Supporting Information

Bipedal DNA nanowalker fueled by catalytic assembly for imaging of base-excision repairing in living cells

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Table of contents:

EXPERIMENTAL DETAILS

Materials and Reagents. Apurinic/apyrimidinic endonuclease 1 (APE1), T7 Exonuclease (T7 Exo), Deoxyribonuclease I (DNase I), Exonuclease III (Exo III), Lambda Exonuclease (λexo) and their corresponding buffers were all purchased from New England Biolabs (NEB, U.K.). Oligonucleotides used in this work were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. All the sequences are listed in Table S1. Lipofectamine 3000 and Opti-MEM were purchased from Invitrogen (MA, USA). HeLa cells (human cervical carcinoma cell line), MCF-7 (human breast adenocarcinoma cell line) were obtained from the cell bank of Central Laboratory at Xiangya Hospital (Changsha, China). RPMI 1640 medium, DMEM high glucose medium, penicillin, streptomycin and 10% heat-inactivated fetal bovine serum were obtained from Thermo Scientific HyClone (MA, USA). All reagents were used as received without further purification. All other chemicals were of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Ultrapure water was obtained through a Millipore Milli-Q water purification system (Billerica, MA, USA) and had an electric resistance >18.25 MΩ.

Instruments. The fluorescence measurements were carried out at room temperature in a quartz cuvette on an FL-7000 spectrometer (Hitachi, Japan). For the Real-time fluorescence intensity analysis, Tecan Finite M1000 (Tecan, Switzerland) was used. Agarose gel was visualized via a Tanon 4200SF gel imaging system (Tanon Science & Technology Co., Ltd., China). Flow cytometric analysis of cells was carried out on a CytoFLEX™ flow cytometer (Beckman Counter, Inc., U.S.A.). All fluorescence images were acquired using an oil dipping objective (60×) on Nikon TI-E+A1 SI confocal laser scanning microscope (Japan).

Synthesis of citrate-modified gold nanoparticles (GNPs) and DNA conjugation. GNPs were synthesized via sodium citrate reduction. Before the experiments, all glassware was cleaned in aqua regia (HCl/HNO_3, 3:1), rinsed with H_2O, and then oven-dried. 30 ml of 0.01% $HAuCl_4$ was heated to boiling under vigorous stirring, and 1 mL trisodium citrate (1%) was added under stirring. The solution color turned from pale yellow to colorless and finally to burgundy. Boiling was continued for an additional 30 min. After the heating source was removed, the colloid was stirred until the solution reached room temperature. Then the GNPs were filtered through a 0.22 μm Millipore membrane filter and stored at 4 °C. The quantitation of GNPs was performed using an approximate extinction coefficient of 2.7×10^8 L/(mol. cm) for GNPs with λ_{max} = 518 nm.

Conjugation of H1 to GNPs was performed following a previously reported protocol. Typically, 15 μL of thiolated DNA1 (50 μM) was treated with 7.5 μL of TCEP (10 mM) (1:100 molar ratio) for 1 h at room temperature in order to cleave the disulfide bond. Then, TCEP-treated DNA1 was mixed with 1 ml of 10 nM GNPs at a molar ratio of 150:1. Subsequently, phosphate buffer (0.1 M; pH = 7.0) were added to the mixture with a final concentration of 0.01 M; the concentration of NaCl of the mixture was slowly increased to 0.3 M over an 8 h period. To remove the unbound DNA, the resulting solution was washed with PBS three times (13 000 rpm, 30 min) and resuspended in 1×PBS and stored in the dark at 4 °C for the following experiments.

Characterization of GNP-H1. Concentrations of GNPs were measured with a UV-Vis spectroscopy (U-2450 UV-Vis spectroscopy, Shimadzu, Tokyo, Japan). Dynamic light scattering (DLS) measurements of GNP, and GNP-H1 were performed at 25 °C on a Zetasizer Nano ZS90 Analyzer (Malvern Instruments, UK). The samples were 0.1 nM GNP, and 0.1 nM GNP-H1 dispersed in ultrapure water before the analysis.

