Supporting information

DNAzyme-powered cascade DNA walkers for sensitive detection of uracil DNA glycosylase activity

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1. Experimental section

1.1. Materials and reagents

All nucleic acid sequences employed in current study (Table S1) were obtained by

Sangon Biotechnology Co., Ltd. (Shanghai, China). Chloroauric acid ($HAuCl_4 \cdot 3H_2O$), dihydrate sodium citrate ($Na_3C_6H_5O_7 \cdot 2H2O$), dithiothreitol (DTT) and tris-(2-carboxyethyl)-phosphine (TCEP) were gained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Sodium chloride (NaCl), magnesium chloride ($MgCl_2$), tris (hydroxymethyl) aminomethane hydrochloride (Tris) and ethylenediamine tetra acetic acid (EDTA) were bought from Macklin Biochemical Co., Ltd. (Shanghai, China). Uracil-DNA glycosylase (UDG), endonuclease IV (Endo.IV), AP endonuclease (APE1), uracil glycosylase inhibitor (UGI), bovine serum albumin (BSA) and human Alkyladenine DNA glycosylase (hAAG) were purchased from New England Biolabs (Beijing, China). Uracil glycosylase ELISA kit was purchased from Ruixin Biotechnology Co., LTD. (Quanzhou, China). Human serum samples were supplied by the Hospital of Shandong University (Shandong, China). The chemical agents used in this experiment are analytical pure grade without further refinement. Ultrapure water (resistivity ≥18.25 $M\Omega\ cm^{-1}$) was used to make up all solutions.

1.2. Apparatus

The UV-2910 ultraviolet spectrophotometer (Hitachi, Japan) was used to measure the UV-Vis absorption spectrum. The fluorescence emission spectra were recorded by F-7000 fluorescence spectrophotometer (Hitachi, Japan). The emission wavelength was collected in the range of 510-620 nm when the excitation wavelength is 488nm.The voltage was set at 700 V and the slit width was 10 nm. The transmission electron microscope (TEM) pictures were taken by JSM-6700F transmission electron microscope (JEOL, Japan). Non-denaturing gel imaging was taken by a GeldocTM

XR+ Imaging Tester (Bio-RAD Laboratories Inc., USA).

1.3. Calculation the number of track strands loading on one AuNP

The loading number of the track strands on AuNPs was determined and calculated according to the DTT substitution method reported in the literature [31]. Briefly, 20 mM DTT and 1 nM functionalized AuNPs were mixed and shaken at normal temperature for 12 h. Then it was separated at 12500 rpm for 30 min. The upper solution was collected for the fluorescence measurement. The concentration of track stands on the AuNPs was calculated according to the standard curve made by the track strand marked with FAM. The mean number of the track stands on each AuNP was assessed by counting the concentration ratio between track stands and AuNPs.

1.4. Non-denatured polyacrylamide gel electrophoresis analysis

In order to verify the feasibility of the designed nucleic acid sequences, 12% non-denatured polyacrylamide gel electrophoresis [TEMED (18 μL), APS (180 μL), 5×TBE buffer (5 mL), 40% acrylamide solution (7.5 mL, 19:1) and 12.5 mL water] was employed for analysis. The gel was run in a 1×TBE buffer (pH 8.3) at a voltage of 500 V and a current of 30 mA at 25 °C for 1h. The gel was dyed in 0.5×TBE solution including SYBR Gold in the dark for 40 min, and then imaging was taken using a gel imaging system.

1.5. Performance evaluation of the cascade DNA walkers

For the control system, UDG were incubated with the AuNPs-(LS-WS1)-TS1-FAM in Tris-HCl solution (100 mM NaCl, 20 mM Tris, pH 8.0) for 1 h at 37 °C to release the pre-locked WS1. Then $MgCl_2$ (5 mM) were entered to the above solution to react at 37

°C for 2 h. After centrifugation, the supernatant was used to determine the fluorescence intensity.

