Supporting Information

CRISPR/Cas12a antifouling nanocomposite electrochemical biosensors enable amplification-free detection of Monkeypox virus in complex biological fluids

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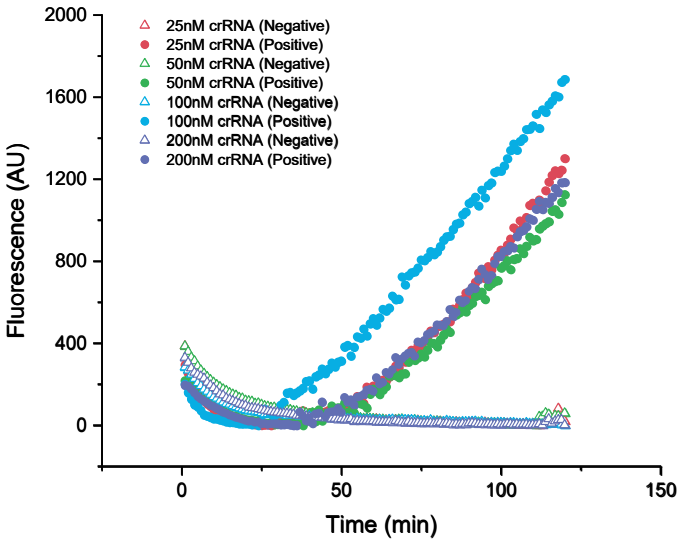

Figure S1. We optimized the crRNA concentration ranging from 25nM to 200nM. Among the tested concentrations, fluorescence intensity was highest at 100nM crRNA; thus we selected this crRNA concentration for further experiments.

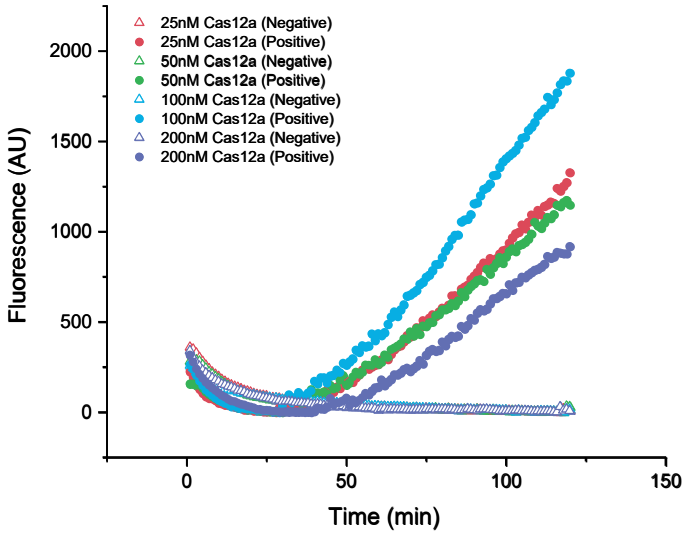

Figure S2. We optimized the Cas12a concentration ranging from 25nM to 200nM. Among the tested concentrations, fluorescence intensity was highest at 100nM Cas12a; thus we selected this Cas12a concentration for further experiments.

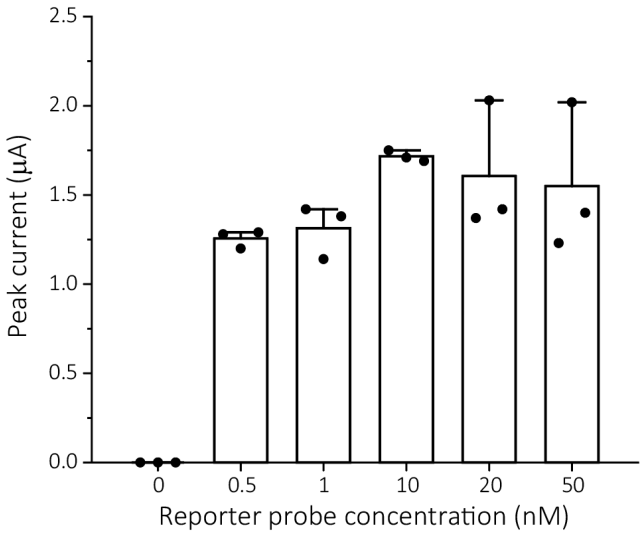

Figure S3. We optimized the concentration of the ssDNA reporter probe, ranging from 0.5nM to 50nM, in electrochemical sensors. Among the tested concentrations, the peak current was highest at 10nM, indicating sufficient binding of the reporter probes to PNA capture probes. Therefore, we selected a 10nM reporter concentration for further experiments.