Quantitation of the DNA hairpins (H1) assembled on the GNPs. To detect H1 loading, DTT (10 mM) was added to the GNP-H1 solution (1 nM) for complete release of H1 from the GNPs. After shaking overnight at room temperature, the sample was centrifuged to separate the released DNA, and the fluorescence of the supernatant was measured. From the fluorescence intensity of the supernatant collected after DTT displacement (Inset of Fig. S4), the concentration of the H1 immobilized on the GNPs (1 nM) was determined to be ~ 45 nM according to the calibration equation: $F = -12.950 + 6.842 \times c_{H1}$ (nM). Therefore, the number of H1 loaded on each GNP was calculated to be ~ 45.

Gel electrophoresis analysis. Gel electrophoresis analysis was carried out on 3% (w/w) agarose gels containing 0.5 μg/mL GoldView and 0.5 μg/mL ethidium bromide running in 0.5×TBE buffer at room temperature. The electrophoresis was performed at a constant potential of 101 V for 2 h after loading 10 μL of each sample into the lanes. After electrophoresis, the gel was visualized via a Tanon 4200SF gel imaging system (Tanon Science & Technology Co., Ltd., China).

Cell culture and cell lysis. MCF-7 cells (Human breast cancer cell line) were grown in DMEM medium supplemented with 10% inactivated fetal bovine serum, 100 U/mL 1% penicillin and streptomycin solution, and HeLa cells (human cervical carcinoma cell line) were grown in RPMI-1640 medium supplemented with 10% inactivated fetal bovine serum, 100 U/mL 1% penicillin and streptomycin. All cells were cultured in a humidified CO2 incubator containing 5% CO2 at 37°C. Cells (1×106) were dispensed in an RNase-free 1.5 mL centrifuge tube, washed twice with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4), centrifuged at 2000 rpm for 3min, and then suspended in 100 μL lysis buffer (10 mM Tris-HCl , pH 8.0, 150 mM NaCl, 1% (w/v) NP-40, 0.25 mM sodium deoxycholate, 1% glycerol and 0.1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride). The lysates were incubated for 30 min on ice, and then centrifuged at 12000 rpm for 20 min at 4 °C. The extract was used immediately for APE1 detection or stored at -80 °C for further research.

Flow cytometry assay of APE1 activity using DNA walker. HeLa cells (1×10^5 cells) were incubated with H0, GNP-H1 and H2 in 500 μL fresh medium at 37 °C for 3 h and washed three times with cold PBS. Then, the cells was detached with 150 μL of 0.25 % trypsin for 3 min and centrifuged for 5 min at 300 g followed by two washes with 500 μL PBS and re-suspension in 1 mL PBS for flow cytometry assay on a FACSVerseTM flow cytometer (BD Biosciences, USA).

TIRF imaging and analysis. For TIRF imaging, 200 μL of reaction mixture containing 0.5 nM H0, 5 nM SNAs and 250 nM H2 were deposited on the 35 mm2 Petri dish with 14 mm well. Images of fluorescently SNAs-based DNA walker were obtained using a commercial TIRF microscopy (Nikon). A 60× oil-immersion objective (NA 1.49) and 640 nm laser was used. The fluorescence intensity changes over time were recorded after addition of APE1.

Fluorescence experiments in vitro. The ability for DNA walker to detect targets was determined using F-7000 fluorescence spectrometer. Cy5 fluorescence emission signal was recorded from 645 to 750 nm in 0.2nm increment, under an excitation wavelength of 620 nm. The concentration of the involved components used for the fluorescence calibration curve assays were 2.0 nM H0, 2 nM GNP-H1 and 100 nM H2 in 1×Nebuffer 1.1 (10 mM Bis Tris Propane-HCl, 10 mM MgCl2, 100 μg/ml BSA pH=7.0), and treated with target of a series of concentrations. The fluorescence signal without target was recorded as the background signal. All experiments were repeated at least three times.

