Electronic Supplementary Information

Dual-amplified CRISPR-Cas12a bioassay for HIV-related nucleic acid

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Experimental section

Materials and Reagents

All oligonucleotides (shown in Table S1) and fetal bovine serum (FBS) were obtained from Sangon Biotechnology Co. Ltd. (Shanghai, China). The Cas12a was obtained from Tolo Biotech Co., Ltd. (Shanghai, China). Dynabeads™ M-280 Streptavidin (10mg/μL) was obtained from Thermo Fisher Scientific Inc. (Massachusetts, USA). Sodium chloride solid (NaCl, for acs, ≥99.9%), sodium phosphate dibasic dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$, AR, ≥99.9%), sodium phosphate monobasic dihydrate ($NaH_2PO_4{\cdot}2H_2O$, ≥99.5%), and sodium Citrate (98%+, Adamas) were obtained from Shanghai Titan Scientific Co. Ltd. (Shanghai, China). Tris-HCl solution (1 M, pH=7.4, sterile), magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_2O$, molecular biology grade), Hepes solution (1 M, Cell Culture Grade), and water-DEPC treated water were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). AuNPs were synthesized in our laboratory and ultrapure water (18.25 $M\Omega.cm^{-1}$) was used for the entire experiment. The normal human serum was obtained from Chengdu 7th People's Hospital.

Instruments

A commercial inductively coupled plasma mass spectrometry produced by PerkinElmer, Inc. (ICPMS, NexION 350, PerkinElmer, Inc.) was employed for measurement, and the working parameters were recorded in Table S2. A Thermo-shaker device used for shake reaction was purchased from Hangzhou Allsheng Instruments Co., Ltd. (Hangzhou, Shanghai). Zeta potential analyzer produced by Malvern Panalytical Ltd. (Zetasizer Nano ZS, Shanghai, China) were employed for the size and zeta potential characterization. TEM images were obtained by Tecnai G2 F20 S-TWIN (FEI, USA). The UV-vis spectra of solutions were measured by Cytation 5 Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc.)

Preparation of gold nanoparticles.

For the preparation of gold nanoparticles, we refer to the method reported in the literature. The reaction was carried out in a three-necked flask in which 100 mL of 0.01% $HAuCl_4$ solution was boiled and refluxed. Then 1 mL of the freshly prepared 3% sodium citrate solution was quickly added, continue heating for 30 minutes and then stop heating until the solution turns wine red, then cool to room temperature and store at 4 ^oC for later use.

Preparation of AuNPs-tagged probe.

First, treat the reporter DNA in a 10 mM TCEP solution for 1 hour, then add 120 μL of 10 μM treated reporter DNA to 1 mL of AuNPs solution, and add 10 μL of 0.1% tween 20 and 10 μL of 0.5 M citric acid sodium (pH=7.0). Subsequently, 200 μL of 3 M NaCl was added 8 times to the final concentration of NaCl is 0.5 M, with an interval of 20 min and ultrasound for 30 s to prevent the accumulation of AuNPs. After the addition, continue to react at room temperature for more than 6 h, so that the reporter DNA anchored on the surface of AuNPs is sufficient. And then, the solution was centrifuged at 16500 rpm for 12 min to remove excess reporter DNA in the supernatant, and then washed 3 times with 20 mM PB buffer (pH=7.4), and finally dispersed in 1 mL 10 mM Tris-HCl buffer (containing 100 mM NaCl) for later use. 20 μL MBs wash washed 3 times by binding buffer (10 mM Tris-HCl, 0.1% PEG 4000, 0.1% tween 20, 50 mM NaCl) and diluted to 40 μL, then added into the 1 mL of above-prepared solution. After reacting at room temperature overnight, the solution was separated magnetically and washed 3 times by 40 mM Hepes solution (containing 100 mM NaCl). Finally, dispersed in 40 μL Hepes solution for later use.

The trans-cleavage reaction of CRISPR-Cas12a on the AuNPs-tagged probe.

The reaction is carried out in 1×TOLO buffer 3, 1.5 mM Mg^{2+} and 0.1 μM crRNA were mixed firstly, and then 30 nM Cas12a, certain concentration of target DNA, 7.5 μL AuNPs-tagged probe solution and diluted in 100 μL with DEPC in final, and the mixture was reacted at 37 ^oC for 40 min. Subsequently, the mixture was separated magnetically, and the supernatant was collected and digested with 50 μL aqua regia for 20 min, then diluted to 4 mL and then sent to ICPMS for detection.

Quantitative detection in complex samples.

Quantitative detection is performed in fetal bovine serum (FBS) and normal human serum. The FBS and normal human serum were first diluted 10-fold, and then directly added different concentrations of target DNA to prepare the test sample. Subsequently, the trans-cleavage reaction of CRISPR-Cas12a and ICPMS measurement followed the procedures described above.

