Supplementary Information

Isotope-Encoded Tetrahedral DNA for Multiple SARS-CoV-2 Variants Diagnosis

Ziyan Li,[a] Jing Zhou, [a] Chaoqun Wang, [a] Rui Liu,[b, *] Jianyu Hu, [c] and Yi Lv[a,b*]

[a] Analytical & Testing Center, Sichuan University, Chengdu 610064, China

[b] Key Laboratory of Green Chemistry & Technology, Ministry of Education, College of Chemistry, Sichuan University, Chengdu, Sichuan 610064, China

[c] Division of Analytical and Environmental Toxicology, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, Alberta T6G 2G3, Canada.

Email: liur@scu.edu.cn (Liu R.); lvy@scu.edu.cn (Lv Y.)

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Apparatus

The detection of stable isotopes was performed by ICPMS using glass concentric atomizer, with collision ball chamber (NexION 350, PerkinElmer, Inc.). The working conditions of ICPMS were optimized as Table S1. Forming of TDN was carried out on a K960 Thermal Cycler (Heal Force Inc., China). Hydration particle size characterization and zeta-potential of lanthanide nanoparticles and magnetic beads was implemented using a Malvern Zetasizer Nano ZS90 (Malvern PANalytical Ltd., Shanghai, China). The energy-dispersive spectrum (EDS) was processed by scanning electron microscopy (SEM, Hitachi, S3400). Transmission electron microscopy (TEM) images was carried out by a JEM-2010 microscope (JEOL Co., Japan) at an accelerating voltage of 200 kV. A Mettler-Toledo ME104 microbalance was used for reagents weighing. Electrospray ionization mass spectrometry (ESI-MS) was recorded on a TSQ Quantum Ultra liquid chromatography-mass spectrometer (Thermo Fisher Scientific, Inc., USA). Isotope-labeling substrates were characterized by an AXIMA Performance matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS, Shimadzu, Japan).

Materials and reagents

Sodium chloride (NaCl), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), sodium hydroxide (NaOH), ammonium acetate (NH_4AC), acetic acid (HOAC), nitric acid (HNO_3) were bought in Chengdu Kelong Chemical Reagent Company (China). Terbium chloride hexahydrate ($TbCl_3 \cdot 6H_2O$), holmium chloride hexahydrate ($HoCl_3 \cdot 6H_2O$) and thulium chloride hexahydrate ($TmCl_3 \cdot 6H_2O$) were purchased from Aladdin Reagent Inc. (Shanghai, China). Tris (2-carboxyethyl) phosphine (TCEP) was obtained from Adamas Reagent, Ltd. (Shanghai, China). 1,4,7,10-Tetraazacyclododecane-1,4,7-tris-acetic acid-10-maleimidoethylacetamide (MMA-DOTA, product no. B-272) was acquired from Macrocyclics, Inc. (USA). Tris (hydroxymethyl) aminomethane (Tris-HCl) stock solution, tris-EDTA buffer (TE

buffer) were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The Streptavidin Magnetic Beads (SA-MBs, 1 μm, 10 mg mL^{-1}) were bought from MedChemExpress (Shanghai, China).

Gel electrophoresis (PAGE)-related reagents containing ammonium persulfate, acrylamide, N,N'-methylenebis(acrylamide), and N,N,N',N'-tetramethyl ethylenediamine were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and used as supplied.

Ultrapure water with 18.24 MΩ cm^{-1} was obtained from a UPURE Sichuan water purification system. All the water used in the RNA hybridization process was nuclease-free under DEPC treatment.

All oligonucleotides used in this study (DNA and RNA oligo) were HPLC-purified and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Their sequences are listed in Table S2.

RNA viral sample collection tubes (inactivated) and EZ-10 spin column viral total RNA extraction kit were purchased in Sangon Biotech Co., Ltd. (Shanghai, China).

A series of buffer was involved in this work, with details listed as follows:

NH_4AC buffer: 0.5 M NH4AC, pH 5.8

5×TBE buffer: 450 mmol L^{-1} Tris, 450 mmol L^{-1} H_3BO_3 and 10 mmol L^{-1} EDTA in DEPC water (pH 8.3).

2×B&W buffer: TE buffer and 2 mol L^{-1} NaCl in DEPC water (pH 8.0).

TE buffer: (pH 6.0): 10 mmol L^{-1} Tris-HCl and 1 mmol L^{-1} EDTA in DEPC water (pH 8.0).

TM buffer: 10 mmol L^{-1} Tris-HCl and 50 mmol L^{-1} $MgCl_2$ in DEPC water (pH 8.0).

TMT buffer: 10 mmol L^{-1} Tris-HCl, 150 mmol L^{-1} NaCl, 50 mmol L^{-1} $MgCl_2$ and 0.05% Tween-20 in DEPC water (pH 7.4).

TMR buffer: 10 mmol L^{-1} Tris-HCl, 150 mmol L^{-1} NaCl and 50 mmol L^{-1} $MgCl_2$ in DEPC water (pH 7.4).

Experimental Details

AFM Analysis.

The ssDNA and TDN were diluted respectively to 50 nM in Tris-Mg buffer in 10 μL, then spotted onto the mica surface and allowed to adsorb for 10 min at room temperature. After that the mica were dried on a desiccator under vacuum for 6 h prior to imaging. Finally, the carried out using a Cypher VRS (Oxford Instruments, UK) with tips AC160 in a tapping mode.

Zeta-potential Analysis

The ssDNA and TDN were diluted respectively to 500 nM in water analyzed using a Zetasizer Nano ZS (Malvern Panalytical, UK). All measurements were determined at 25 °C.

