Supporting Information

A DNAzyme-driven random biped DNA walking nanomachine for sensitive detection of Uracil-DNA glycosylase activity

Table S1. Oligonucleotides sequences used in the random DNA walking nanomachine based on DNAzyme for UDG detection.

Name	Sequences (5'-3')
B1	GGTCCACGACCTGCAGACCGGACCTTGACCCACTA/ideoxyU/ TTCGCTGTTTT
B2	CAACCGACCGAGAAGTCTGACCTTGCGGTCTGCAGGTCG /ideoxyU/GGACCTTTTTTTTTTTTTTT-SH
S	AGACTTCTCCGAGCCGGTCGAAATAGTGGGTCAAGGTCTTTTT T TTTTTTTTTTTTTCAAGGTCAGACTTCTCCGAGCCGGTCGAAA T AGTGGGT
D	FAM-ACCCACTAT /rA /GGAAGTCTTTTTTTTTT-SH

AuNP Preparation Method

First heat $HAuCl_4$ (0.25 mM) to boiling, and then quickly add trisodium citrate (34 mM) under vigorous stirring. After the color of the solution changes from blue to bright red, continue heating the solution at high temperature boiling 15 min, then the solution was naturally cooled to room temperature under continuous vigorous stirring, and finally the solution was centrifuged at 10000 rpm for 30 min, and the supernatant was removed, the remaining solution was collected and stored at 4° C for subsequent use.

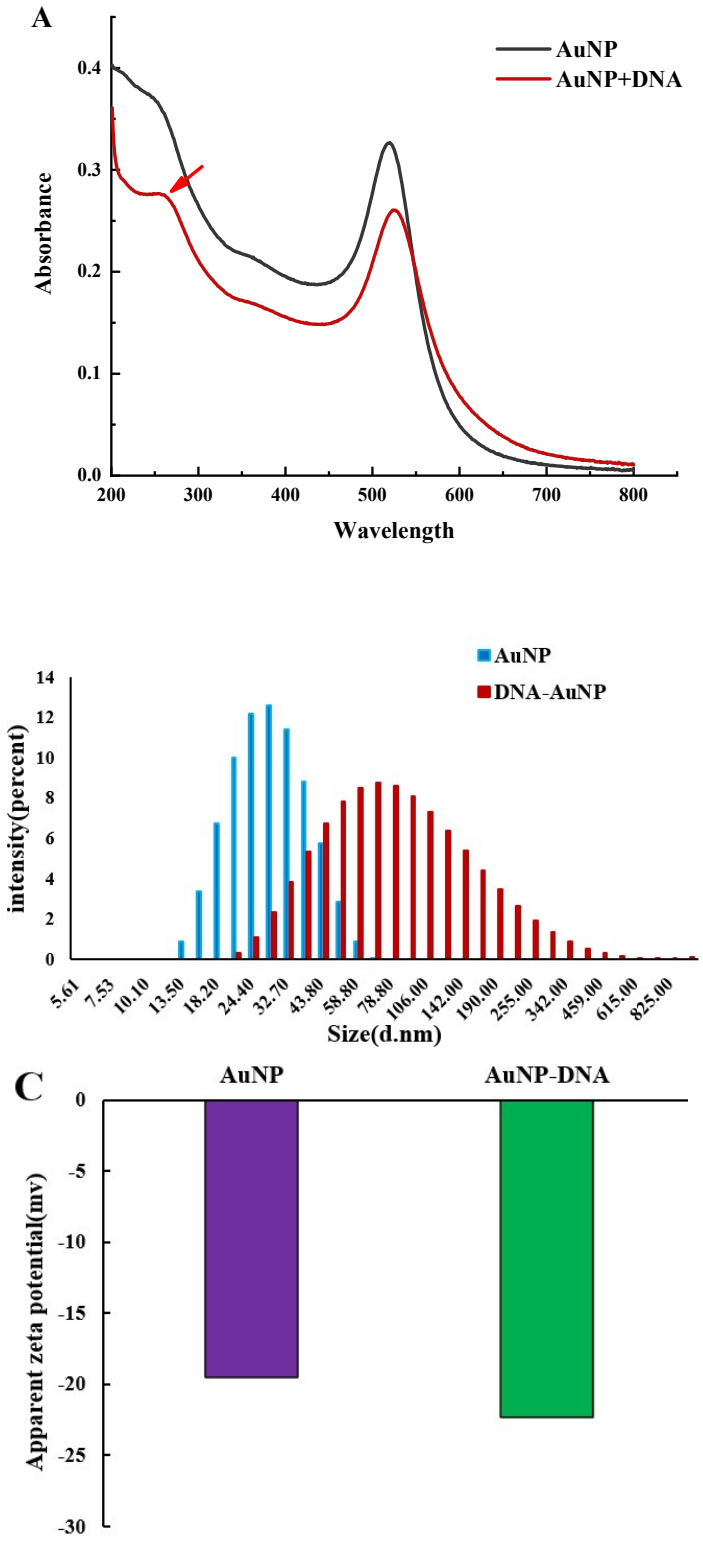
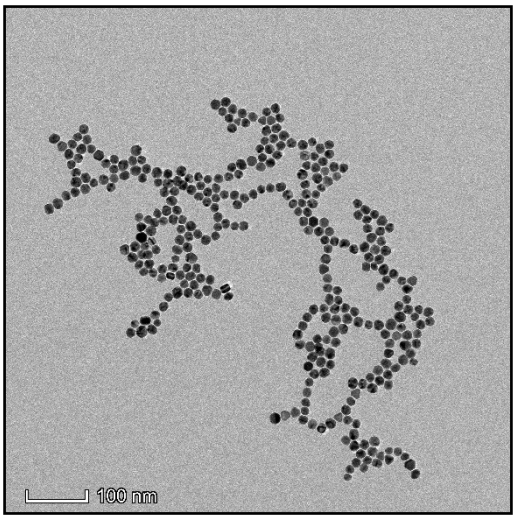


