhibitors, RNase-free water, and DEPC-treated water were provided by Sangon Biotech Co., Ltd.

1.2. Experimental instruments

Microplate analyzer (PerkinElmer, USA) was used for signal readout from the PC array. LS-55 Fluorescence Spectrometer (PerkinElmer, USA) was employed to obtain fluorescence spectra. The field emission scanning electron microscope (FE-SEM, JSM-7500F, Hitachi, Japan) was employed to characterize the morphologies of the PC. Zetasizer pro (Malvern, UK) was utilized for dynamic light scattering (DLS) of the nanospheres.

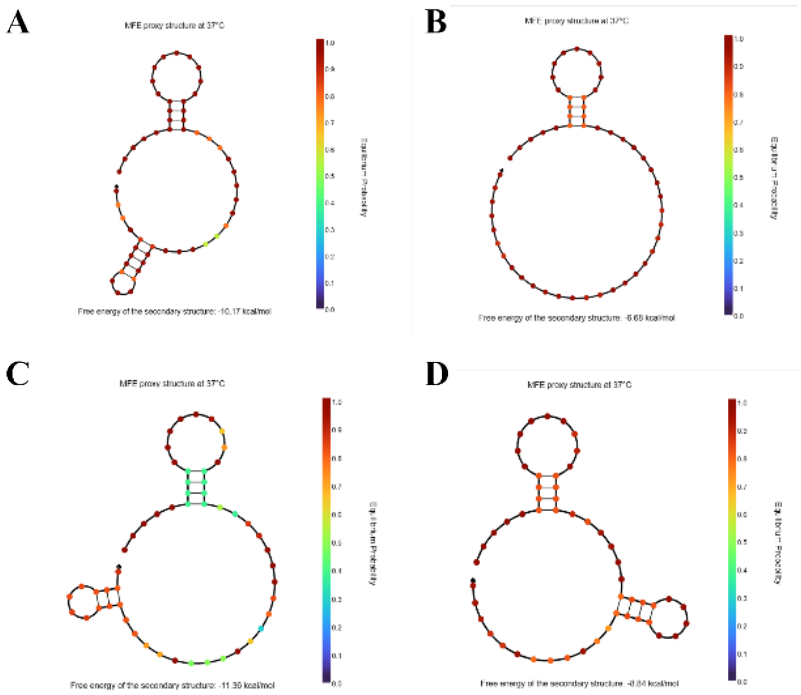
1.3. Fluorescence analysis

Fluorescence spectroscopy was used to assess the feasibility of the designed PC bionic array based on tandem CRISPR/Cas13a system and optimize the key experimental parameters. First, the crRNAs were annealed at 95 °C for 5 minutes,

followed by slowly cooling to room temperature for subsequent use. Then, 50 nM Cas13a, 20 nM crRNA (containing different crRNA combinations), 400 nM RNA fluorescent probes, and target CVA6 RNA with different concentrations were mixed in reaction buffer and incubated at 37 °C for 30 minutes. The reaction products (total volume 50 μL) were transferred to a cuvette, and the fluorescence intensity was measured using a fluorescence spectrophotometer. The excitation wavelength was set to 497 nm, and the emission spectra were recorded in the range of 500 to 700 nm. Both the excitation and emission grating widths were set to 8 nm.

1.4. Recovery experiment of the spiked serum samples

Healthy serum samples were obtained from Fourth Hospital of West China (ethics number: HXSY-EC-2020074). Firstly, the samples were heated at 95 °C for 5 minutes to minimize the interference from protein matrix. After the samples were centrifuged at 12,000 rpm for 5 minutes, the supernatant was collected and diluted to 5% with reaction buffer. Next, different concentrations of CVA6 RNA were added to the diluted serum samples. Subsequently, 5 μL of spiked serum sample, 2 μL of Cas13a (500 nM), 2 μL of crRNA1 (500 nM), 2 μL of crRNA3 (500 nM), and 5 μL of crRNA1 (4 μM) were mixed in reaction buffer (total volume of 50 μL, with final MT concentrations of 0.1 pM, 0.5 pM, and 1.0 pM, respectively). After incubation at 37 °C for 40 minutes, 2 μL of the product was loaded onto the PC array, and signal reading was performed using the microplate analyzer.



2. Results and Discussion

Fig. S1. Secondary structure prediction results of four crRNAs. (A) crRNA1; (B) crRNA2; (C) crRNA3; (D)crRNA4.