Preparation of HV69/70 del, K417N, T478K-substrate-Tb, Ho, Tm

MMA-DOTA and lanthanide metal salts were dissolved in 0.5 M NH_4AC buffer (pH 5.8) to 10mM and 5 mM separately. Next, 10 μL of 10 mM lanthanide and 10 μL of 5 mM MMA-DOTA were mixed and incubated at 37 °C for 1 h with gently shaking. After that, 10 mM EDTA was added to the reaction and incubated for 15 min to chelate the excess lanthanide (III) ions. The prepared lanthanide complexes were characterized by electrospray ionization mass spectrometry (ESI-MS). Before the reaction of lanthanide complexes with the thiolated ssDNA dissolved in 0.5 M NH_4AC buffer (pH 5.8) at 100 μM , was performed in the following way. The 3′ end thiolated ssDNA were dissolved in 0.5 M NH_4AC buffer (pH 5.8) at 100 μM. Then, 60 μL of tracking strands were incubated with 10 mM TCEP at 37 °C for 10 min, and were purified using ultra-filtration tubes. And then, the chelate complex was added to the reaction solution, and incubated for 2 h at 37 °C. Isotope-labeled tracking strands were purified using ultra-filtration tubes. Ultra-filtration was performed according to manufacturer's guidelines. Briefly, the device was centrifuged at 16500rpm for 8 min, and then the sample was washed five times with washing buffer. Finally, the sample was centrifuged at 1000g for 2 min to move the purified specimen to a new pipe, and the solution was diluted to 120 μL with Tris-HCl buffer.

PAGE Electrophoresis.

Each DNA samples were prepared to 2μM mixed with 6 × loading buffer. The 5% nondenaturing polyacrylamide gel electrophoresis (PAGE) was employed to characterize formation step of TDN structure and the remain TDN after a single/double/triple component MNAzyme catalyzation reaction. This experiment was performed in standard 1× TBE buffer at room temperature for 0.5h at 70V and then 1.5 h at a 120 V constant voltage. Eventually, the gel was imaged by Fluorchem M.

Recovery of viral RNA from real samples

The real sample matrix of recovery experiment was obtained by the RNA viral sample collection tubes (inactivated) and EZ-10 spin column viral total RNA extraction kit: Three human throat swab samples were firstly collected. After sampling, these swabs were put into the tube containing preservative solution 1 mL avoiding contact with other parts. Break the swab tip, discard stick, and cap the tube containing samples. Each swab sample preservation solution was fully shaken on a vortex for half a minute, and then 0.2 mL was transferred to a 1.5 mL plastic centrifuge tube for the second step. Add 0.6 mL Buffer Rlysis-VG to the 0.2 mL sample obtained in the first step, shake for 30 s, mix well, and place at room temperature for 10 min. Add 0.6 mL of absolute ethanol, cap the tube and vortex for 15 sec. After a short centrifugation, transfer 700 μL of the solution to a centrifugal adsorption column and leave it at room temperature for 2 minutes. Centrifuge at 12,000 rpm for 1 min at room temperature, discard the penetrant, and put the adsorption column back into the collection tube. Transfer the remaining solution to the centrifugal adsorption column and leave it at room temperature for 2 minutes. Centrifuge at 12,000 rpm for 1 min at room temperature, discard the penetrant, and put the centrifugal adsorption column back into the collection tube. Add 500 μL RPE solution to the centrifugal adsorption column, centrifuge at 12,000 rpm for 1 min at room temperature, discard the penetrant, and put the centrifugal adsorption column back into the collection tube. Repeat last step once. Centrifuge at 12,000 rpm for 2 minutes at room temperature. Discard the centrifuge tube containing the penetrant. Put the centrifugal adsorption column into a new self-prepared RNase-free 1.5 mL

centrifuge tube and add 120 μL to the middle of the filter membrane of the centrifugal adsorption column DEPC-treated H_2O, then let it stand at room temperature for 2 minutes. Centrifuge at 12,000 rpm at room temperature for 2 minutes. The sample in the collection tube at last was added certain amount of target RNA fragments. 20 μL spiked sample was added in each reaction system.

Supplementary Tables & Figures

Table S1. Operating parameters of ICP-MS

Parameters	Settings
RF power (W)	1300
Plasma gas flow (L/min)	18
Auxiliary gas flow (L/min)	1.20
Nebulizer gas flow (L/min)	0.96
Vacuum pressure (Torr)	1.02×10^{-6}
Sweeps per reading	120
Sample uptake rate (L/min)	0.25
Dwell time (ms)	25
Dead time (ns)	35
Isotope monitored	$^{159}Tb/^{165}Ho/^{169}Tm$

Table S2. DNA and RNA sequences in this work

Name	Sequence (5'-3')
HV69/70 del	UUGGUCCCAGAGAUAGCAU
K417N	AAUCAGCAAUAUUUCCAGUUUG
T478K	UACAAGGUUUGCUACCGG
t-Sub-HV69/70 del	ACATTCCTAAGTCTGAAACATTACAGC
	TTGCTACACGAGAAGAGCCGCCATAGTA
	TTTTTGACTCACTAT/rA/GGAAGAGATGA-C_6-HS-SH
PA-HV69/70 del	ATGCTATCTCGGTCGAAATAGTGAGTC
PB-HV69/70 del	TCATCTCTTCTCCGAGCCTGGGACCAA
t-Sub-K417N	TATCACCAGGCAGTTGACAGTGTAGCAA
	GCTGTAATAGATGCGAGGGTCCAATAC
	TTTTTACGAGTACCT/rA/GATCTATGGAA-C_6-HS-SH
PA-K417N	CAAACTGGAAACGGTCGAAAGGTACTCGT
PB-K417N	TTCCATAGATTCCGAGCTATTGCTGATT
t-Sub-T478K	TCAACTGCCTGGTGATAAAACGACACT
	ACGTGGGAATCTACTATGGCGGCTCTTC
	TTTTTCACAGAGTAT/rA/GGATATCAGAT-C_6-HS-SH
PA-T478K	CCGGTAGCACGGTCGAAATACTCTGTG
PB-T478K	ATCTGATATCTCCGAGCAACCTTGTA
t-toehold	Biotin-TTTTTCAGACTTAGGAATGTGCTTCCCA
	CGTAGTGTCGTTTGTATTGGACCCTCGCAT
ss-Sub-HV6970	Biotin-TTTTTTTTTTGACTCACTAT/rA/
	GGAAGAGATGA-C_6-HS-SH
ss-Sub- K417N	Biotin-TTTTTTTTTTACGAGTACCT/rA/
	GATCTATGGAA-C_6-HS-SH
ss-Sub-T478K	Biotin-TTTTTTTTTTCACAGAGTAT/rA/
	GGATATCAGAT-C_6-HS-SH
HV69/70 del SM	UUGGUCCCAGAGAUACCAU