Table S1. Sequences of the Oligonucleotides.

Note	Sequences (5'→3')
crRNA	UAAUUUUCUACUAAGUGUAGAUAUGUGGAAAAUCUCUAGCAG
Reporter DNA (T75)	Biotin-T75-(CH2)6-SH
HIV-DNA	ACTGCTAGAGATTTTCCACAT
HAV	HAVGGACTTGATACCTCACCGCC
HBV	ATACCACATCATCCATATAACTGAAAGCCA
HCV	ATCTCCAGGCATTGAGCGGGTTTATCCAGGA
HPV-16	AATATGTCATTATGTGCTGCCATATCTACTTCAGAAACT

Table S2. Working parameters of ICPMS.

Parameters	settings
Nebulizer Gas Flow (L/min)	0.93
Auxiliary Gas Flow (L/min)	1.2
Plasma Gas Flow (L/min)	18
ICP RF Power (w)	1300
Dwell time (ms)	25
Dead time (ns)	35
Sweeps per reading	120
Isotope monitored	^{197}Au

Table S3 Some nucleic acid amplification-free CRISPR-Cas biosensors.

Detection means	Pricinple	LOD	Time	Target	Ref.
Fluorescence	A size-selective DNA nanocage-based activatable CRISPR/Cas12a system	10 pM	2-3 h	miRNA-21	1
	A spherical nucleic acid (SNA) reporter for stable and sensitive CRISPR-Dx biosensing	10 fM	1 h	HPV-16	2
Electrochemical	a CRISPR-Cas12a based electrochemical biosensor	50 pM	3 h	HPV-16	3
Microvolume	Solid-State CRISPR-Cas12a-Assisted Nanopores	10 nM	1 h	HIV-1 DNA	4
This work	AuNPs-tagging base CRISPR-Cas12a bioassay	1.05 amol (10 fM)	40 min	HIV-DNA	-

Table S4. Spike recovery experiment of HIV-DNA in fetal bovine serum and normal human serum.

Sample	Added (fmol)	Measured (fmol)	Recovery (%)	RSD (%)
FBS	1.00	1.10	110	2.33
	10.0	9.37	93.7	1.56
	100	91.1	91.1	4.43
Normal human serum	1.00	1.05	105	3.84
	10.0	10.2	102	1.81
	100	95.0	95.0	2.93

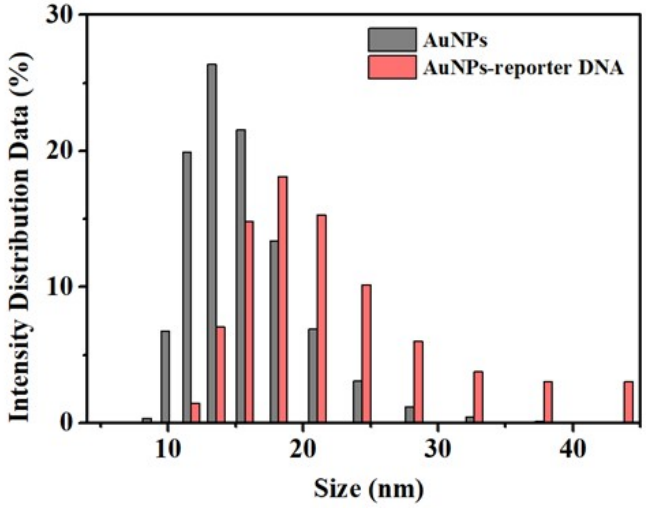

Fig. S1. DLS data of AuNPs and AuNPs-reporter.

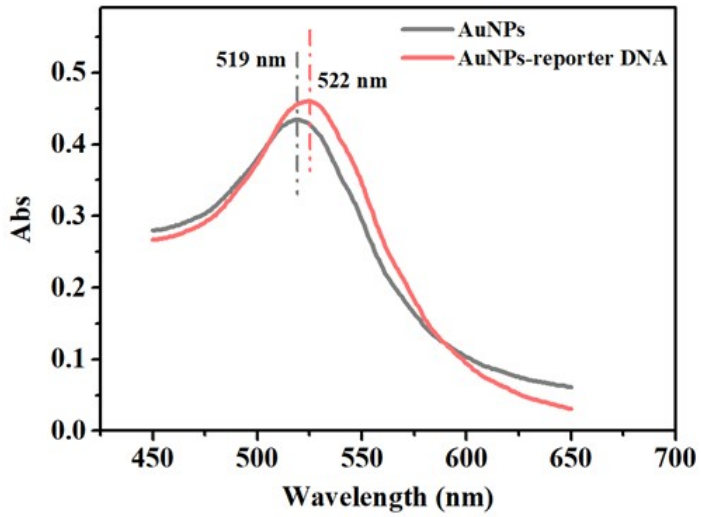

Fig. S2 UV-vis spectra of AuNPs and AuNPs-reporter.