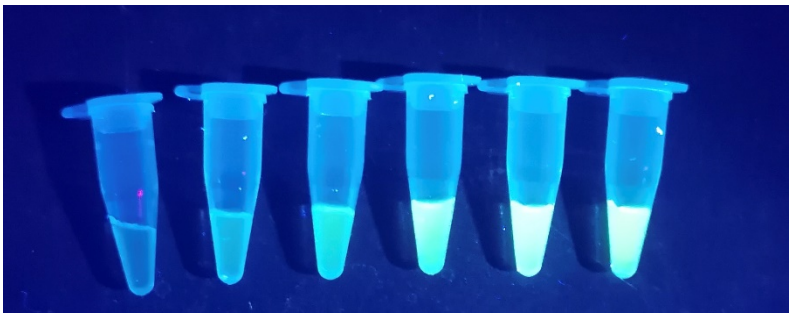


Fig. S2. A physical image of the CRISPR/Cas13a reaction system under UV light.

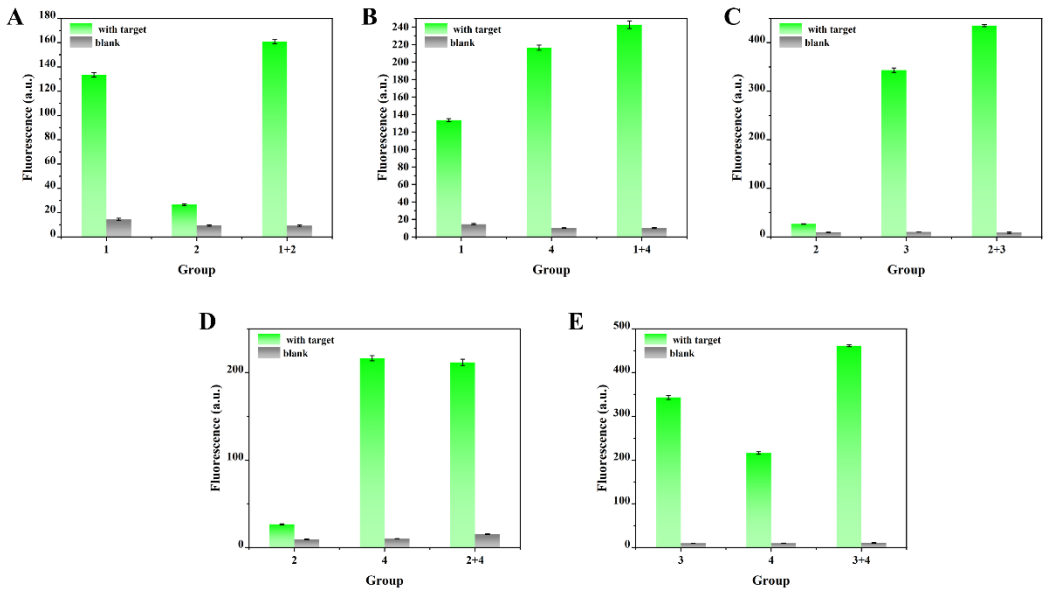


Fig. S3. Comparison of fluorescence signals generated by crRNA combinations and crRNA alone. (A) crRNA1 and crRNA2; (B) crRNA1 and crRNA4; (C) crRNA2 and crRNA3; (D) crRNA2 and crRNA4; (E) crRNA3 and crRNA4.