HV69/70 del TM	UUGGUGGCAGAGAUACCAU
K417N SM	AAUCAGCAAUAUUUCCACUUUG
K417N TM	AAUCACGAAUAUUUCCACUUUG
T478K SM	UACAAGGUUUGCUAGCGG
T478K TM	UACAACCUUUGCUAGCGG
NC1(miR-223)	UGUCAGUUUGUCAAAUACCCCA
NC2(miR-141)	UAACACUGUCUGGUAAAGAUGG

Table S3. Multicomponent detection method for SARS-CoV-2 variants

Target	Method	Analytical Performance	Simultaneous detection	Ref.
E gene RdRP gene HV69/70 del N501Y	quantum dot barcode FL	10^6 to 10^2 copies per reaction	No	[1]
S Protein of Delta Omicron	SERS	nM level	No	[2]
N501Y K417N T478K H655Y 49*X*	RT-RPA-Cas12a-based assay	50 to 100 copies per reaction	No	[3]
HV69/70 del I82T	qPCR-HRM	-	Yes	[4]
S gene E gene N gene	Nanopore	-	Yes	[5]
HV69/70 del K417N T478K	qTDN Mass Spectrometry	1.1-1.5 fmol	Yes	This Work

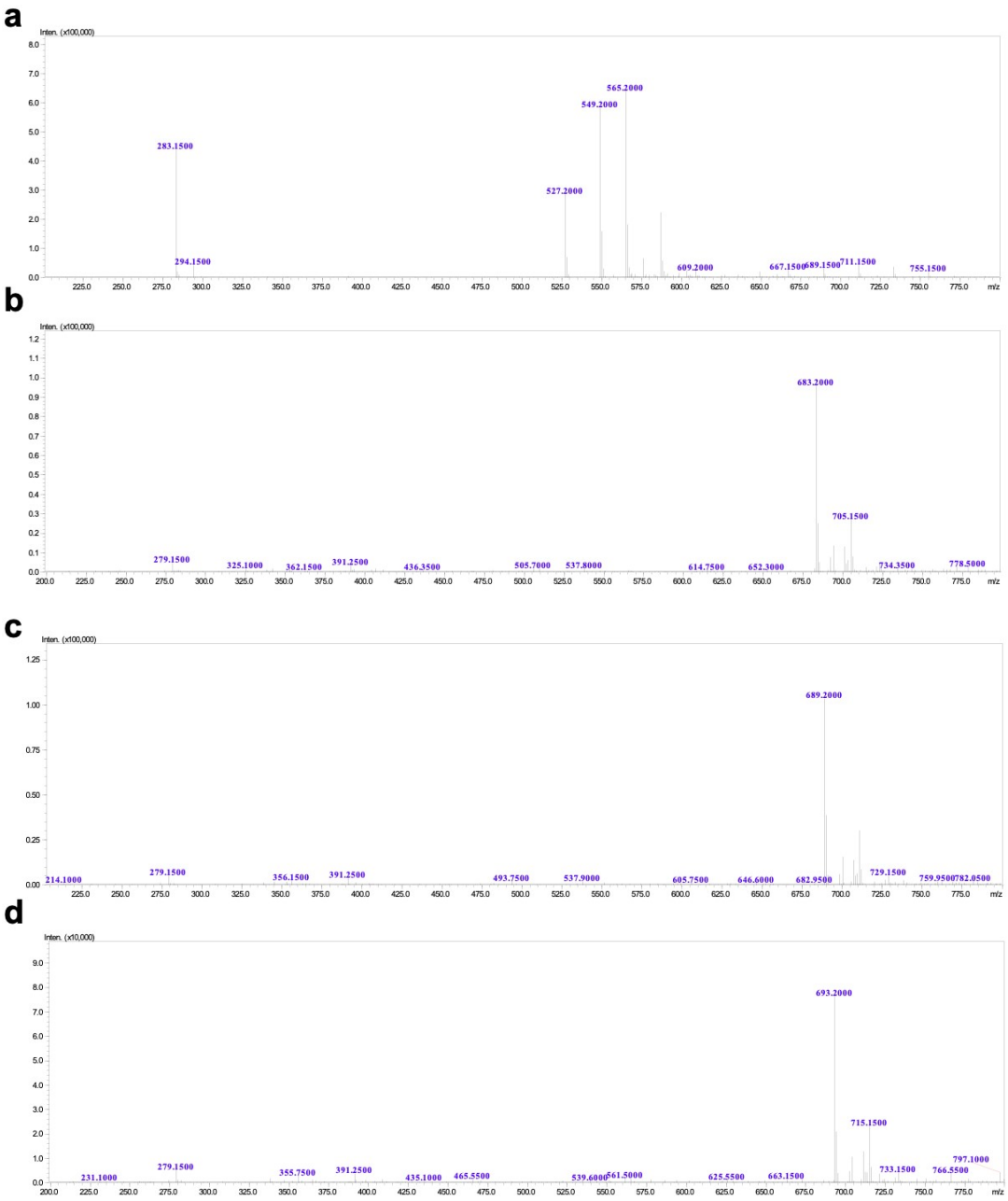

Fig. S1 ESI-MS characterization of MMA-DOTA and its chelation with Ln^{3+}. (a)MMA-DOTA, ESI-MS, m/z, found: 527.2 ($[M^+H]^+$), 549.2 ($[M^+Na]^+$); calc.: 527.5 ($[M^+H]^+$). (b) MMA-DOTA-Tb^{3+},ESI-MS, m/z, found: 683.1 ($[M^+H]^+$), 705.1 ($[M^+Na]^+$); calc.: 683.4 ($[M^+H]^+$). (c) MMA-DOTA-Ho^{3+}, ESI-MS, m/z, found: 689.1 ($[M^+H]^+$), 711.1 ($[M^+Na]^+$); calc.: 689.4 ($[M^+H]^+$). (d) MMA-DOTA-Tm^{3+}. ESI-MS, m/z, found: 693.1 ($[M^+H]^+$), 715.1 ($[M^+Na]^+$); calc.: 693.4 ($[M^+H]^+$).