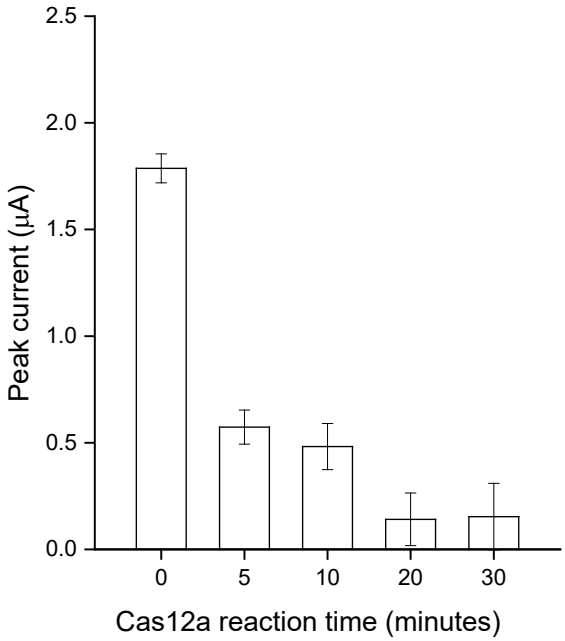

Figure S4. We optimized the Cas12a reaction time ranging from 0 to 30 minutes in electrochemical sensor. Among tested reaction time, the highest cleavage efficiency of Cas12a was observed at reaction times exceeding 20 minutes; thus, we selected Cas12a reaction time of 20 minutes for further experiments.

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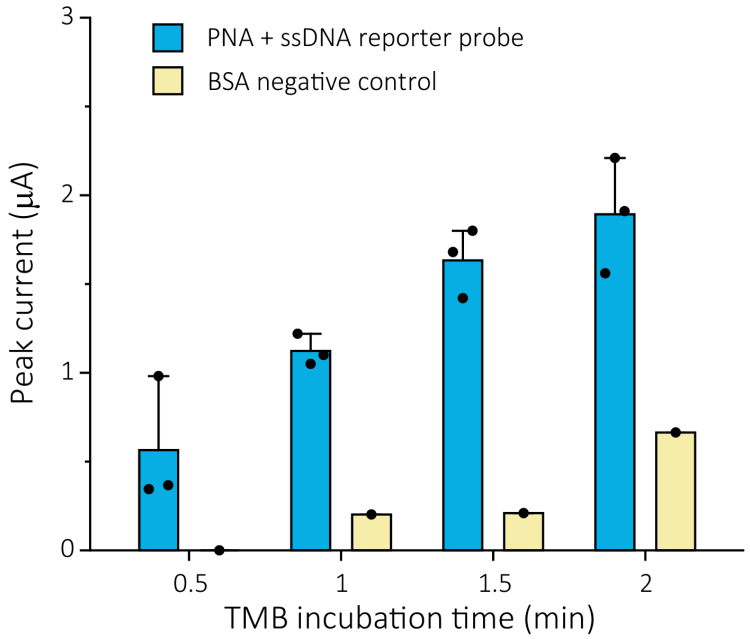

Figure S5. We optimized the concentration of the TMB incubation times, ranging from 0.5 to 20 minutes. Prolonged incubation leads to TMB precipitation extending beyond the electrode with the PNA probe, causing non-specific signals even in the BSA control. Among the tested incubation time, the peak current was highest at 1.5 minutes with negligible non-specific signal from BSA negative control; thus, we selected a TMB incubation time of 1.5 minutes for further experiments.

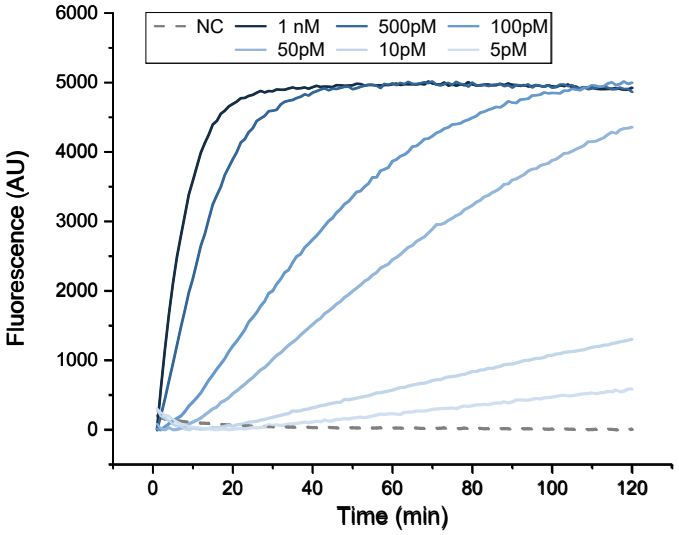

Figure S6. Fluorescence detection of synthetic F3L gene of MPXV in PBS. Combination of crRNA 4+5+6 was utilized to detect serially diluted F3L gene. Signal of negative control (NC) was recorded in the absence of the target F3L gene.

