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Electronic Supplementary Information

Double amplification bioassay based on CRISPR-Cas12a and 3D nanomachine for HPV-16 sensitive detection

Yueli Hu,[a] Liwei Liu, [b] Chaoqun Wang, [c] Jing Zhou, [c] Rui Liu,[a*] and Yi Lv [a,c*]

[a] Key Laboratory of Green Chemistry and Technology of Ministry of Education, College of Chemistry, Sichuan University, Chengdu 610064, China.

[b] State Key Laboratory of Vanadium and Titanium Resources Comprehensive Utilization, Pangang Group Research Institute Co, Ltd, Panzhihua 617000, China.

[c] Analytical & Testing Centre, Sichuan University, Chengdu 610064, China.

Email: liur@scu.edu.cn (R. Liu); lvy@scu.edu.cn (Y. Lv)
Tel. and Fax: +86-28-8541-2798.

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Experimental section

Reagents and Materials

Streptavidin-coated magnetic micro spheres (MBs) were Dynabeads™ M-280 Streptavidin (10mg/μL) were purchased from Thermo Fisher Scientific Inc. (USA). 1,4,7,10-Tetraazacyclododecane-1,4,7-tris-aceticacid-10-maleimidoethylacetamide (MMA-DOTA) was purchased from Macrocyclics, Inc. (USA). Terbium chloride hexahydrate ($TbCl_3 \cdot 6H_2O$) were purchased from Aladdin Reagent Inc. (Shanghai, China). Tris (2-carboxyethyl) phosphine (TCEP) was obtained from Adamas Reagent, Ltd. (Shanghai, China). Tris (hydroxymethyl) aminomethane (Tris-HCl) stock solution, ethylenediaminetetraacetic Acid (EDTA) and DEPC-treated water were purchased from Shanghai Sangon Biotech Co. Ltd. (Shanghai, China). Sodium chloride (NaCl), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), ammonium acetate (NH_4AC), acetic acid (HOAC), nitric acid (HNO_3) were bought in Chengdu Kelong Chemical Reagent Company (China). 3K Amicon Ultra-0.5 NMWL spin filters was purchased from Merck Millipore (Germany). Ultrapure water (UPW, 18.2 MΩ cm^{-1}) was produced using a ULUPURE (Chengdu, China) water purification system.

All the oligonucleotides listed in Table S1 were synthesized and HPLC-purified by Shanghai Sangon Biotech Co. Ltd. (Shanghai, China). The clinical serum samples were collected by Chengdu Seventh People's Hospital.

Apparatus

A NexION 350 commercial inductively coupled plasma mass spectrometry (ICP-MS) from PerkinElmer Co., Ltd. was used for ^{159}Tb detection and the working condition of the instrument was exhibited in Table S3. Electrospray ionization mass spectrometry (ESI-MS) was recorded on a TSQ Quantum Ultra liquid chromatography-mass spectrometer (Thermo Fisher Scientific, Inc., USA). Element-labeling substrates were characterized by an AXIMA Performance matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS, Shimadzu, Japan). Zeta potential of magnetic beads were measured using a Zetasizer Nano ZS nanoparticle size and zeta potential analyzers (Malvern Panalytical, United Kingdom). The energy-dispersive spectrum (EDS) was processed by scanning electron microscopy (SEM, Hitachi, S3400).

Buffers

a. NH_4Ac buffer: 0.5 M NH_4Ac, pH 5.8
b. Wash buffer: 10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 7.4
c. Tris-HCl buffer: 20 mM Tris-HCl, 150 mM NaCl, pH 7.4
d. Binding and Washing (B&W) buffer: 5 mM Tris-HCl, 1 M NaCl, 0.5 mM EDTA, pH 7.4
e. 2×B&W buffer：10 mM Tris-HCl, 2 M NaCl, 1 mM EDTA, pH 7.4
f. NEBuffer r2.1: 10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 100 μg/ml Recombinant Albumin, pH 7.9

Labeling DNA substrate with Tb

The labeling process is carried out on the basis of the literature of Zhang's group,[1] and the specific experimental steps are shown in the Figure S1. In brief, the $TbCl_3$ and 1,4,7,10-Tetraazacyclododecane-1,4,7-tris-aceticacid-10-maleimidoethylacetamide (MMA-DOTA) were dissolved in NH_4AC buffer (pH 5.8). The same volume of 5 mM MMA-DOTA chelates with 10 mM Tb^{3+} at 37 ℃ for 1 h with gentle shaking. At the same time, the DNA substrate with sulfhydrylation dissolved in 0.5 M NH_4AC buffer (pH 5.8) reacted with excess TCEP at 37 ℃ for 30 min, then were purified using ultra-filtration tubes. The ultra-filtration involves centrifuging at 14000 rpm for 15 min with wash buffer to remove excess TCEP and then centrifuging at 1000 g for 2 min to transfer the product to a new centrifuge tube. The prepared DOTA-Tb is added and incubated at 37 ℃ for 2h with violent shaking. After centrifugation with wash buffer for five times to remove excess Tb^{3+} and with Tris-HCl buffer for the sixth time, the production is diluted to 1220 μL and stored at 4 ℃ for later use.