Fig. S1. Characterization of AuNP and DNA-AuNP. (A) UV-Vis spectra of AuNP(Black) and DNA-AuNP (Red). (B) The particle size distributions of AuNP(Blue) and DNA-AuNP (Red). (C)The zeta potentials of AuNP and DNA-AuNP.

A



B

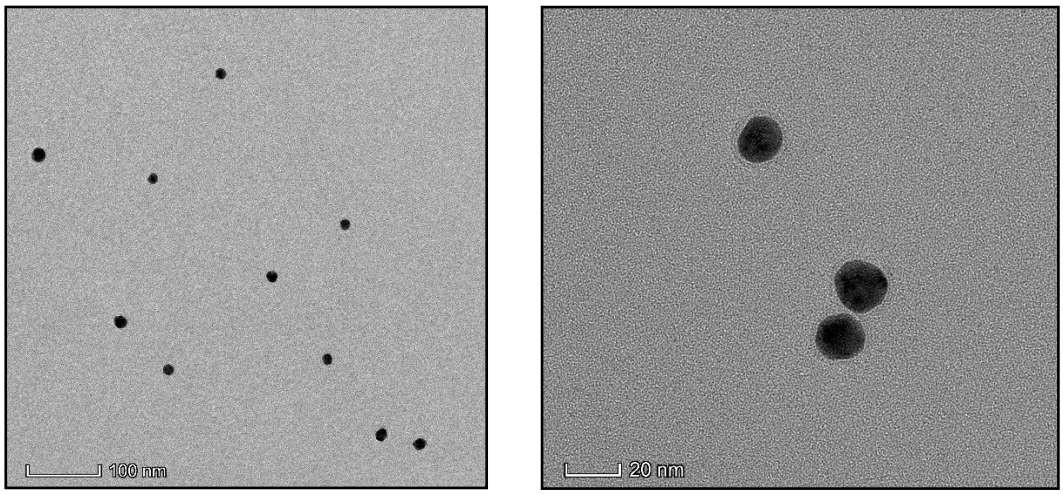


Fig. S2. TEM imaging of AuNP(A) and DNA-AuNP (B).

Determination of oligonucleotide chain loading on AuNP

According to the standard curve method, the fluorescence intensity of DNA modified by FAM at a series of different concentrations was determined and calibration curve was obtained, then the conjugated loaded DNA was released from the AuNP by DTT (20 mM), We collected the supernatant and measured its fluorescence intensity[1]. The concentration of conjugated oligonucleotide on AuNP can be determined according to the calibration curve and the fluorescence intensity of the supernatant. Finally, the concentration of the oligonucleotide chain is divided by the concentration of AuNP to obtain the average loading of oligonucleotide on AuNP[2].