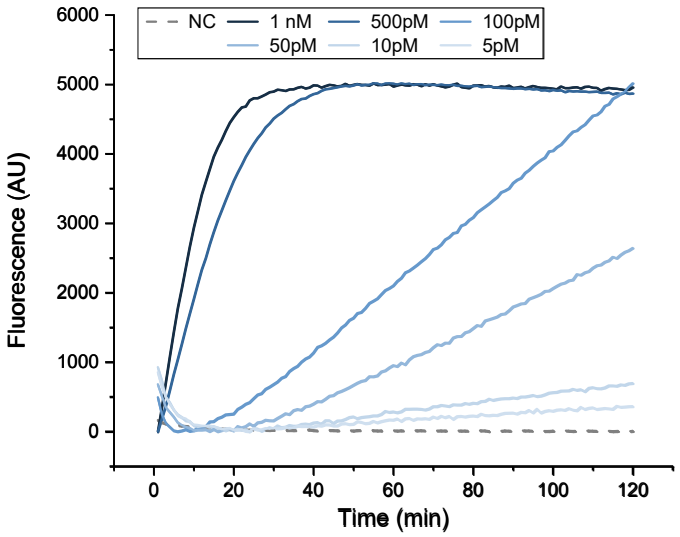

Figure S7. Fluorescence detection of F3L gene spiked in human serum. Combination of crRNA 4+5+6 was utilized to detect serially diluted F3L gene. Signal of negative control (NC) was recorded in the absence of the target F3L gene.

Table S1. Sequences of oligonucleotide used in this experiment.

Type	Source	Description	Sequence (5' to 3')
crRNA	IDT	crRNA 1	rUrA rArUrU rUrCrU rArCrU rArArG rUrGrU rArGrA rUrUrG rArUrG rUrCrU rArGrU rUrUrC rGrUrA rArUrA
crRNA	IDT	crRNA 2	rUrA rArUrU rUrCrU rArCrU rArArG rUrGrU rArGrA rUrCrA rUrCrU rGrCrC rUrUrA rUrCrG rArArU rArCrU
crRNA	IDT	crRNA 3	rUrA rArUrU rUrCrU rArCrU rArArG rUrGrU rArGrA rUrArC rArCrU rArUrU rUrUrC rCrGrU rCrArA rUrUrG
crRNA	IDT	crRNA 4	rUrA rArUrU rUrCrU rArCrU rArArG rUrGrU rArGrA rUrGrC rGrGrG rArUrA rCrArU rCrArU rCrUrA rUrUrA
crRNA	IDT	crRNA 5	rUrA rArUrU rUrCrU rArCrU rArArG rUrGrU rArGrA rUrGrA rUrArU rArUrG rUrUrC rCrArA rCrUrC rUrGrU
crRNA	IDT	crRNA 6	rUrA rArUrU rUrCrU rArCrU rArArG rUrGrU rArGrA rUrArC rCrGrG rArArU rArArC rArUrC rArUrC rArArA
PNA	Panagene	Capture probe	amine-ACAACAACAACAACA
DNA	IDT	Reporter probe	Biotin-AT TAT TAT TAT TAT TTG TTG TTG TTG TTG T

*r: ribonucleotide

Table S2. Comparison of our technology with previous CRISPR-based electrochemical diangnostics.

	CRISPR/Cas	Target	LOD	Turnaround time	Target amplification	Antifouling activities
This work	Cas12a	MPXV	682 fM ($4*10^5$ copies/μl)	< 35 min	X	O
[1]	Cas12a	HPV16	30 pM	60 min	X	X
[2]	Cas12a	HPV16	50 pM	> 60 min	O	X
[3]	Cas12a	HPV18	$1.2*10^4$ copies/μl	2-3 hours	O	X
[4]	Cas12a	SARS-CoV-2	77 pg/ml	> 60 min	O	X
[5]	Cas13a	microRNA	10 pM	< 4 hours	X	X
[6]	Cas12a	SARS-CoV-2	2.3 copies/μl	50 min	O	O
[7]	Cas13a	microRNA	50 aM	36 min	O	X

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