Isotope labels on t-Sub-DNA were characterized by MALDI-TOF-MS. For determination, a kind of matrix consists of 3-hydroxypicolinic acid (3-HPA) and DHCA (volume ratio 3:2) was applied through all the test with equal volume adding to the samples. 1.5 μL of the mixture is dropped on the target plate and air-dried naturally at room temperature. Firstly, a calibration curve of standard DNA with certain molecular weight was made. Std1(Mw)=17801.56, std1(Mw)=20481.32, std1(Mw)= 27842.17. Next, unlabeled DNA and with MMA-DOTA-Ln^{3+} were investigated. As what are shown in Fig. S2, the detected molecular weight of t-Sub-HV69/70 del-Tb^{3+}, t-Sub-K417N-Ho^{3+}, t-Sub-T478K-Tm^{3+} are obviously greater than their original ones, demonstrating the successfully modification of isotope labels.

Instrument conditions

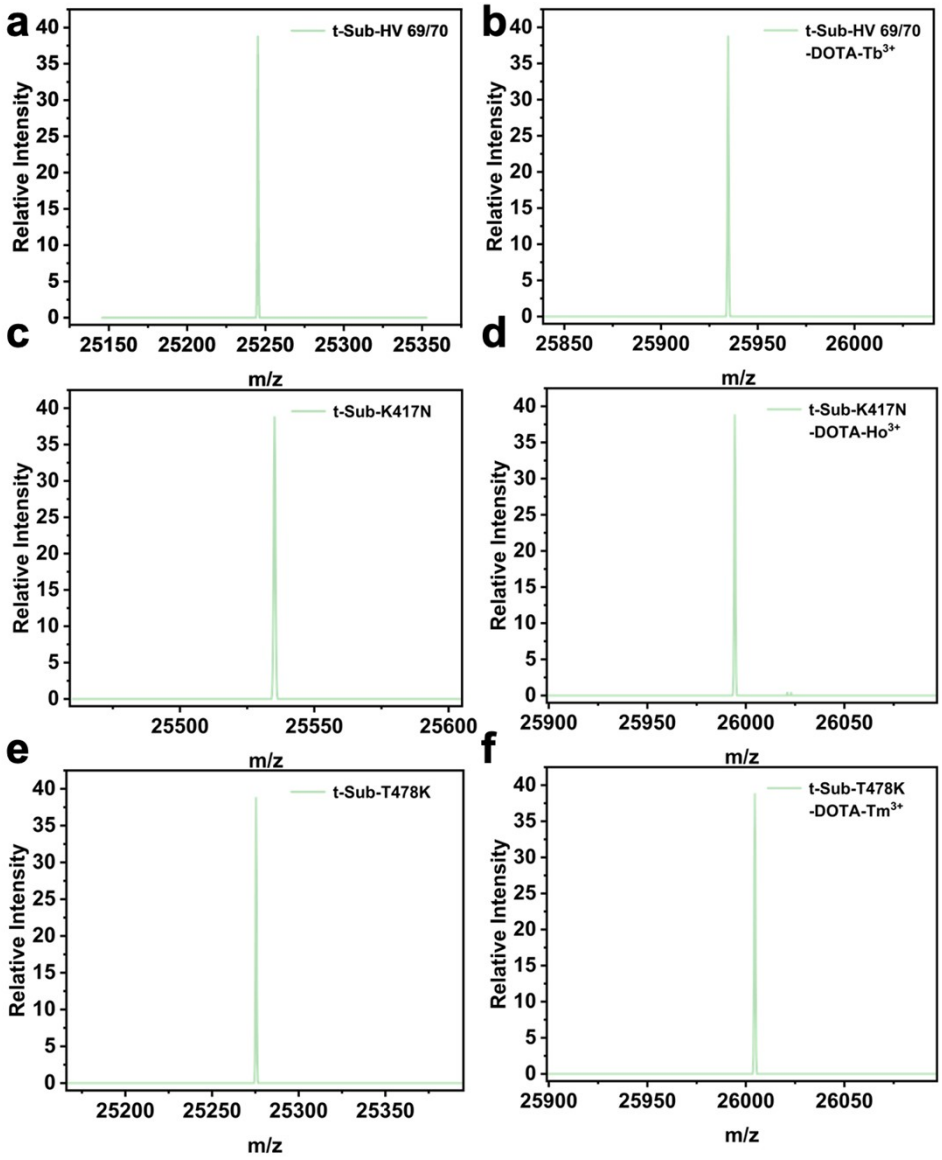

Fig. S2 MALDI-TOF-MS characterization of t-Sub-DNA labeled with MMA-DOTA-Ln^{3+}，(a) t-Sub-HV69/70 del, (b) t-Sub-HV69/70 del-DOTA-Tb3+ (c) t-Sub-K417N.(d) t-Sub-K417N-DOTA-Ho3+, (e) t-Sub-T478K, (f) t-Sub-T478K-DOTA-Tm3+.