1.6. Inhibition assay

Before adding 1 U/mL UDG, UGI at different concentrations were reacted with fixed walker at 37 °C for 30 min, and then the action was implemented in accordance with the above experimental procedures. The inhibition effect of UGI was evaluated based on the UDG relative activity RA = (Fi-F0)/(Ft-F0) ×100%, where F0 represented the fluorescence intensity without UDG, Ft represented the fluorescence intensity at 1 U/mL UDG, and Fi represented the fluorescence intensity with the coexistence of 1 U/mL UDG and UGI at various concentrations.

1.7. Cell culture and sample preparation

HeLa cells were cultivated in DMEM medium, which contained 10% fetal bovine serum and 1% penicillin/streptomycin in 37°C cell incubator with 5% CO_2. The cell lysate was prepared by using logarithmic phase cells. The cells were washed twice with PBS solution and digested with trypsin until the single-cell suspension was obtained. Then trypsin was removed and added to the culture medium for counting. HeLa cells (1×10^6) were placed in a centrifugal tube, and the supernatant was abandoned after separation for 5 min at 3000 rpm. Next 100 μL 10 mM low permeability Tris-HCl solution was added and then the mixture was blowing to dissolve and disperse with pipettor to make the cells swell and rupture. Next, the tube placed on ice for 30 min, and the cells were rotated at 3000 rpm every 10 min for 15 s to fully cells lyse. At last, it was separated in the condition of 12,000 rpm at 4°C for 20 min, and the supernatant

was collected, which was stored at -80°C for later use.

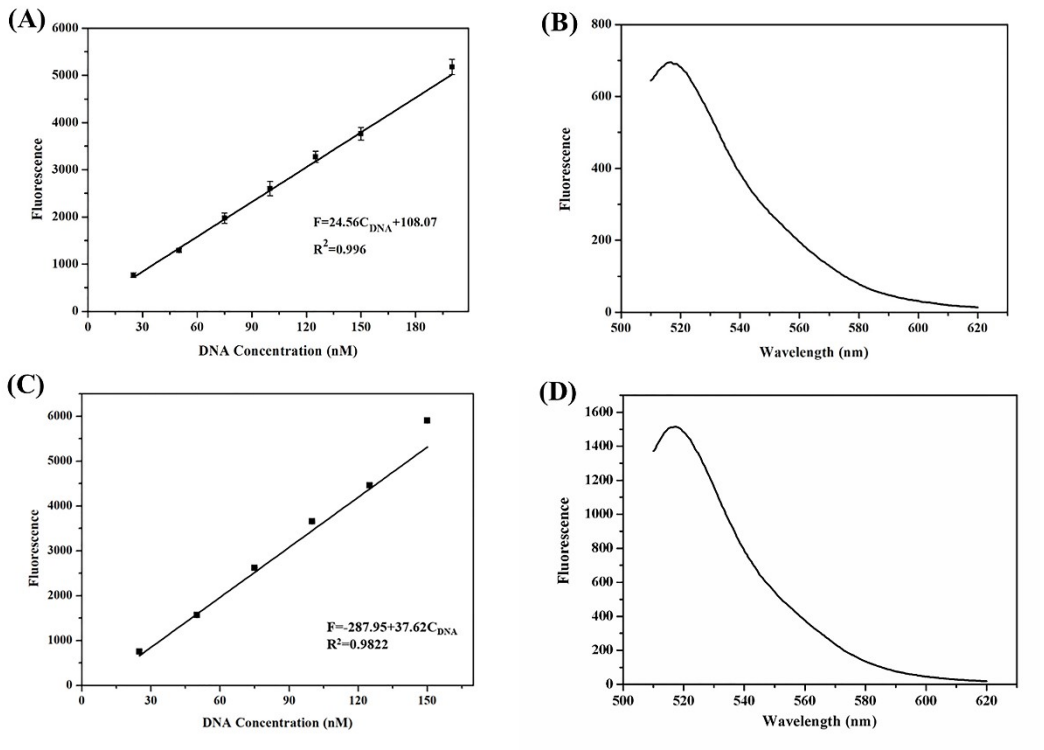

Fig. S1. (A) Standard linear calibration curves of FAM-labeled TS1. (B) Fluorescence spectra of AuNPs-(LS-WS1)-(FAM-TS1) after incubation with DTT. (C) Standard linear calibration curves of FAM-labeled TS2. (D) Fluorescence spectra of AuNPs-(FAM-TS2) after incubation with DTT. Error bars show the standard deviations of three experiments.

