Supporting Information

Recognition-induced three-dimensional bipedal DNA walker for highly sensitive detection of APE1

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1. Materials and reagents

Apurinic/apyrimidinic endonuclease 1 (APE1), bovine serum albumin (BSA), 8-oxoguanine DNA glycosylase (OGG1), human alkyl adenine DNA glycosylase (hAAG), uracil DNA glycosylase (UDG) and 8-oxoGDNA glycosylase (FPG) were supplied by New England Biolabs (Beijing, China). Exonuclease III (Exo III) were obtained from Takara (Beijing, China). Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was procured from Sinopharm Chemical Reagent (Shanghai, China). Trisodium citrate was purchased from Damao Chemical Reagent Factory (Tianjin, China). Mercaptoethanol and dithiothreitol were acquired from Dingguo Biotech (Beijing, China). Tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). All oligonucleotides were synthesized by Hippo Biotechnology Co., Ltd. (Huzhou, China), and the sequence information of oligonucleotides were provided in Table S1. Michigan Cancer Foundation-7 (MCF-7), RAW 264.7, minimum essential medium (MEM), fetal bovine serum (FBS) and penicillin-streptomycin were purchased from Procell Life Science &Technology Co., Ltd (Wuhan, China). 7-nitroindole-2-car-boxylic acid (NCA) was purchased from Med Chem Express (New Jersey, USA), and it structure information is detailed in Table S2.

2. Preparation of 13 nm AuNPs

Citrate-stabilized Au nanoparticles (AuNPs) with a size of 13 nm were synthesized using citrate stabilization, following established techniques.[1, 2] Briefly immerse all glassware in the prepared solution - HCl/HNO_3 (3:1), then washed many times with Milli-Q water, and subsequently dried before to the experiments. Then, 3 mL of 1%

trisodium citrate was quickly injected into a boiling solution composed of 50 mL of 0.01 % $HAuCl_4$ while stirring. The solution was then refluxed for an additional 15 min after color changed. The AuNPs were stored at 4 °C for further analysis.

3. Preparation of nanoflares

Before nanoflares immobilization, 15 μL of 50 μM thiol modified H1 was incubated with 7.5 μL of 10 mM TCEP at room temperature for 1 h to prevent disulfide bond formation. Subsequently, the solution was mixed with 10 nM AuNPs and shaken overnight. After 16 h, during salt aging, different volumes of NaCl were gradually added to the mixture over 8 h. The above solution was centrifuged at 13 000 rpm for 30 min and then washed with PBS to remove unmodified H1. The resulting nanoflares were re-suspended in PBS buffer and stored in a dark environment at 4 °C.

4. Assemble of the block-DNA/bipedal walker

The block-DNA/bipedal walker involves a bipedal walker with the walking strands enclosed by block-DNA. Initially, bipedal walker and excess block-DNA are added to Mops buffer, reacted at 37 °C for 30 min, and then annealed. Given that block-DNA contains AP site, the denaturation temperature during annealing was set to 80°C for 5 min for denaturation, followed by gradual cooling to room temperature to establish a stable double-stranded structure.

5. Cell culture and cell lysate

MCF-7 cell and RAW 264.7 cell were cultured in MEM medium. All cells were maintained in a humidified CO_2 incubator (5%) at 37°C. The cells were transferred to RNase-free 1.5 mL centrifuge tubes, washed twice with PBS, centrifuged at 2000 rpm

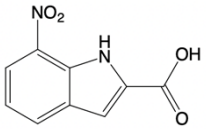
for 3 min, and then suspended in 100 μL of lysis buffer. After a 40 min incubation on ice, the lysate was centrifuged at 12000 rpm for 20 min at 4 °C and the resulting extract immediately employed for APE1 detection. During the experimental reaction process, APE1 was substituted with the cell lysate, and the remaining reactions were carried out under identical conditions to measure its fluorescence signal.

