Electronic Supplementary Material (ESI) for Analyst.

Supplementary Materials

One-tube dual-readout biosensor for detection of nucleic acids and non-nucleic acids using CRISPR-ALP tandem assay

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Native polyacrylamide gel electrophoresis (20%) was conducted to characterize the cleaved products of hyDNA. Prepared samples were mixed with 6 X loading buffer and the electrophoresis was performed at a voltage of 100V for 30 to 60 min. 0.5 X TBE buffer was used as the electrophoresis buffer in the experiment. The gel was visualized with Gel Doc XR (BioRad Company, USA).

Preparation of MB@hyDNA

HyDNA sense strand (400 nM) and anti-sense strand (400 nM) were dissolved in buffer (pH 7.4, 20 mM Tris-HCl, 300 mM NaCl) and denatured at 95 °C for 10 min. Then, they were cooled at room temperature for 1 h to form hyDNA. Then, streptavidin-MB beads (4 μL) were washed with 100 μL buffer (pH 7.4, 20 mM Tris-HCl, 300 mM NaCl) for three times and incubated with 400 nM hyDNA for 30 min. Afterwards, the supernatant was removed and washed three times with 100 μL PBS buffer.

Preparation of tyramine-ALP conjugates

10 μL of 10 mM biotin-tyramine was incubated with 2 μL of 0.4 mg/mL streptavidin-ALP in 100 μL buffer (pH 8.0, 20 mM Tris-HCl) for 30 min. After that, 4 μL of 50 mM biotin was added to block the remaining streptavidin sites. Afterwards, the resultant mixtures were centrifuged (13500 x g) for 15 min at 4 °C to remove the extra biotin and biotin-tyramine for three times. Finally, the obtained tyramine-ALP conjugates were resuspended in 50 μL of PBS buffer and stored at 4 °C for further use.

RPA assay

RaPure virus RNA/DNA kit was used to extract nucleic acids of pseudoviruses. Specific targets of SARS-CoV-2 and Monkeypox Virus were amplified by using RPA and RT-RPA kit. The reaction buffer contained 1 μL of 10 μM each primer, 12.5 μL reaction buffer V, 4.0 μL water and 5.0 μL template. 1.5 μL of 280 mM MgOAc were then added to the tube and the mixture was incubated at 37°C for 15 min. The amplification products were subsequently analyzed by 2% agarose gel electrophoresis. The experiment was conducted in 0.5 X TBE buffer. The gel was visualized by Gel Doc XR (BioRad Company, USA).

Preparation of ATP solution

ATP-activator (5 μM), probe-1(10 μM), and probe-2 (10 μM) were dissolved in buffer (pH 7.4, 40 mM HEPES, 100 mM NaCl, 20 mM $MgCl_2$) and heated to 95 °C for 10 min to form the locked activator. 5 μL of different concentrations of ATP were mixed with 5 μL of locked activator for 10 min to obtain ATP solution for ATP detection.

Supplementary figures

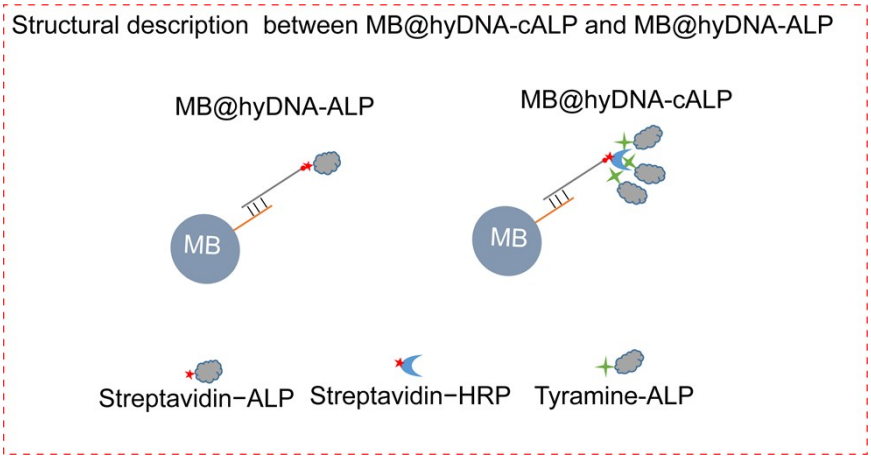

Fig. S1 The detailed structural description MB@hyDNA-cALP and MB@hyDNA-ALP.