Confocal fluorescence imaging. MCF-7 cells and HeLa cells were added into a 35 mm2 Petri dish with 10 mm well in 1 mL of culture medium at 37 °C for 24 h to reach 80% confluency and then were washed with PBS buffer. Next, H0 (2.0 nM), GNP-H1 (2 nM) and H2 (100 nM) were delivered into cell by liposome transfection in 500 μL fresh medium and incubated for 3 h. In the inhibitor experiment, HeLa cells were pretreated with 2 μM NCA for 3 h, and then incubated with fresh DMEM containing lipofectamine-3000, H0, GNP-H1 and H2 for another 3 h. After washing three times with 1 mL of PBS, the cells were incubated with 1 mL of fresh medium at 37 °C before imaging. All cellular fluorescent images were collected with a Nikon TI-E+A1 SI confocal laser scanning microscope, and the images were acquired using 60× oil immersion objective lens.

Calculation of number of walking steps. 2 nM H0/Hs, 2 nM SNAs and 100 nM H2 were incubated with 20 U/mL APE1. Because of the stoichiometric ratio of catalyst and SNAs is 1:1, the catalyst was supposed to be irreversibly released after walking all over the SNAs surface. Fluorescence over time corresponding to the walking behavior for unipedal and bipedal DNA walker were recorded, respectively. In unipedal case, H2 was omitted whether without (line 1 in Fig. 2b) or with catalyst (line 3 in Fig. 2b) in the non-catalytic reaction. While in the catalytic turnover, H2 was added with H1-particles (line 2 in Fig. 2b) or catalyst-H1-particle (line 4 in Fig. 2b). Left schematic in Scheme S2 showed the final state of the non-catalytic reaction, the difference of fluorescence value between line 3 and line 1 ($F_{non\text{-}cat}$ =F_{line3}-F_{line1}=26) reports the number of catalyst on the SNAs surface. While the right one showed the final state of the catalytic case and the difference of fluorescence value between line 4 and line 2 (Fcat=F_{line4}-F_{line2}=315) reports the H1:H2 complex generated by the catalyst. The ratio of F_{cat} to $F_{non\text{-}cat}$ reports the amplification times (AT_S= F_{cat}/$F_{non\text{-}cat}$=315/26=12.1). The walking steps of 1 single catalyst on the SNAs surface in the non-catalytic case was set to 1, so 1 single catalyst walks 12 steps in the catalytic case. In bipedal case, the same procedure was used and the amplification times was calculated to be 17.5 (AT_D= F_{cat}/$F_{non\text{-}cat}$=1141/65=17.5). Because the walking steps of 1 double catalyst on the SNAs surface in the non-catalytic case was set to 2, so 1 double catalyst walks 35 steps in the catalytic case.

Table S1. Sequences of oligonucleotides used in this work.

Name	sequences (5'-3')
H0	CGACATCTAACCTAGCTCACGGATCCGTGACAACCTCACGGTCTATTA CACACCACGACATCTAACCTAGCTCACGGAXCCGTGAGC
H_T	CGACATCTAACCTAGCTCACGGATCCGTGACAACCTCACGGTCTATTA CACACCACGACATCTAACCTAGCTCACGGATCCGTGAGC
H_S	CGACTCATGTATCCGTGACAACCTCACGGTCTATTACACACCACGACA TCTAACCTAGCTCACGGAXACATGAGTCG
H1	ttttttttttTCCGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATCTAA CCTAGC
H1-Cy5	SH-ttttttttttTCCGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATCTAA CCTAGC(Cy5)
H1-FQ	ttttttttttTCCGTGAGCT(BHQ3)AGGTTAGATGTCGCCATGTGTAGACGACA TCTAACCTAGC(Cy5)
H2	AGATGTCGTCTACACATGGCGACATCTAACCTAGCCCATGTG TAGAC

Helper hairpin H0 includes the initiator sequence (blue) and the spacer sequence (black). X and A in red represent the abasic site and its opposite base. Underlined sequences indicate complementary regions of the probes to form hairpin structure.