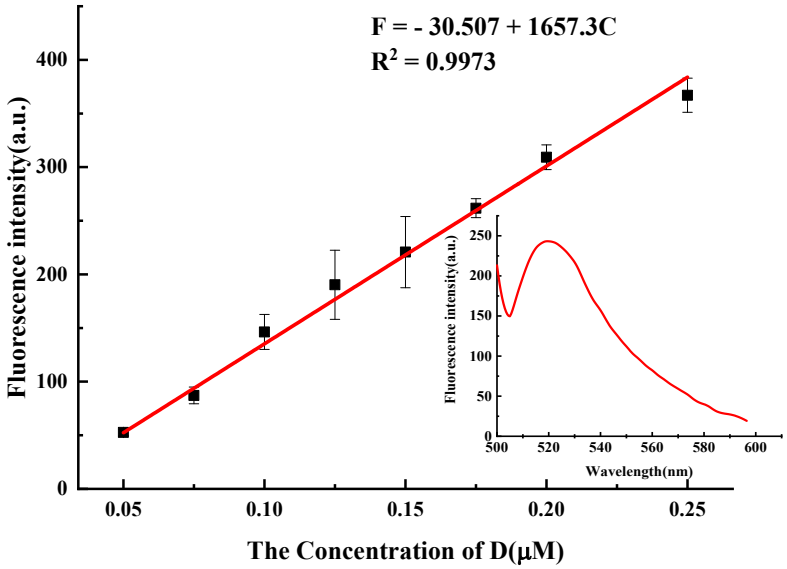
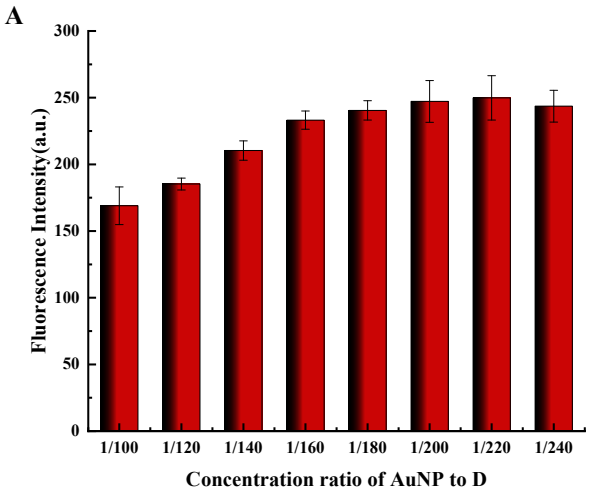
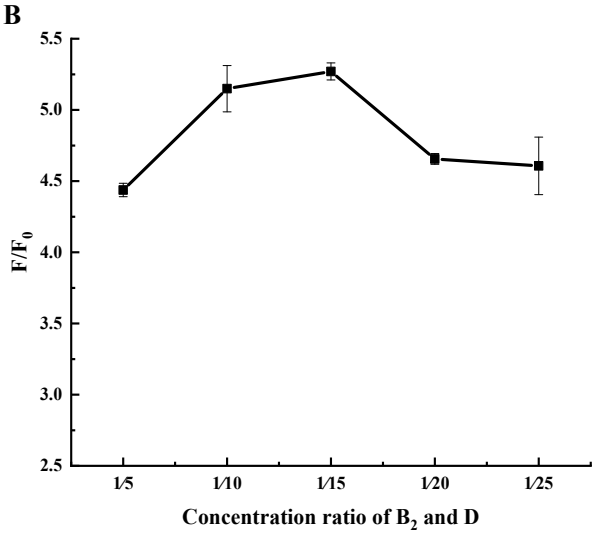


Fig. S3. Determination of oligonucleotides loading on AuNP and the fluorescence emission intensity of substrate strand D at different concentrations(0.050, 0.075, 0.100,0.125, 0.150, 0.175, 0.200, 0.250 μM). The inset is the fluorescence emission spectrum of the oligonucleotides on AuNP treated with DTT.

Pretreatment of Hela cell samples

Cultivate the purchased living cells in a cell incubator containing 5% CO_2 at 37 °C for 4-6 hours. After the cells are attached, they are digested with trypsin. Then the Hela cells obtained by the digestion are centrifuged at 8000 rpm and 4 °C for 5 min to remove the supernatant solution, then resuspended in sterile PBS buffer. Repeat this three times to completely remove the excess cell culture fluid and other impurities. Finally, it was resuspended in 1 mL Tris-HCl buffer, a part of which was reserved for cell counting experiments to determine the cell density,the remaining part was sonicated in cold water for 10 min with an sonicator to obtain cell lysate for actual sample analysis.