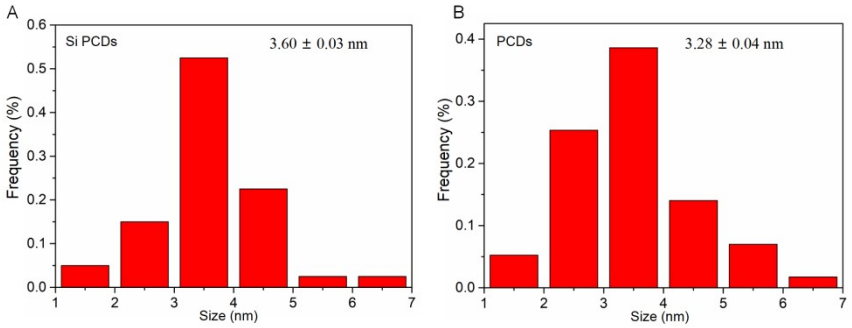

Fig. S2 The frequency versus particle size of Si PCDs (A) and PCDs (B).

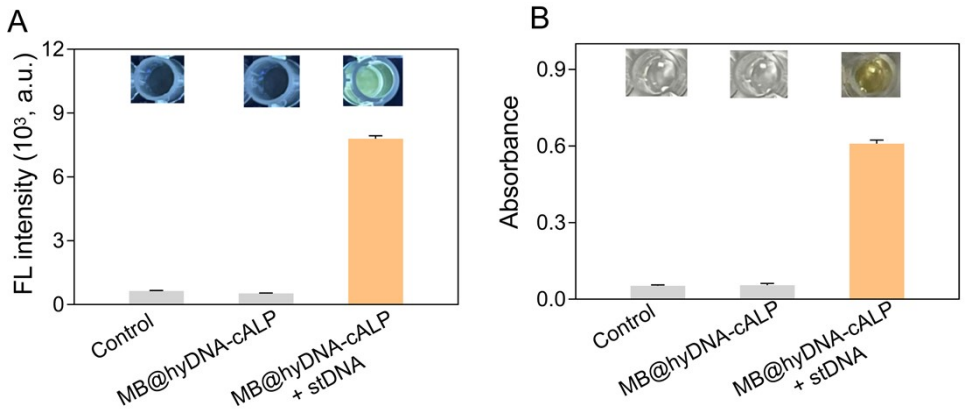

Fig. S3 The feasibility of the established Calpin-CRISPR platform for FL (A) and absorbance (B) intensity detection of stDNA target based on PCDs. The insets in (A) and (B) show the visual images under UV and white light, respectively.