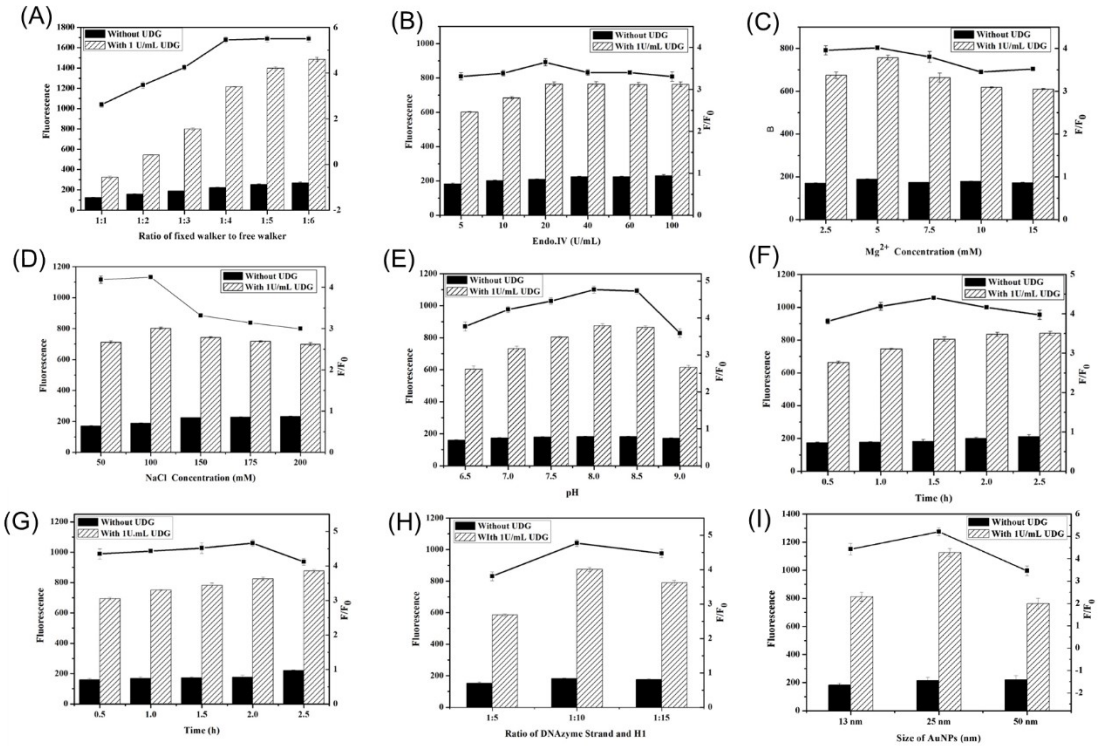

Fig. S2. Effect of different experimental conditions on fluorescence intensity and F/F_0. F and F_0 represent fluorescence intensity in the presence and absence of UDG, respectively. (A) Ratio of AuNPs-(LS-WS1)-TS1 to AuNPs-TS2, (B) Endo.IV concentration, (C) Mg^{2+} concentration, (D) NaCl concentration, (E) pH, (F) Fixed walker operation time, (G) Free walker operation time, (H) Ratio of WS1 and TS1, (I) Size of AuNPs. Error bars show the standard deviations of three experiments.