In the AFM image, ssDNA is dispersed and soft, while tTDN has a certain rigidity and maintains a three-dimensional structure

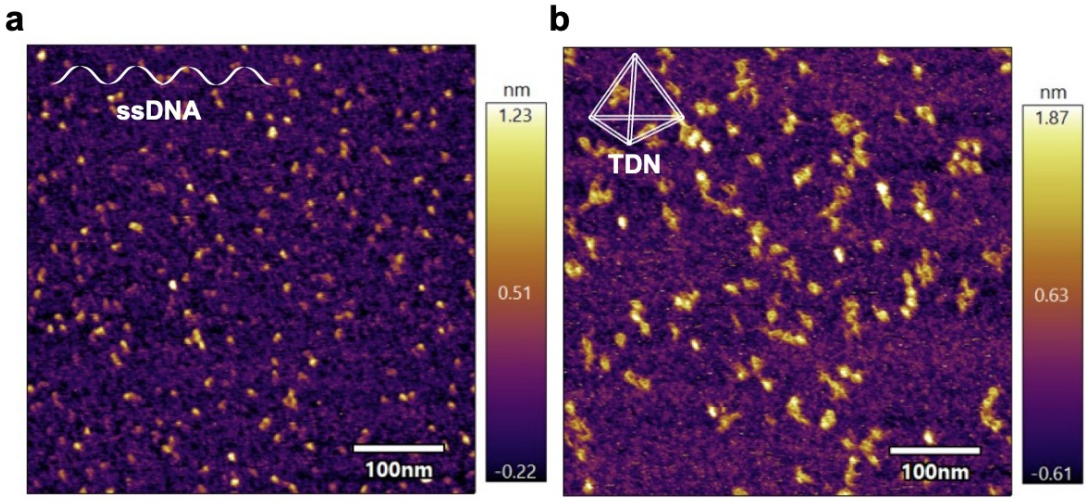

Fig. S3 Characterization of ssDNA and TDN using AFM with the inserted image shows a 2D view of the TDN.

The particle size distribution indicates that the synthesized tTDN is comparatively pure and homogeneous. Evidence demonstrates the formation of tTDN from a more intuitive perspective.

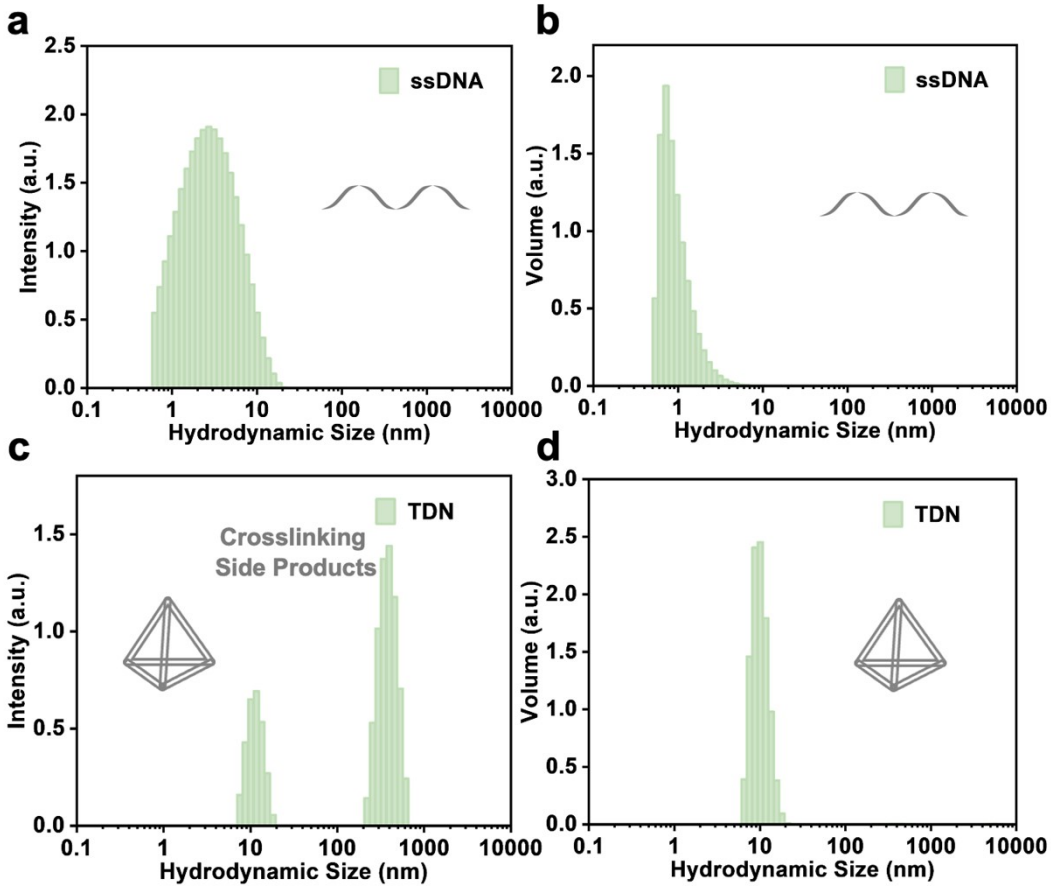

Fig. S4 DLS analysis of ssDNA and TDN prepared using 500 nM compositional strands.

MBs are used as substance to construct a MNAzyme-TDN nanosystem with a particle size of about 1 μm.

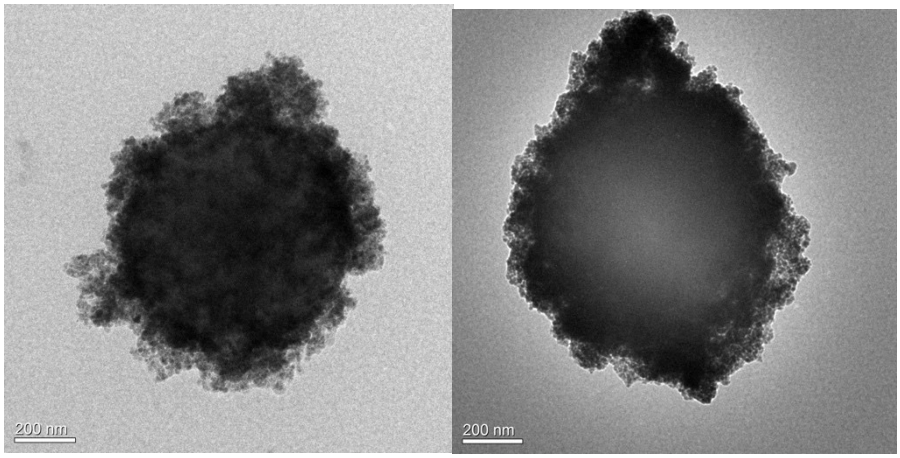

Fig. S5 Magnetic beads used in this work without/with TDN modified shown as the TEM image.