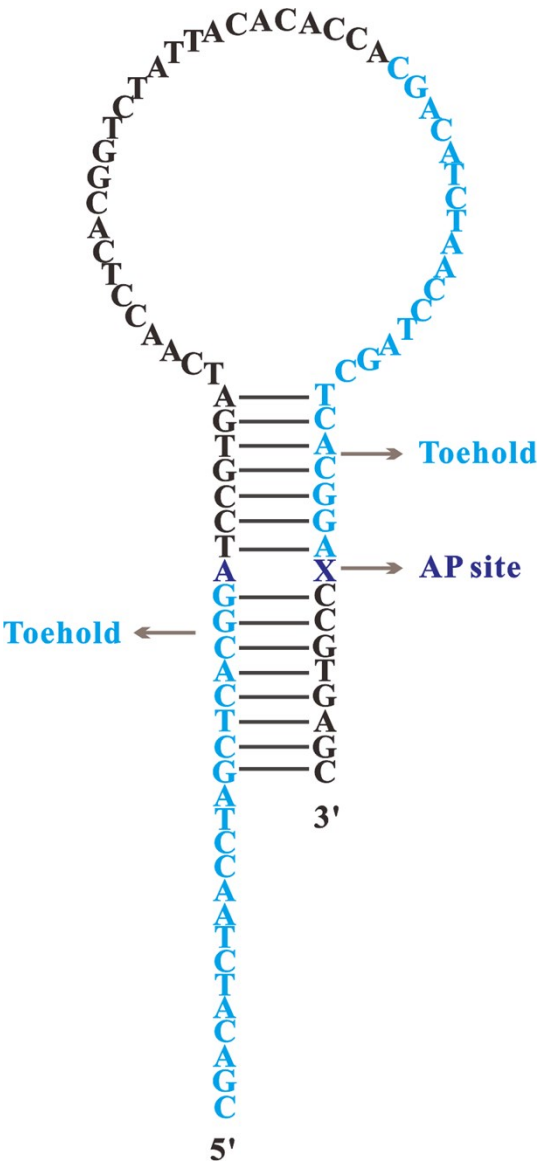


Fig. S1 Structure of the self-hybridized helper hairpin (H0). ΔG = -13.8 kcal•mole^{-1}, ΔH = -117.4 kcal•mole^{-1}, ΔS = -334.0 cal•K^{-1}mole^{-1}, Tm = 78.3°C. The data was calculated with UNAfold using 10 mM Mg^{2+}.

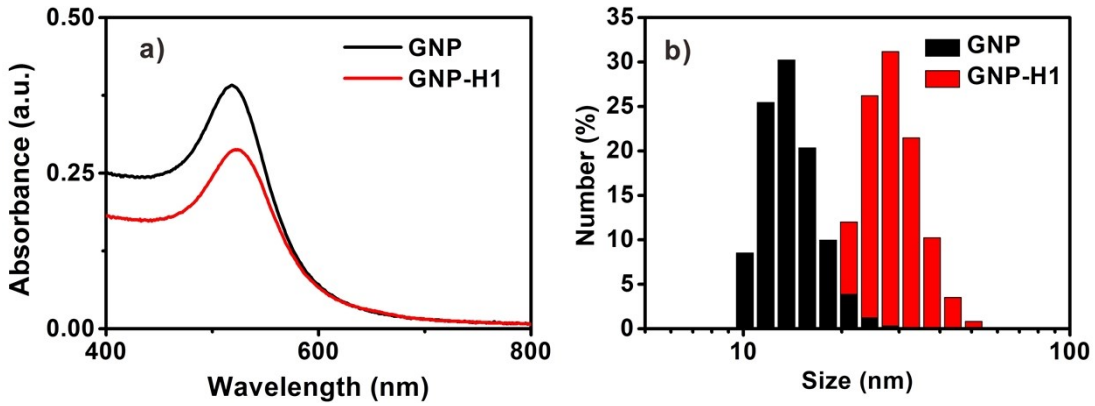


Fig. S2 Characterization of GNP-H1 conjugation. (a) Absorption spectra show that the maximal absorption of GNPs shifted from 518 nm to 522 nm after the conjugation of H1. (b) Dynamic light scattering measurements show that the hydrodynamic sizes of GNPs increased after conjugation with H1.