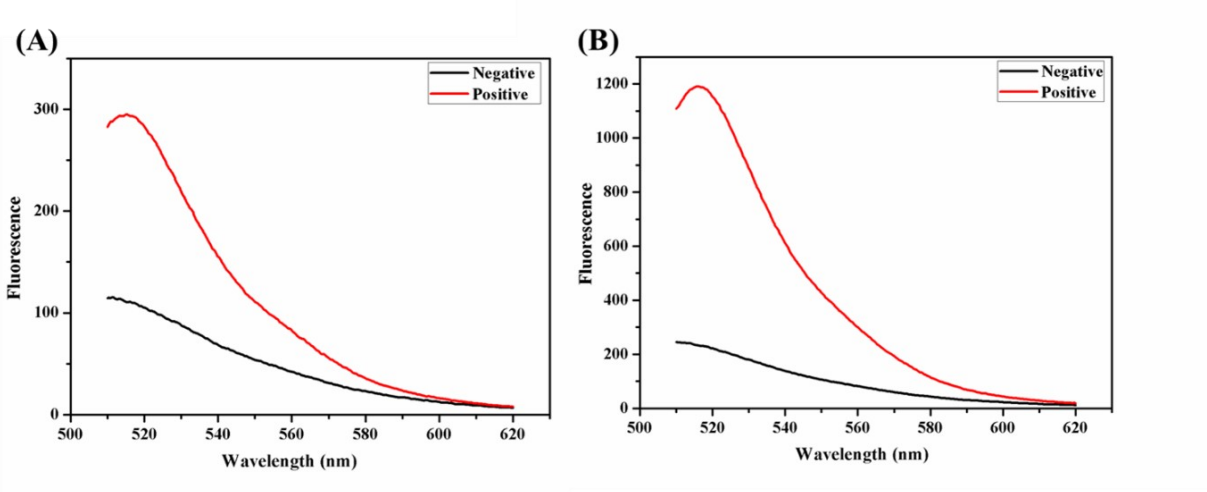

Fig. S3. (A) Negative (black curve) and positive (red curve) fluorescence spectra of signal walker. (B) Negative (black curve) and positive (red curve) fluorescence spectra of cascade walker.

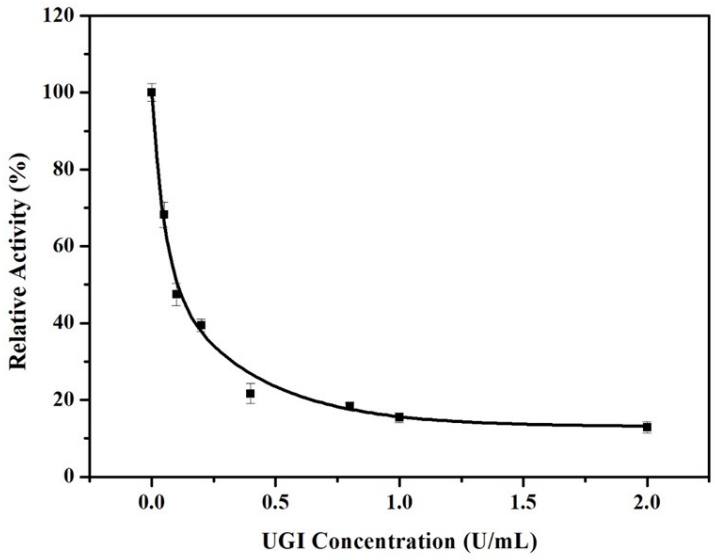

Fig. S4. Variance of relative activity of UDG in response to different concentrations of UGI. The concentration of UDG is 1 U/mL. Error bars show the standard deviation of three experiments.

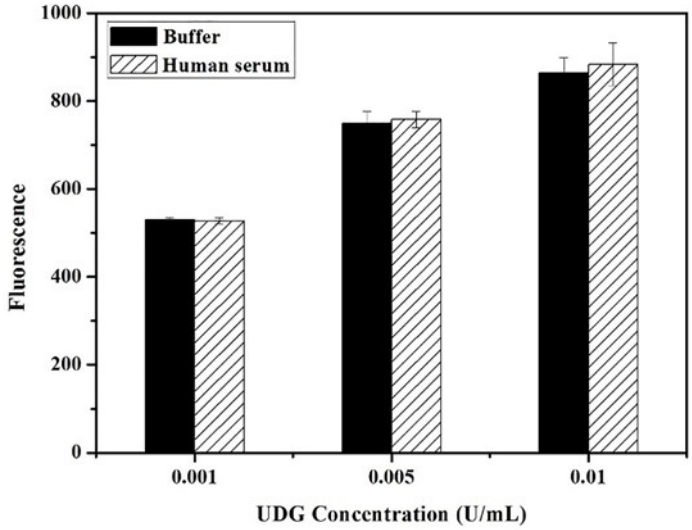

Fig. S5. Comparison of fluorescence intensity of different concentrations of UDG in buffer and human serum. Error bars show the standard deviations of three experiments.