Preparation of the DNA Nanomachine

After washing 80 μL MBs with BW buffer for three times, 400 μL Tb^{3+}-labeled DNA is added and incubated at 25 ℃ for two hours. The successfully prepared nanomachine is washed with Tris-HCl buffer four times to remove excess Tb^{3+}-labeled DNA, and finally diluted to 1000 μL for use.

Analysis of target through double amplification

The trans-cleavage reaction of CRISPR-Cas12a is realized by mixing 10 μL 150 nM DNAzyme, 10 μL 20 nM Cas 12a and 20 μL different concentrations of target in a PCR tube and reacting at 37℃ for 60 min. After inactivating at 85 ℃ for 5 min, 20 μL 20 mM Mg^{2+} and 20 μL MBs are added to the PCR tube to walk at 25 ℃ for 40 min to release the Tb^{3+} on MBs. Finally, 70 μL supernatant is absorbed by magnetic separation and added to 1 mL 1%HNO_3 for ICPMS analysis.

Table S1 The DNA and RNA sequences used in this work.

Name	Sequences (5'→3')
crRNA	UAAUUUCUACUAAGUGUAGAUAGGGCGUAACCGAAAUCGGU
HPV-16	ACCGATTTCGGTTACGCCCT
Substrate	Biotin-T15CTCACTAT/rA/GGAAGAGATGT5-SH
DNAzyme-E5	CATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT
DNAzyme-E1	CTCCGAGCCGGTCGAAA
DNAzyme-E2	TTCTCCGAGCCGGTCGAAATA
DNAzyme-E3	TCTTCTCCGAGCCGGTCGAAATAGT
DNAzyme-E4	TCTCTTCTCCGAGCCGGTCGAAATAGTGA
HPV-18	GGAATGCTCGAAGGTCGTCT
HPV-31	GGTGAACCGAAAACGGTTGG
HPV-51	TCTGCTGTACAACGCGAAGG
HPV-58	ACAGCTAGGGCACACAATGG

Table S2 The comparison of analytical methods for HPV-16.

analytical method	linear range	LOD	references
Double amplification based on CRISPR-Cas12a and 3D nanomachine	10-400 pM	1.2 pM	this work
Fluorescent DNA-templated silver nanoclusters and hairpin-blocked DNAzyme-assisted cascade amplification	10 pM-10 nM	5.7 pM	2
CRISPR-Cas12a-Derived Photoelectrochemical Biosensor	5-5000 pM	1.2 pM	3
CRISPR/Cas12a-mediated liposome-amplified strategy	0.01-100 nM	1.6 pM	4
Electrochemical Resistive DNA Biosensor	5-20 nM	2.39 nM	5

Table S3 The working condition of ICPMS.

Parameter	Setting
Vacuum Pressure (Torr)	3.50×10^{-7}
ICP RF Power (W)	1300
Nebulizer Gas Flow (L/min)	0.94
Auxiliary Gas Flow (L/min)	1.2
Plasma Gas Flow (L/min)	18
Dwell Time (ms)	50
Dead Time (ns)	35
Sweeps per reading	120
Isotope monitored	^{159}Tb

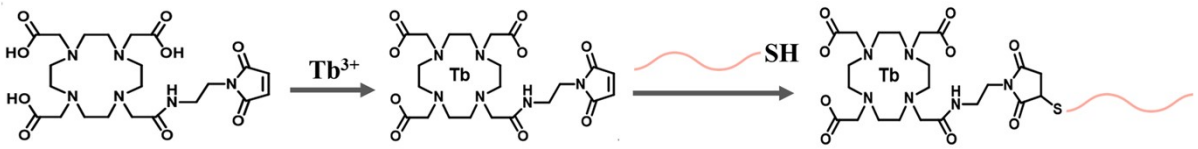


Fig. S1 Labeling process of DNA substrate with DOTA-Tb.