A
Fluorescence Intensity(a.u.)
300
250
200
150
100
50
0
1/100
1/120
1/140
1/160
1/180
1/200
1/220
1/240
Concentration ratio of AuNP to D


B
F/F0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
1/5
1/10
1/15
1/20
1/25
Concentration ratio of B2 and D

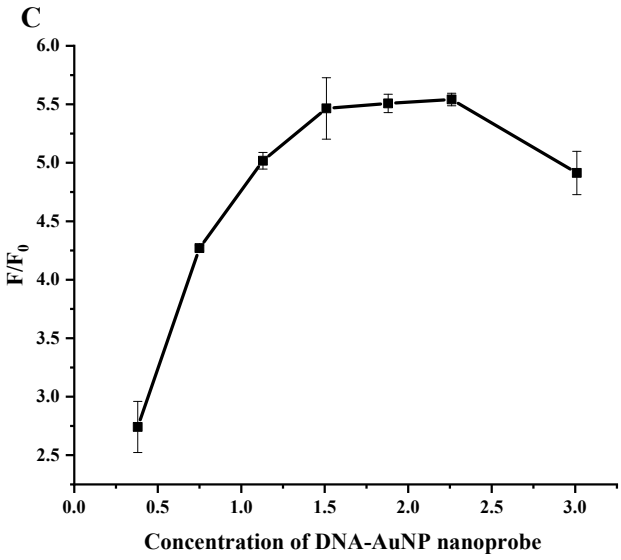

C
F/F0
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Concentration of DNA-AuNP nanoprobe

Fig. S4. (A) Concentration ratio of AuNP to D(3.01nM DNA-AuNP, DTT(20 mM); (B)The concentration ratio optimization of B2 and D(3.01nM DNA-AuNP, 0.1 U μL^{-1} Endo-Ⅳ, 1 U μL^{-1} Exo-Ⅲ, 50 U mL^{-1} UDG; (C)Optimization of nanoprobe dosage(0.1 U μL^{-1} Endo-Ⅳ, 1 U μL^{-1} Exo-Ⅲ, 50 U mL^{-1} UDG).

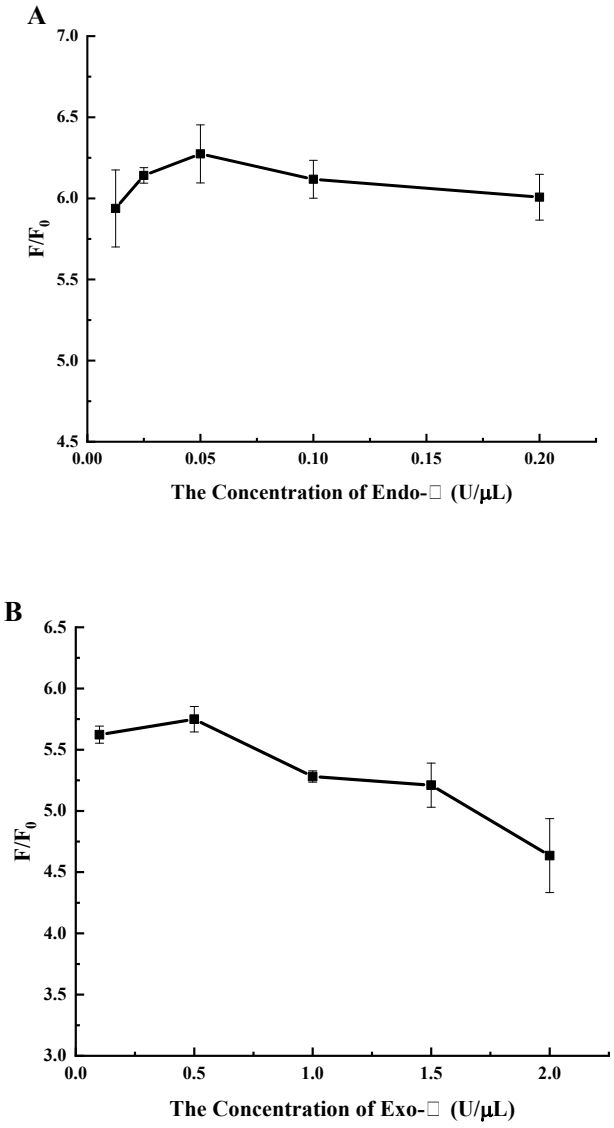


Fig. S5. (A) Endo-Ⅳ concentration of optimization (1.88nM DNA-AuNP, 1 U μL^{-1} Exo-Ⅲ, 50 U mL^{-1} UDG); (B)Optimization of Exo-Ⅲ concentration(1.88nM DNA-AuNP, 0.05 U μL^{-1} Endo-Ⅳ, 50 U mL^{-1} UDG).