The sphere becomes positively charged overall thanks to SA, and after tTDN was attached, the negative charge of it (essentially as DNA) replaces the positive charge on the particle surface.

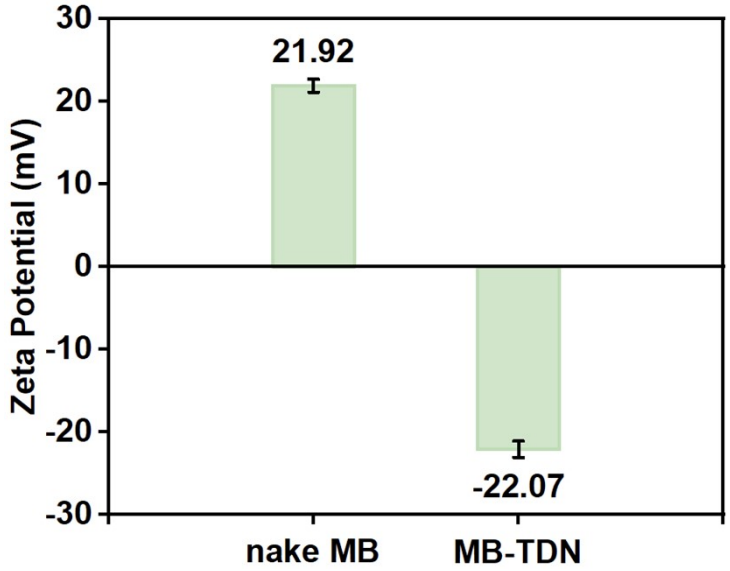

Fig. S6 Zeta-potential for MB and MB-TDN.

In isotope analysis by energy dispersive spectroscopy (EDS), MBs contain a large amount of C, N, O, P, S and Fe elements. When the isotope labeled tTDN were coupled to the MBs, we discovered new emerged elements: Mg, Tb, Ho and Tm.

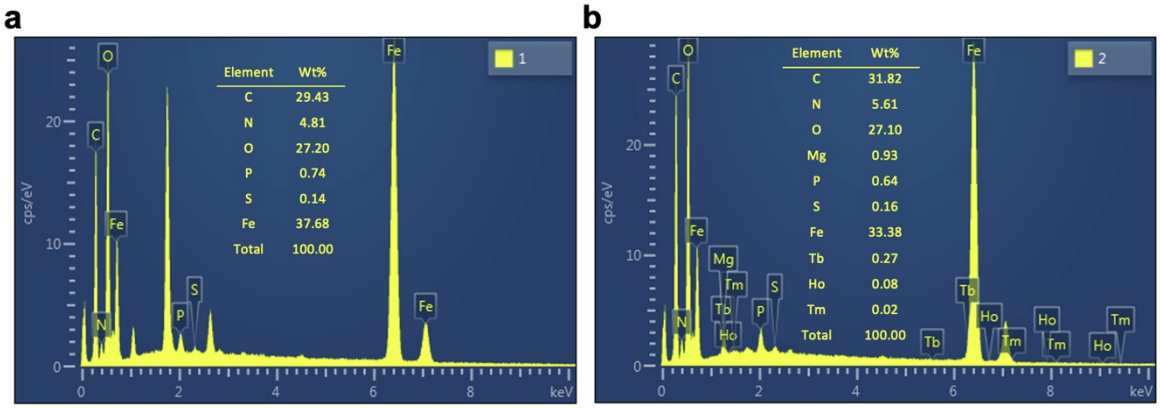

Fig. S7 Energy dispersive X-ray spectrum (EDS) of the MBs and TDN-MNAzyme nanosystem attached on MBs.

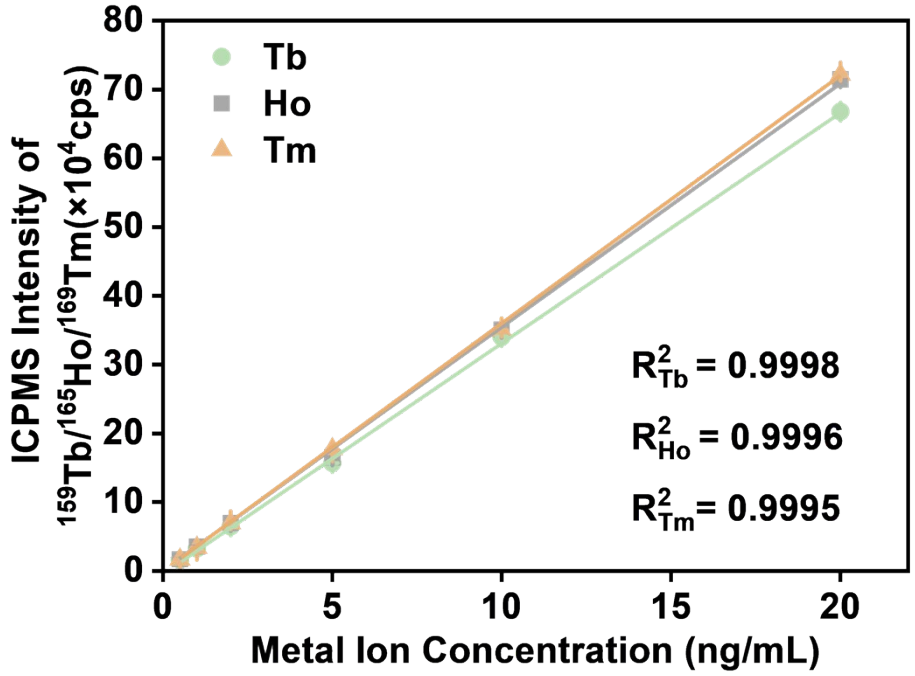

Fig. S8 Calibration curve of isotopes ^{159}Tb, ^{165}Ho and ^{169}Tm simultaneously detected by ICPMS

Under treated without/with certain amount of DNase I and subsequently a PAGE experiment, we found that tTDN prepared by thermal annealing protocol exhibits better stability.