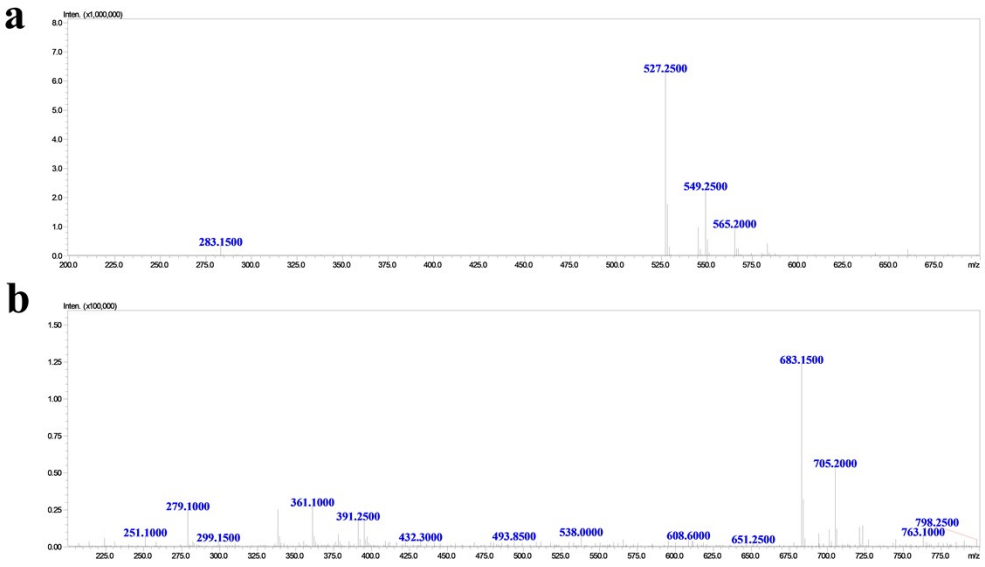


Fig. S2 ESI-MS of MMA-DOTA and DOTA-Tb. (a)MMA-DOTA, ESI-MS, m/z, found: 527.2 ($[M+H]^+$), 549.2 ($[M+Na]^+$); calc.: 527.5 ($[M+H]^+$). (b) DOTA-Tb, ESI-MS, m/z, found: 683.1 ($[M+H]^+$), 705.2 ($[M+Na]^+$); calc.: 683.4 ($[M+H]^+$).

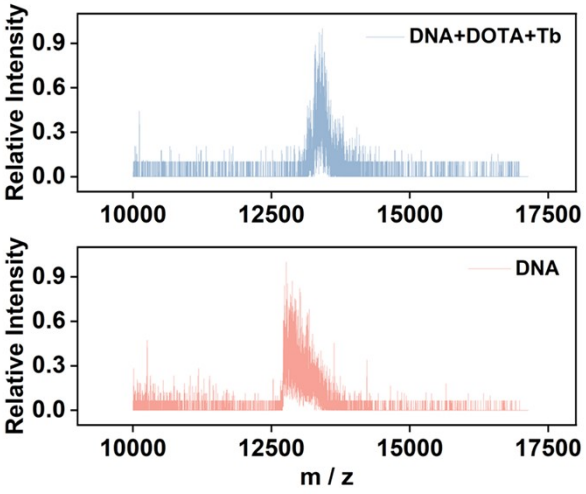
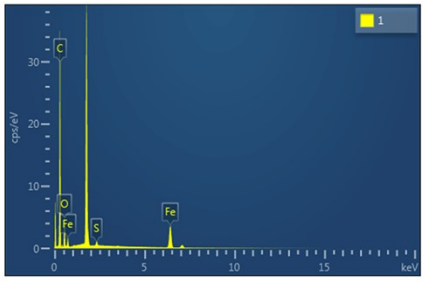


Fig. S3 MALDI-TOF of DNA and DNA+DOTA+Tb.

a



b

Element	Wt %
C	67.64
O	18.68
S	0.5
Fe	13.18
Total	100

Fig. S4 (a) EDS of bare MBs and (b) weight content distribution in EDS.

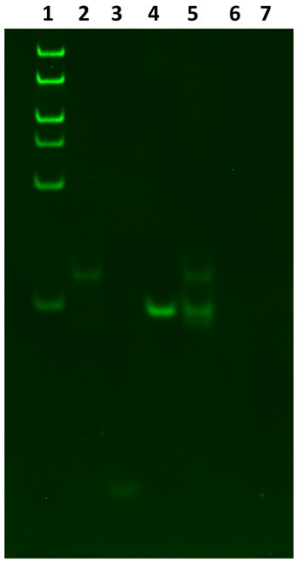


Fig. S5 PAGE image of DNA ladder (lane 1), 400 nM Cas12a/crRNA (lane 2), 4 μM HPV-16 (lane 3), 4 μM DNzyme (lane 4), 400 nM Cas12a/crRNA and 4 μM DNAzyme (lane 5), 400 nM Cas12a/crRNA and 4 μM HPV-16 (lane 6), 400 nM Cas12a/crRNA and 4 μM DNAzyme and 4 μM HPV-16 (lane 7).

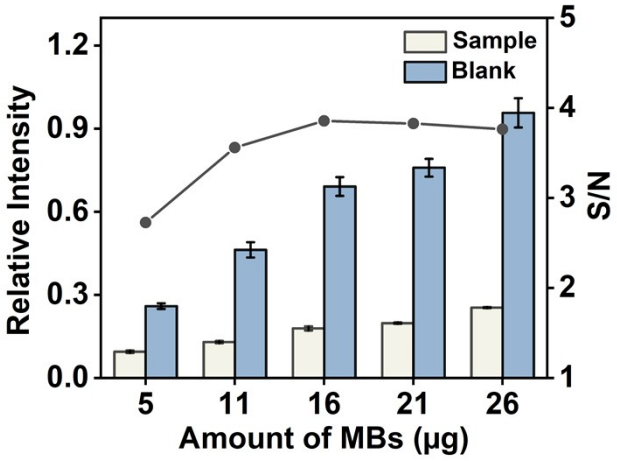


Fig. S6 Optimization of amount of MBs.

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