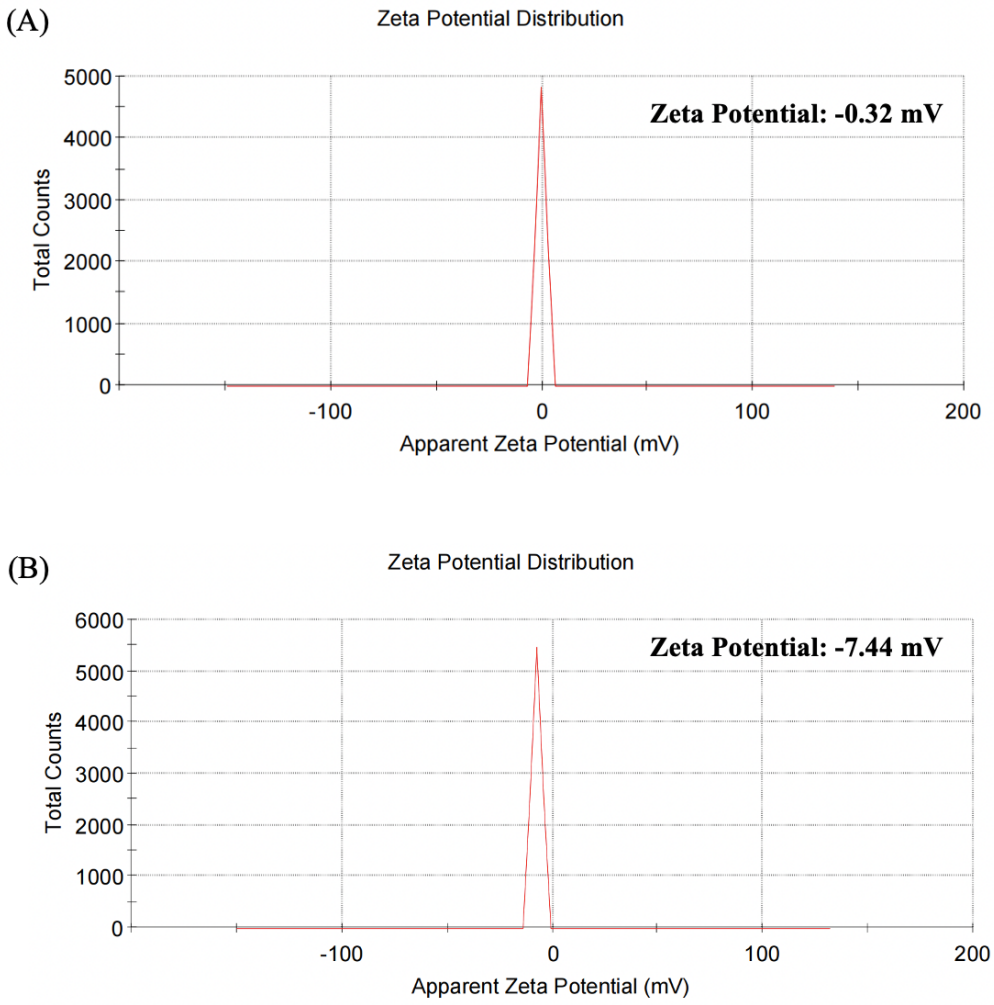
Table S1 Sequence information of the oligonucleotides

Name	Sequence (5' - 3')
bipedal walker	AGG CAC TCG ATC CAA TCA TCA GCT TTT TTT TTT TTT TTT TTT TTT TTT TTT AGG CAC TCG ATC CAA TCA TCA GC
unipedal walker	AGG CAC TCG ATC CAA TCA TCA GCT TTT TTT TTT TTT TTT TTT TTT TTT TTT
block-DNA	GAT TGG ATX GAG TGC CT
block'-DNA	GAT TGG ATC GAG TGC CT
H1	SH-TTT TTT TTC GAT CCA ATC ATC AGC CTA GCT CCG AAT TCC GCT GAT GAT TGG ATC GAG TGC CT-FAM

Note: X represents the AP site recognized by APE1. The blue colored sections are the functional regions of the respective DNA sequences.

Table S2 The structure information of NCA

Code Name	Molecular Formula	Structure
NCA	$C_9H_6N_2O_4$	



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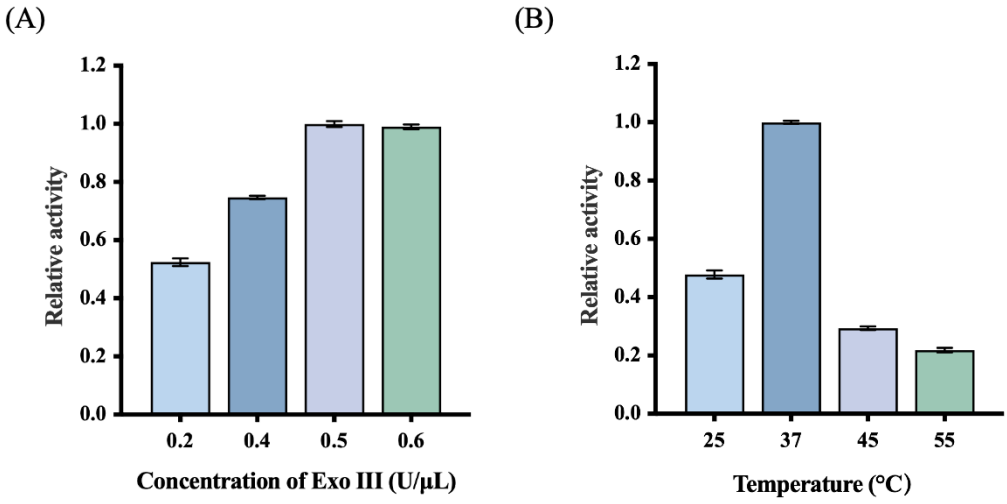
96 Fig. S1. Zeta potential distributions of (A) AuNPs and (B) nanoflares.



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98 Fig. S2. Optimization of assay conditions. (A) Concentration of Exo III. (B) Reaction temperature.

99 Error bars represent standard deviations based on three replicate measurements.

References

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2. J. Shen, L. Liang, M. Xiao, X. Xie, F. Wang, Q. Li, Z. Ge, J. Li, J. Shi and L. Wang, *J. Am. Chem. Soc.*, 2019, **141**, 11938-11946.