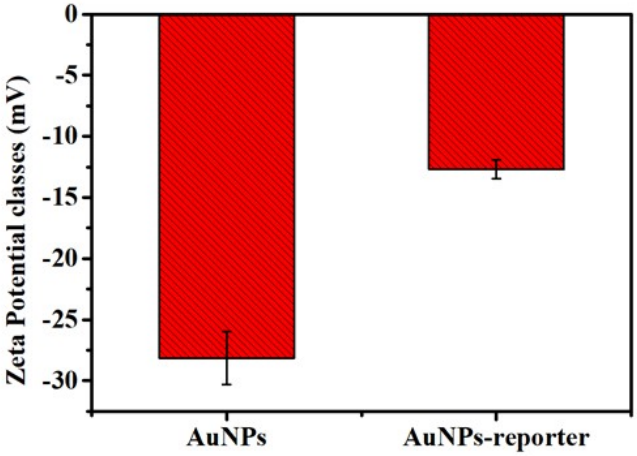

Fig. S3 Zeta potentials of AuNPs and AuNPs-reporter.

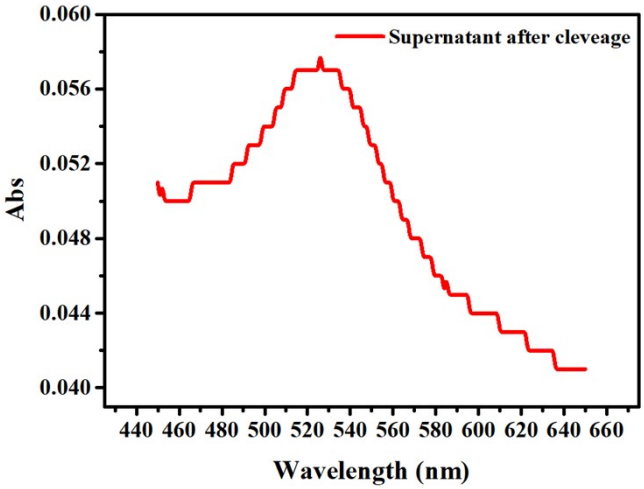

Fig. S4 UV-vis spectra of the supernatant after the trans-cleavage reaction. (The supernatant is the solution after trans-cleavage of CRISPR-Cas12a in 100 nM HIV-DNA.)

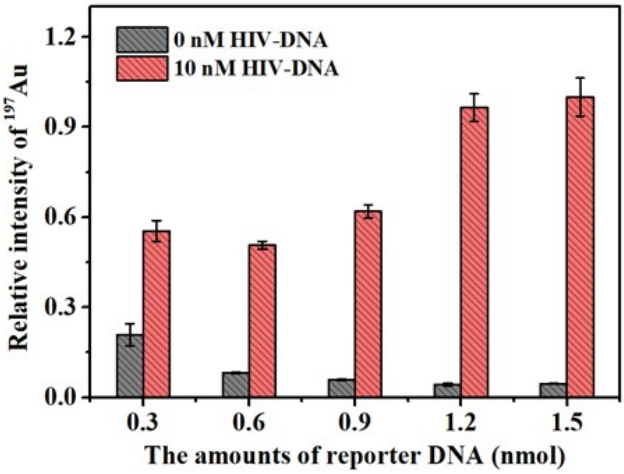

Fig. S5 The effect of amount of reporter DNA on the AuNPs-tagging based CRISPR-Cas12a bioassay. (Conditions: 1.5 mM Mg^{2+}, 30 mM Cas12a, crRNA: Cas12a=4:1, 2.25 μM AuNPs-tagged probe, 37 °C, 40 min.)

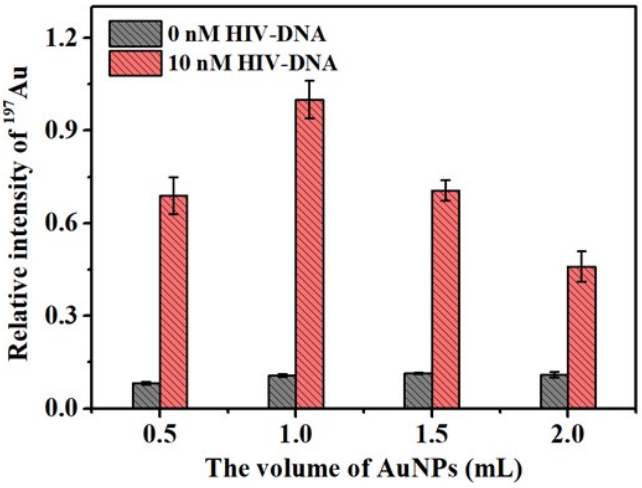

Fig. S6 The effect of AuNPs volume on AuNPs-tagging based CRISPR-Cas12a bioassay. (Conditions: 1.5 mM Mg^{2+}, 30 mM Cas12a, crRNA: Cas12a=4:1, 2.25 μM AuNPs-tagged probe, 37 °C, 40 min.)