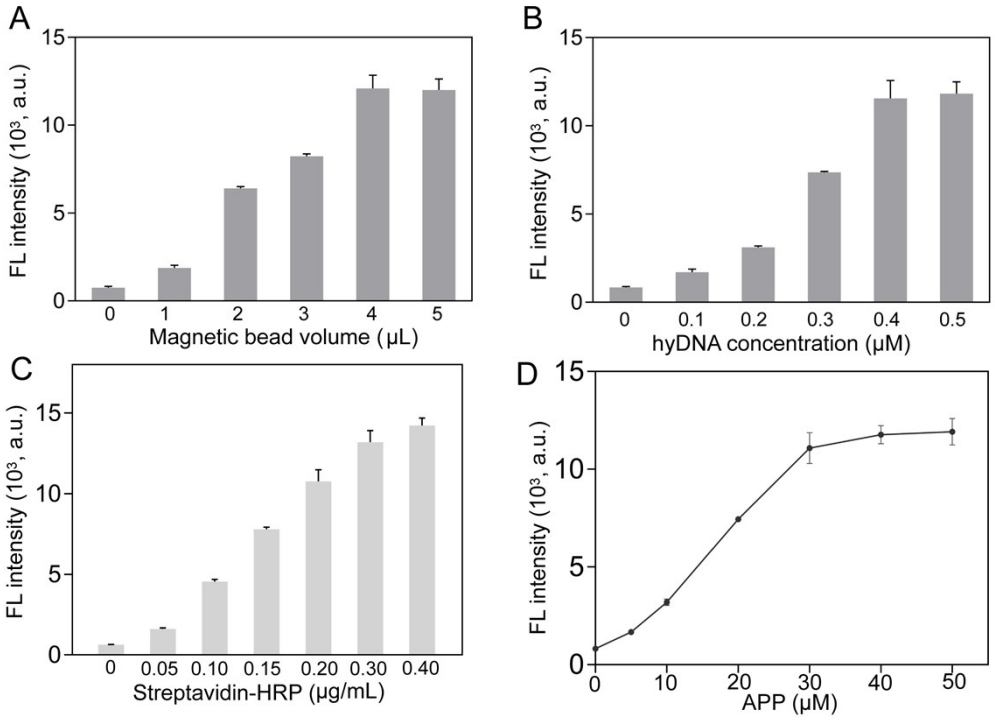

Fig. S4 (A) The FL intensity versus different volumes of MB. (B) The FL intensity versus different concentrations of hyDNA. (C) The FL intensity versus different concentrations of streptavidin-HRP. (D) The FL intensity as a function of APP concentration.

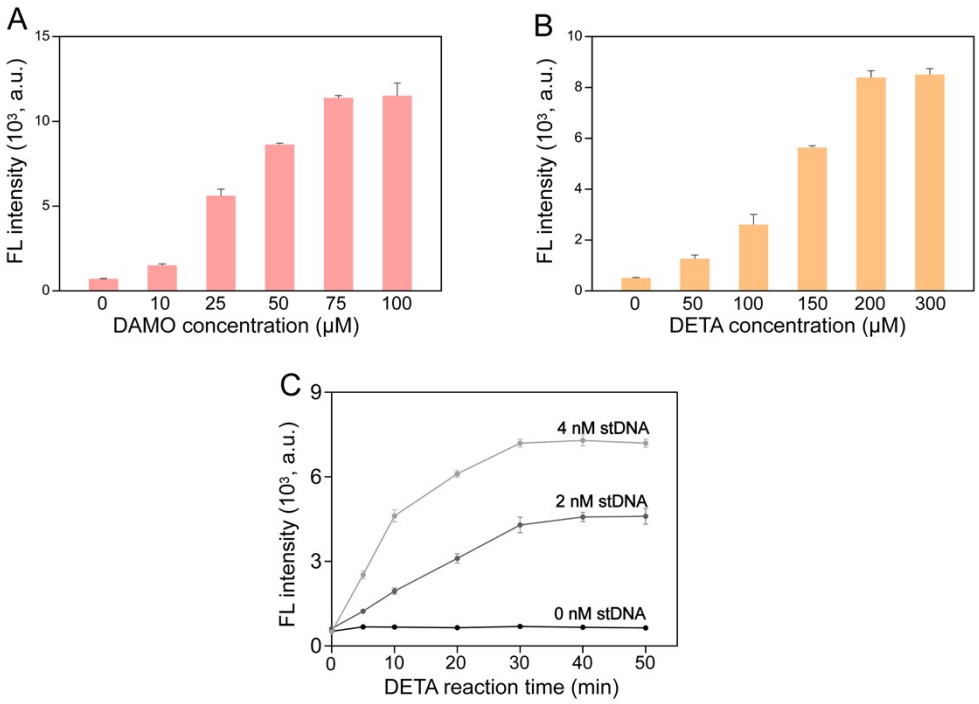

Fig. S5 (A) The FL intensity versus different concentrations of DAMO. (B) The FL intensity versus different concentrations of DETA. (C) The FL intensity as a function of DETA reaction time.