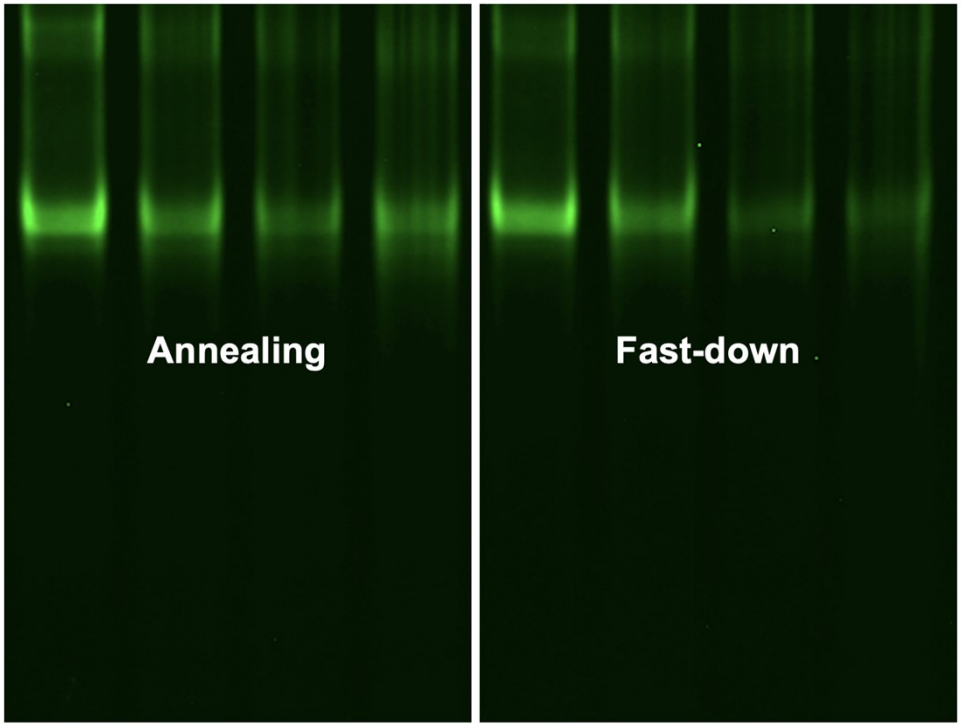

Fig. S9 Optimization of preparation process of TDN.

Under the same conditions, the magnetic beads have a larger specific surface area, which is more conducive to the spatial dispersion of tTDN with a rigid structure and more binding.

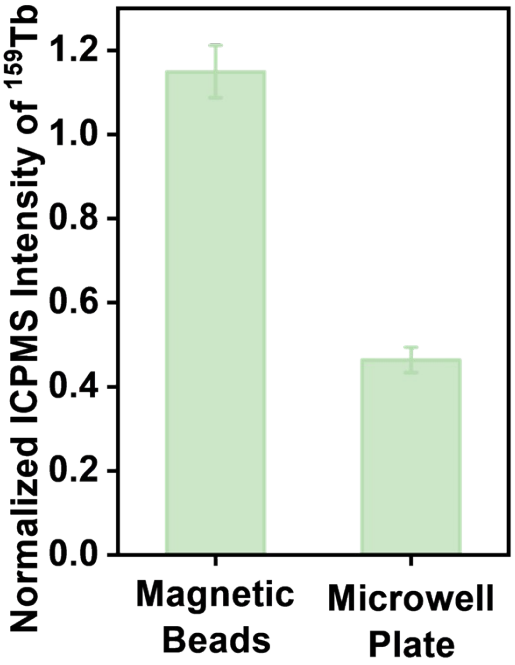

Fig. S10 Optimization of reaction platform.

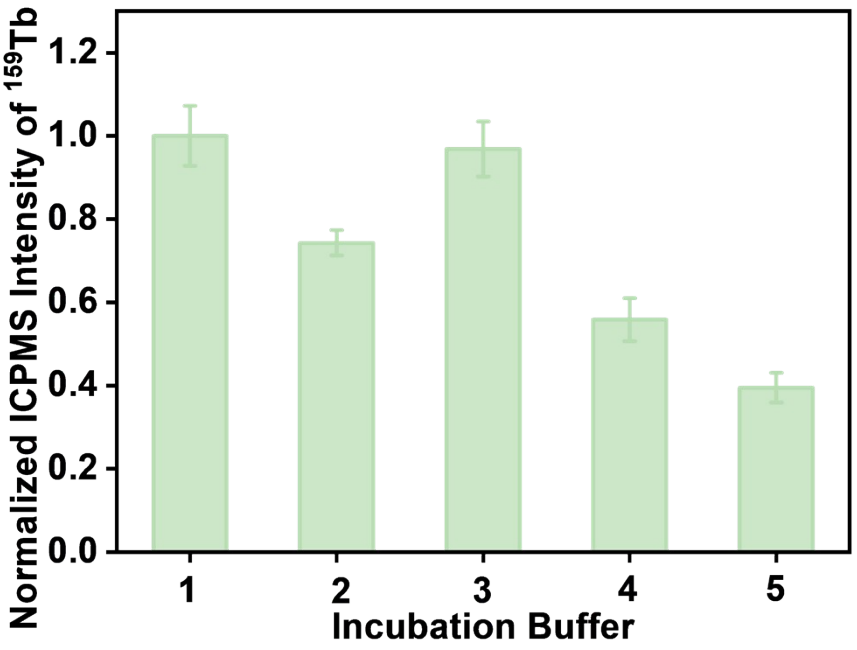

Fig. S11 Optimization of incubation buffer of TDN-MBs formation. (1.TM; 2.TM with 0.1M NaCl; 3.TMT; 4. TM with 1M NaCl; 5. TE with 2M NaCl)