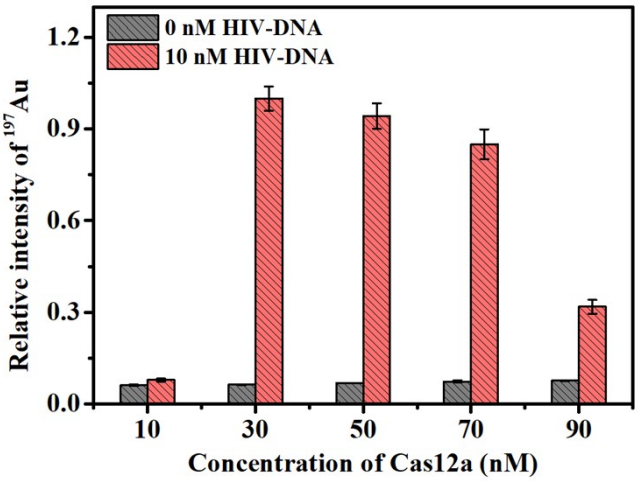

Fig. S7 The effect of concentration of Cas12a on the AuNPs-tagging based CRISPR-Cas12a bioassay. (Conditions: 1.5 mM Mg^{2+}, crRNA: Cas12a=4:1, 2.25 μM AuNPs-tagged probe, 37 °C, 40 min.)

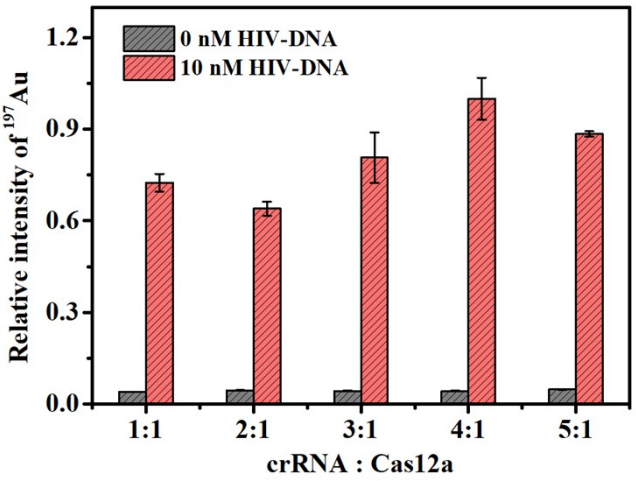

Fig. S8 The effect of different ratios of crRNA: Cas12a on AuNPs-tagging based CRISPR-Cas12a bioassay. (Conditions: 1.5 mM Mg^{2+}, 30 mM Cas12a, 2.25 μM AuNPs-tagged probe, 37 °C, 40 min.)

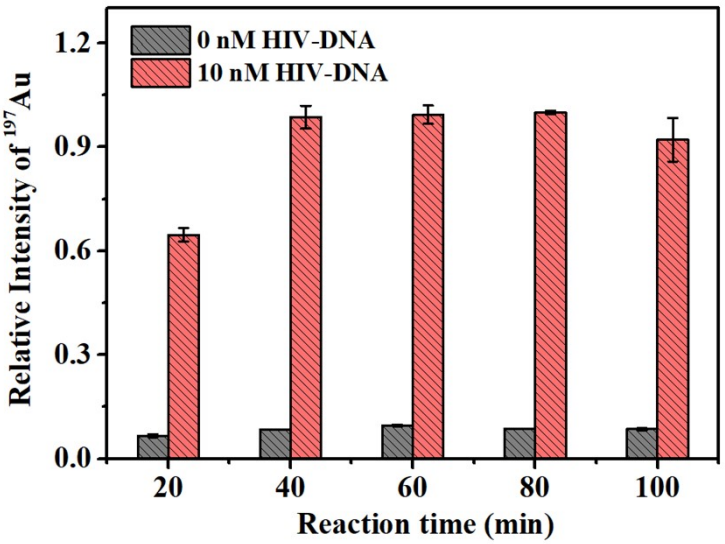

Fig. S9 The effect of different reaction times on AuNPs-tagging based CRISPR-Cas12a bioassay. (Conditions: 1.5 mM Mg^{2+}, 30 mM Cas12a, crRNA: Cas12a=4:1, 2.25 μM AuNPs-tagged probe, 37 °C.)

References

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