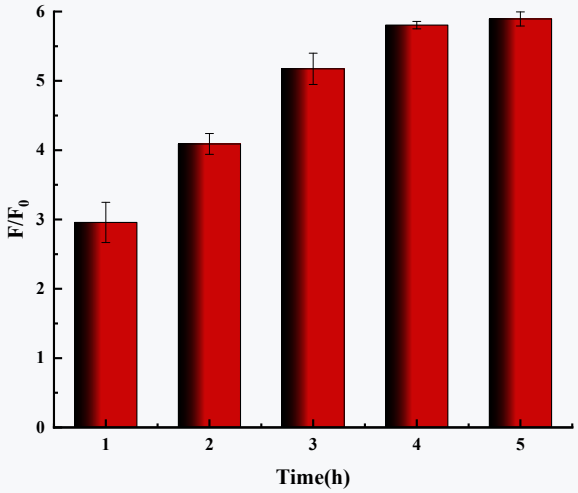


Fig. S6. The effect of reaction time on the system (1.88nM DNA-AuNP, 0.05 U μL^{-1} Endo-Ⅳ, 0.5 U μL^{-1} Exo-Ⅲ, 50 U mL^{-1} UDG).

Table S2. The comparison between UDG methods was detected based on different amplification strategies

Detection method	Amplification technique	Linear range (U mL^{-1})	Detection limit (U mL^{-1})	Reference
Fluorescence	TdT assisted	5.0×10^{-4}~1.0×10^{-2}	1.5×10^{-4}	3
Fluorescence	HCR	2.5×10^{-3}~0.25	2.4×10^{-4}	4
Colorimetry	Nb.BbvCI assisted	6.0×10^{-2}~8.0	2.0×10^{-2}	5
Fluorescence	RCA	5.0×10^{-5}~1.25×10^{-3}	1.7×10^{-5}	6
Fluorescence	Polymearase assisted	1.0×10^{-4}~0.1	1.0×10^{-4}	7
Fluorescence	CRISPR/Cas12a assisted and SDA	5.0×10^{-5}~0.1	3.1×10^{-5}	8
SERS	——	1.0×10^{-3}~10.0	4.29×10^{-4}	9
Fluorescence	DNAzyme assisted	2.0×10^{-5}~1.2×10^{-4}	3.69×10^{-6}	This work

Table S3. Recovery experiment of UDG in Hela cell lysate (n =3)

Sample	Added(U/mL)	Found((U/mL)	Recovery(%)	RSD(%)
1	0	0.316×10^{-4}		
2	0.300×10^{-4}	0.654×10^{-4}	112.79	2.46
3	0.500×10^{-4}	0.829×10^{-4}	102.70	7.93
4	0.700×10^{-4}	1.088×10^{-4}	110.32	1.49

References

1. S. J. Hurst, A. K. R. Lytton Jean and C. A. Mirkin, *Analytical Chemistry*, 2006, **78**, 8313-8318.
2. H. Pang, X. Xu and W. Jiang, *Sensors and Actuators B: Chemical*, 2020, **314**, 128053.
3. Y. C. Du, Y. X. Cui, X. Y. Li, G. Y. Sun, Y. P. Zhang, A. N. Tang, K. Kim and D. M. Kong, *Analytical Chemistry*, 2018, **90**, 8629-8634.
4. X. F. Zhang, N. Li, Y. Ling, N. B. Li and H. Q. Luo, *Sensors and Actuators B: Chemical*, 2018, **271**, 9-14.
5. X. J. Liu, M. Q. Chen, T. Hou, X. Z. Wang, S. F. Liu and F. Li, *Biosensors and Bioelectronics*, 2014, **54**, 598-602.
6. L. Dong, X. G. Zhang, Y. Li, F. J. E, J. Y. Zhang and Y. Q. Cheng, *ACS Omega*, 2019, **4**, 3881-3886.
7. L. J. Wang, M. Ren, Q. Y. Zhang, B. Tang and C. Y. Zhang, *Analytical Chemistry*, 2017, **89**, 4488-4494.
8. X. L. Chen, Y. W. Wu, G. H. Cao, X. F. Wang, Z. Ji, D. Q. Huo, F. L. Xu and C. Hou, *ACS Sensors*, 2020, **5**, 1615-1623.
9. H. W. Wang, Y. F. Cui, J. F. Wang, S. Liu, X. Zhang, X. L. Song, Y. Wang, J. D. Huang and J. H. Yu, *Sensors and Actuators B: Chemical*, 2019, **287**, 535-543.