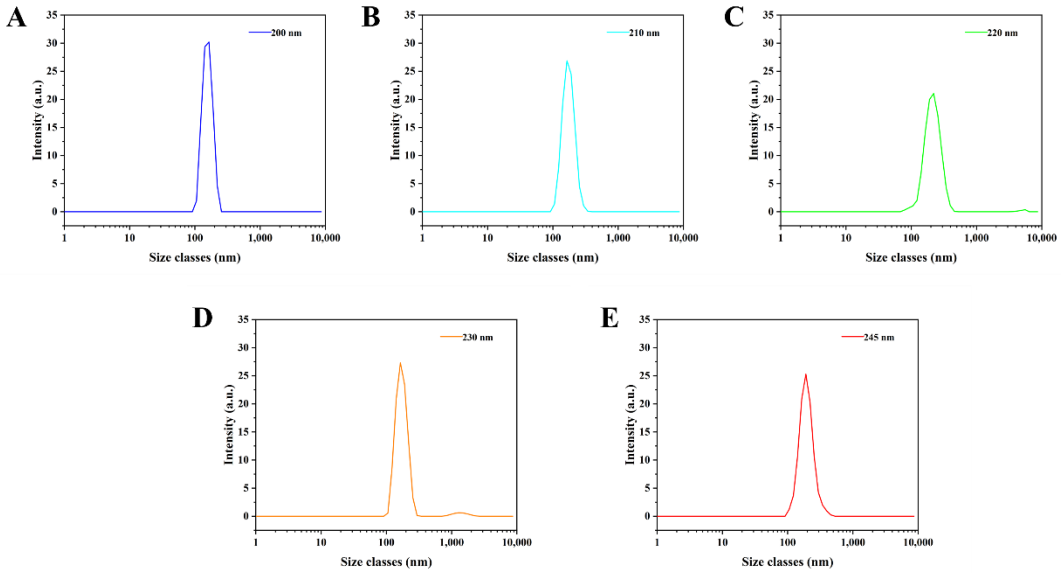


Fig. S4. DLS result of PS nanospheres with different sizes. (A) 200 nm; (B) 210 nm; (C) 220 nm; (D) 230 nm; (E) 245 nm.

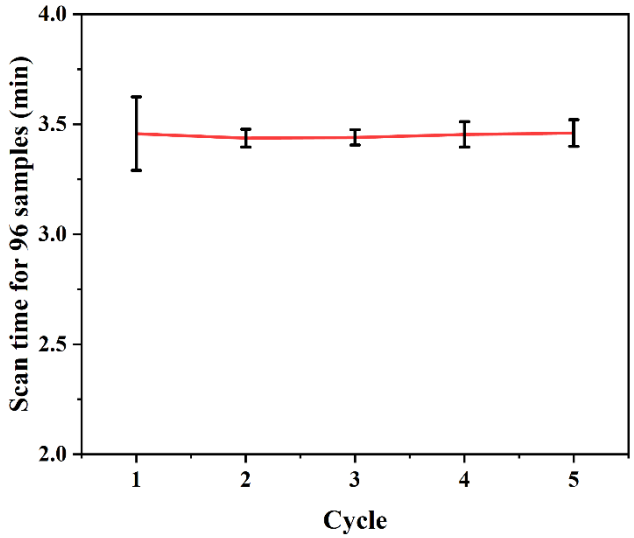


Fig. S5. Scanning rate of the proposed PCs array method. The error bar for each cycle represented the standard deviation of three parallel tests.

Table S1 List of the enterovirus genes from GeneBank for target sequences screening.

Enterovirus	GeneBank serial number	Year	Country/Region
CVA6	MG385831.1	2017	China
CVA6	FJ525951.1	2009	United Kingdom
CVA6	MH544999.1	2019	China
CVA6	MT577721.1	2018	India
CVA6	MN712205.1	2019	China
CVA6	MN233836.1	2017	China
CVA6	MN336326.1	2017	China
CVA6	KY211736.1	2013	China
CVA6	KY211721.1	2015	China
CVA6	KU958491.1	2014	China
CVA6	KX212485.1	2014	Sweden
CVA6	KP143073.1	2004	China
CVA6	KP129344.1	2008	Finland
CVA6	KM079513.1	2009	China
EV71	MT861101.1	2012	China
CVA16	AB542221.1	2010	China
Poliovirus	GQ329726.1	1999	China

Table S2 RNA sequences in this work.

Name	Sequence	Length	Position on the target
Tandem CRISPR/Cas13a and PC analysis			
CVA6	GGCGAGUGUAGAACACUUUUACUCUCG CGCAGGGCUGGUAGGAGUUGUGGAGGU GAAGGACUCGGGCACUAGCCUGGAUGG GUACACAGUUUGGCCCAUAGAUGUGAU G	109	-
crRNA1	GACCACCCCAAAAAUGAAGGGGACUAA AACAGUAAAAGUGUUCUACACUCGCC	53	57-79
crRNA2	GACCACCCCAAAAAUGAAGGGGACUAA AACCUCCACAACUCCUACCAGCCCUG	53	86-108
crRNA3	GACCACCCCAAAAAUGAAGGGGACUAA AACUGUACCCAUCCAGGCUAGUGCCC	53	120-142
crRNA4	GACCACCCCAAAAAUGAAGGGGACUAA AACCAUCACAUCUAUGGGCCAAACUG	53	143-165
ssRNA probe	FAM-UUUUUU-BHQ	6	-
Specificity analysis			
EV71	UACGACGGAUACCCCACAUUCGGAGA	26	-
CVA16	UGUACCAGCACUGCAAGCCGCGGAGA	26	-
poliovirus	UGUAGGGGUAAAUUUUCUUUAAUUCGG	27	-