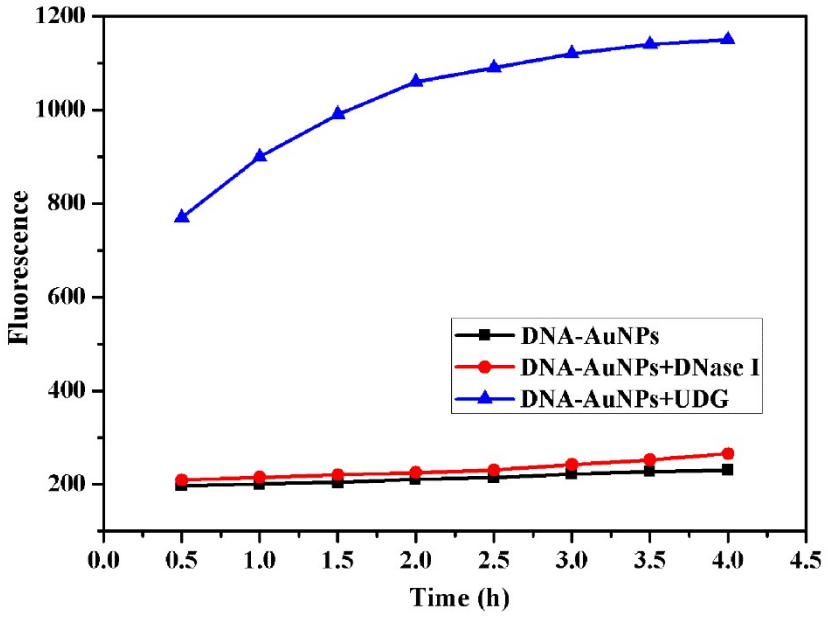

Fig. S6. Effect of different experimental conditions on DNA-AuNPs over time.

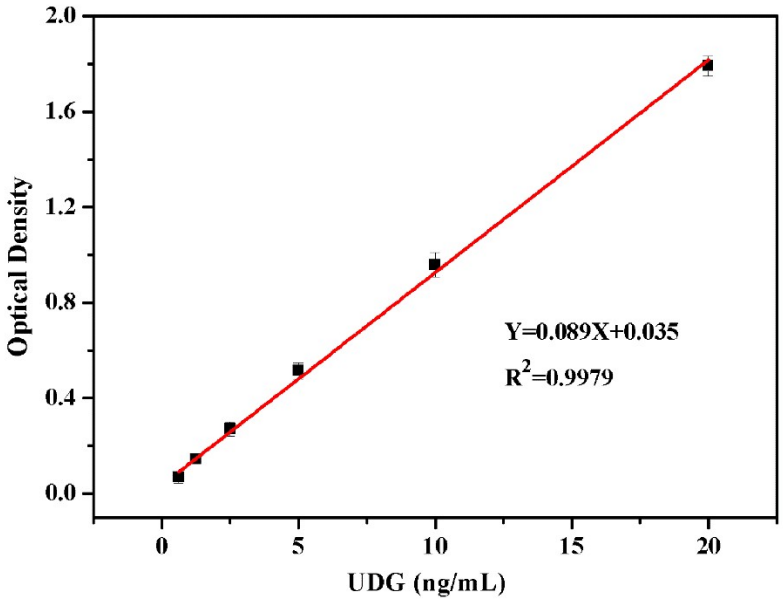

Figure S7. the linear relationship between optical density and the UDG concentration by ELISA kit.