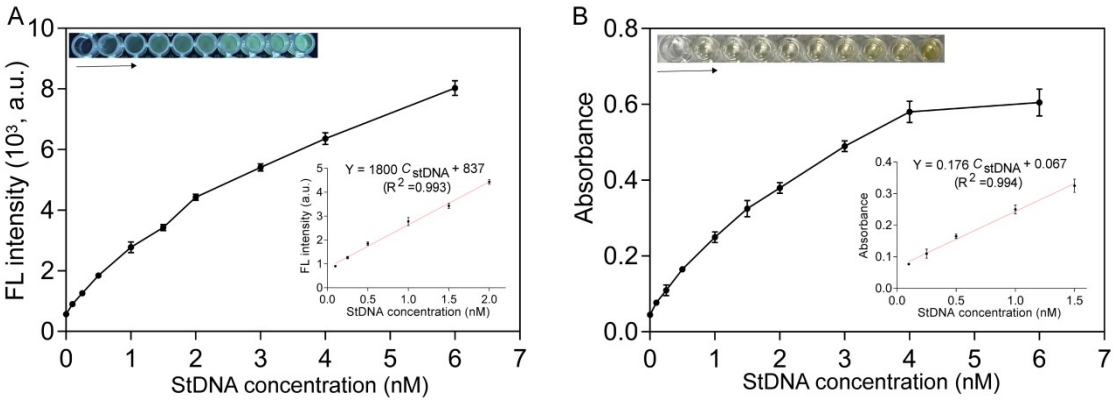

Fig. S6 (A) The fluorescence intensity versus different concentrations of stDNA (0, 0.1, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 nM) based on PCDs (Top inset: visual image changes of increasing concentration of stDNA target under 365 nm UV light; right inset: a calibration curve over the range of 0.1-2 nM). (B) The absorbance intensity versus different concentrations of stDNA (0, 0.1, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6 nM) based on PCDs (Top inset: visual image changes of increasing concentration of stDNA target under natural light; right inset: a calibration curve over the range of 0.1-1.5 nM)

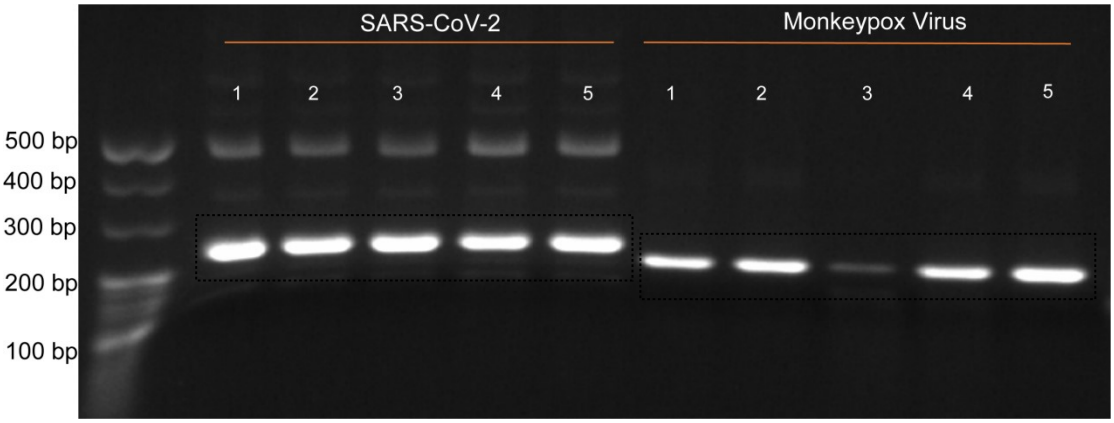

Fig. S7 Agarose gel detection of the amplified products of pseudovirus corresponding to SARS-CoV-2 and Monkeypox Virus-F3L gene by RPA. Red and blue frames represent five repeats experiments.