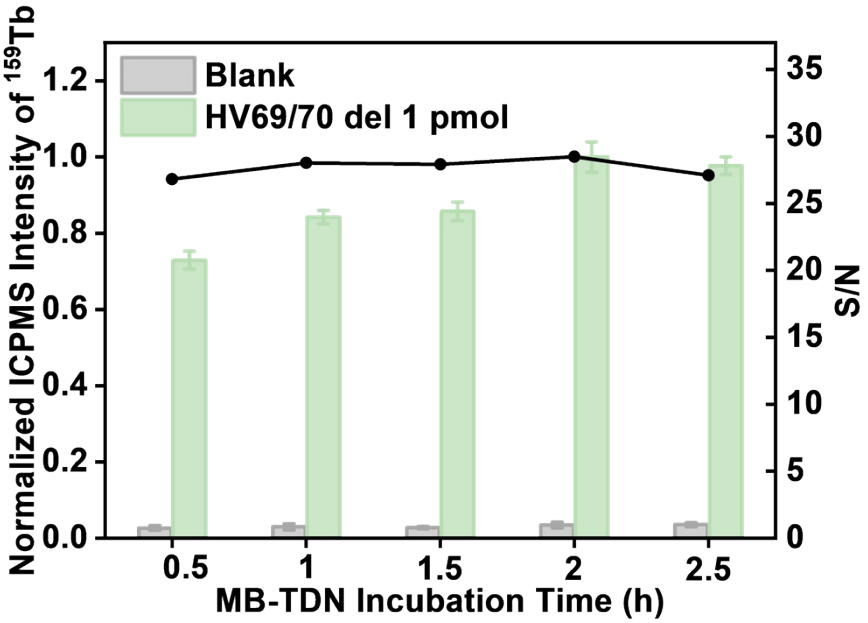

Fig. S12 Effects of the as-prepared MB-capture amount per tube of a sample.

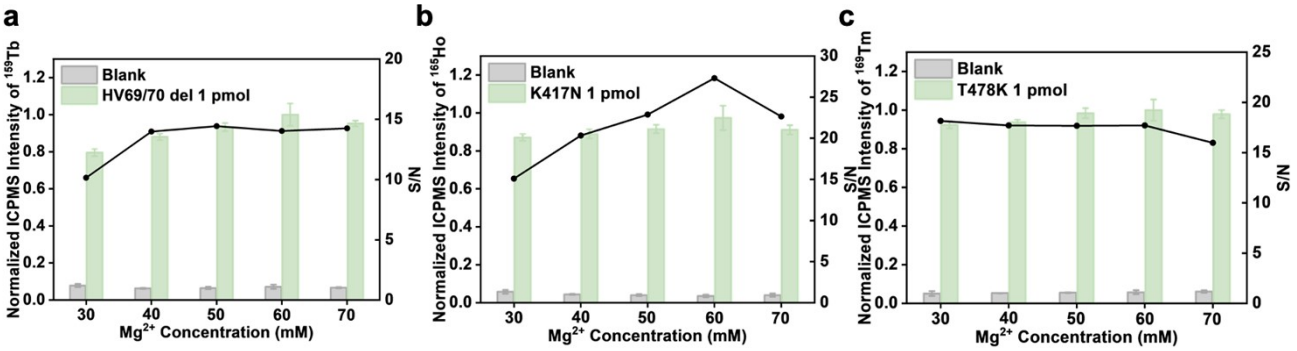

Fig. S13 Optimization of Mg^{2+} concentration for the simultaneous cleavage of TDN-Sub-HV69/70 del, K417N and T478K.

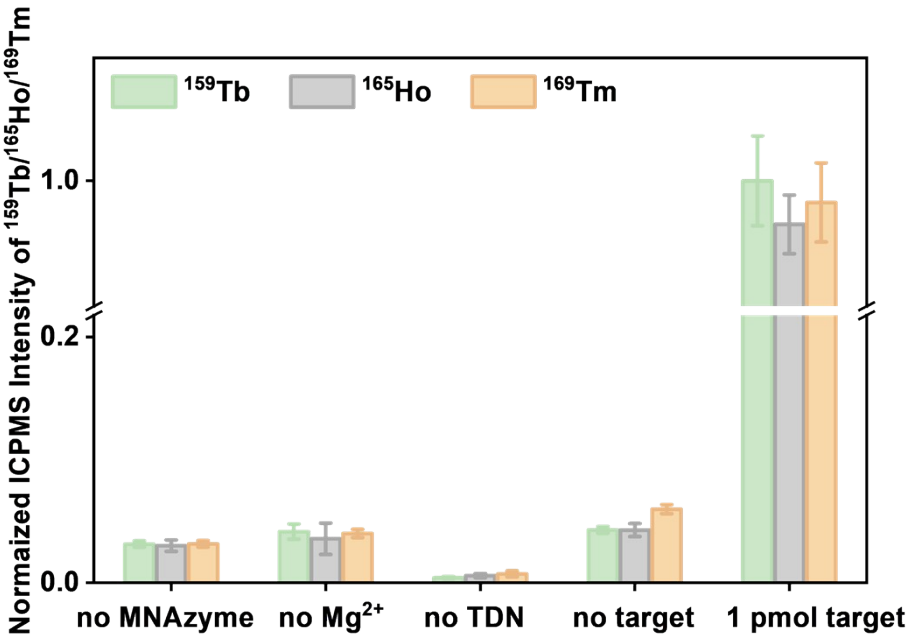

Fig. S14 ICPMS detection under different conditions.

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