Table S1. Sequences of oligonucleotides used in present work.

oligonucleotide	sequence (5′→3′)
Walking Strand 1	HS-$(T)_{42}$ GGG AAA GGA TCA GAA CTC CGA GCC GGT CGA AAT AGT GCG T
Track Strand 1	HS-$(T)_{12}$ *TTT CGA CCG* CCA TCC ACG CAC TAT /rA/GG TTC TGA TCT CTT CTC CGA GC*C GGT CGA AA*T AGT GGG T
Track Strand 2	HS-$(T)_{12}$ *CCA CCA C*AT TGA AAT TGA CCC ACT AT/rA/ GGA AGA GAT CTT ACG AGG CG*G TGG TGG*-FAM
Locking Strand	ACCGGCUCGGAGTT

1) The italicized portion of the track strand 1 and 2 represents the complementary sequence. 2) The underlined parts of the track strands 1 and 2 represent the binding sites of the corresponding walking strands. 3) rA in the track strands 1 and 2 represents adenine ribonucleic acid. 4) The red parts of walking strand 1 and track strand 1 represent the catalytic core of 8-17 DNAzyme. 5) “FAM” represents 6-carboxyfluorescein.

Table S2: Comparison of the detection performance for UDG activity with some reported works.

Method	Linear range ($U\ mL^{-1}$)	Detection limit ($U\ mL^{-1}$)	Reference
SERS platform using plasmonic nanoparticles coupling	1×10^{-3}~10	4.29×10^{-4}	[1]
electrochemiluminescent biosensor with triple signal amplification	5×10^{-4}~1	2.5×10^{-4}	[2]
fluorescent method based on TdT and T7 Exo-aided amplification strategy	5×10^{-4}~0.01	1.5×10^{-4}	[3]
fluorescent method with smart catalyzed hairpin assembly-induced DNAzyme nanosystem	1×10^{-3}~1	6.8×10^{-4}	[4]
Single-Nanoparticle ICP-MS method	1×10^{-3}~10	3×10^{-4}	[5]
DNAzyme powered cascade walker	1×10^{-4}~0.01	8.5×10^{-5}	This work

Table S3. The results of UDG assay precision test.

Concentration ($U\ mL^{-1}$)	Sample 1 ($U\ mL^{-1}$)	Sample 2 ($U\ mL^{-1}$)	Sample 3 ($U\ mL^{-1}$)	RSD (n=3)
1.0×10^{-3}	1.0×10^{-3}	1.1×10^{-3}	1.1×10^{-3}	5.4%
5.0×10^{-3}	4.9×10^{-3}	5.1×10^{-3}	5.3×10^{-3}	3.9%
1.0×10^{-2}	9.8×10^{-3}	1.0×10^{-2}	9.9×10^{-3}	1.0%

Table S4. The results of UDG assay reproducibility test.

Concentration ($U\ mL^{-1}$)	Sample 1 ($U\ mL^{-1}$)	Sample 2 ($U\ mL^{-1}$)	Sample 3 ($U\ mL^{-1}$)	RSD (n=3)
1.0×10^{-3}	9.9×10^{-4}	1.0×10^{-3}	1.0×10^{-3}	5.8%
5.0×10^{-3}	5.1×10^{-3}	5.3×10^{-3}	5.0×10^{-3}	3.0%
1.0×10^{-2}	9.8×10^{-3}	1.0×10^{-2}	9.7×10^{-3}	1.6%

Table S5. Recovery tests of UDG detection in human serum samples.

Sample Number	Added UDG ($U\ mL^{-1}$)	Detected UDG ($U\ mL^{-1}$)	Recovery ($U\ mL^{-1}$)	RSD (n=3)
1	1.0×10^{-3}	9.8×10^{-4}	98%	3.6%
2	5.0×10^{-3}	5.2×10^{-3}	104%	2.4%
3	1.0×10^{-2}	1.04×10^{-2}	104%	5.5%

Table S6: Comparison of UDG activity in Hela cells detected by cascade DNA walker and ELISA kit.

Method	Cascade DNA walker	ELISA kit
Cell number	10^6	10^6
Test value	1250	0.21
UDG activity (ng/mL)	1.82	1.95

Table S7: Comparison of the performance for UDG activity in human serum with some reported works.

Method	Recovery	Reference
TdT combined CRISPR-Cas12a amplification strategy	98%-104%	[6]
Enzyme-free and substrate-free electrochemical biosensor	98%-107%	[7]
Catalyzed hairpin assembly-induced DNAzyme nanosystem	85%-117%	[8]
Cascade DNAzyme walker	98%-104%	This work

Reference

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