Fig. S8 Design of the ATP aptamer and "Locked" Activator.

Table. S1 DNA and RNA sequences used in this study.

Name	Sequence (5'-3')	Purpose
HyDNA sense strand	Biotin-AAAAAAAAAAAAAAAAAAACACAACCATCA	For hyDNA
HyDNA anti-sense strand	Bio-AAAAAAAAAATGATGGTTGTG	For hyDNA
FAM-hyDNA sense strand	FAM-AAAAAAAAAAAAAAAAAAACACAACCATCA	Analysis of hyDNA cleavage
FAM-hyDNA antisense strand	FAM-AAAAAAAAAATGATGGTTGTG	Analysis of hyDNA cleavag
COV-crRNA	UAAUUUCUACUAAGUGUAGAUCUGCUGCUUGACAG AUUGAACCA	Target recognition
MV-crRNA	UAAUUUCUAAGUGUAGAUCAUCUGCCUUAUCGAAU ACUCUU	Target recognition
RPA -(COV)-forward primer	GAAATTCAACTCCAGGCAGCAGTAGGGGAAC	RPA
RPA -(COV)-reverse primer	GAAAGCTTGTGTTACATTGTATGCTTTAGTG	RPA
RPA -(MV)-forward primer	CTAAGAAGTTTATCTACAGCCAATTTAGCT	RT-RPA
RPA -(MV)-reverse primer	GTCACCGTTATTAATGAGTACTGTCAAATA	RT-RPA
Target (COV) -sense strand	TGATGCTGCTCTTGCTTTGCTGCTGCTTGACAGATTGA ACCAGCTTGAGAGC	Target generation
Target (COV) -antisense strand	GCTCTCAAGCTGGTTCAATCTGTCAAGCAGCAGCAAA GCAAGAGCAGCATCA	Target Generation
Target (FLU) -sense strand	GAGCGTTTTGATGAAGGACATTCAAAGCCAATTCGAG CA	Target generation
Target (FLU) -antisense strand	TGCTCGAATTGGCTTTGAATGTCCTTCATCAAAACGC TC	Target Generation
Target (RSV) -sense strand	GAGTATTTTGTGACACAATGAACAGTTTAACATTACC AA	Target generation
Target (RSV) -antisense strand	TTGGTAATGTTAAACTGTTCATTGTGTCACAAAATAC TC	Target Generation
ATP-crRNA	UAAUUUCUACUAAGUGUAGAUAAGGUUUGUGUGUU UACCUG	For ATP detection
ATP-activator	CCCAGGTAAACACACAAACCTT	For ATP detection
Probe-1	ACCTGGGGGAGTATTGCGGAGGAAGGTTTGTGT	For ATP detection
Probe-2	GTTTACCTGGGGGAGTATTGCGGAGGAAGGT	For ATP detection

Table. S2 Comparison of the Calpin-CRISPR platform with other Cas12a-based single-readout biosensors for nucleic acids and ATP.

Target	Methods	LOD	Ref.
ATP	Fluorescence	400 nM	(Shu et al. 2022)
	chemiluminescence	18 nM	(Li et al. 2020)
	Fluorescence	104 nM	(Niu et al. 2021)
	Fluorescence	4.75 μM	(Xiong et al. 2020)
	Colorimetry	1 μM	(Samanta et al. 2022)
	Colorimetry	50 μM	(Tang et al. 2022)
	Fluorescent and colorimetric dual-mode	10 μM	This work
DNA/RNA	Fluorescence	150 fM	(Wang et al. 2022)
	Electrochemistry	30 pM	(Zhang et al. 2022)
	Colorimetry	1 nM	(Ma et al. 2021)
	Colorimetry	40 pM	(Li et al. 2019)
	Electrochemistry	50 pM	(Dai et al. 2019)
	Electrochemiluminescence	2.8 pM	(Liu et al. 2022)
	Fluorescence	10 nM	(Chen et al. 2020)
	Fluorescent and colorimetric dual-mode	23 pM and 48 pM	This work

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