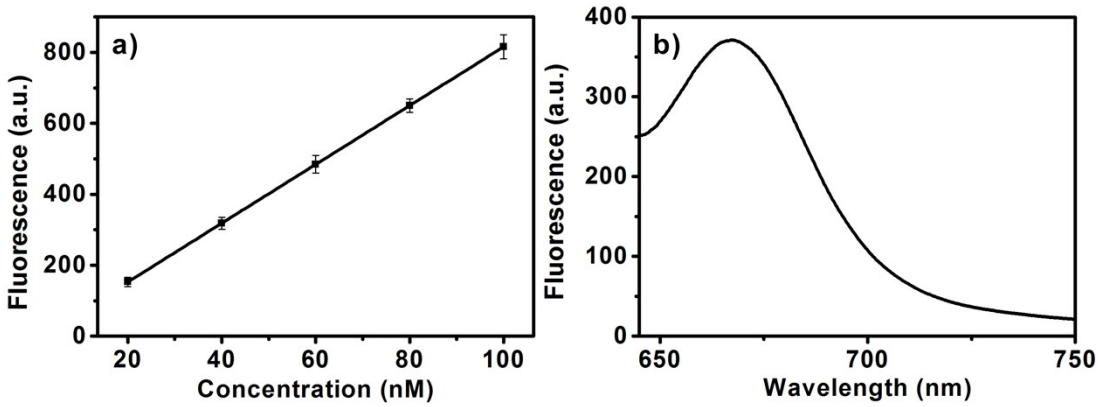


Fig. S3 Quantitation of the DNA hairpins (H1) assembled on the GNPs. (a) Standard linear calibration curve of the fluorescence signal against the concentration of the H1. (b) Fluorescence spectrum of the supernatant after the treatment of the GNP-H1 with DTT to release the hairpins. Solutions were excited at 620 nm (Cy5) and emission spectra were collected from 645 nm to 750 nm.

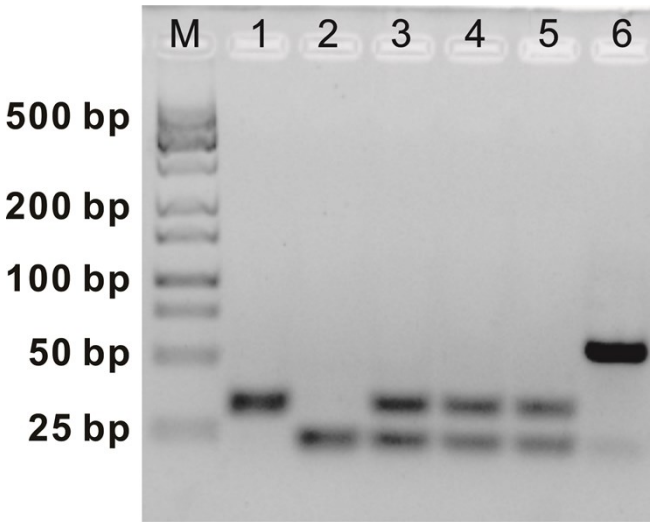
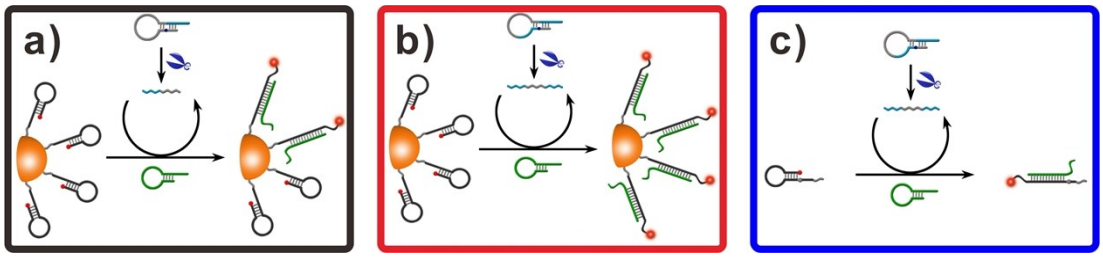
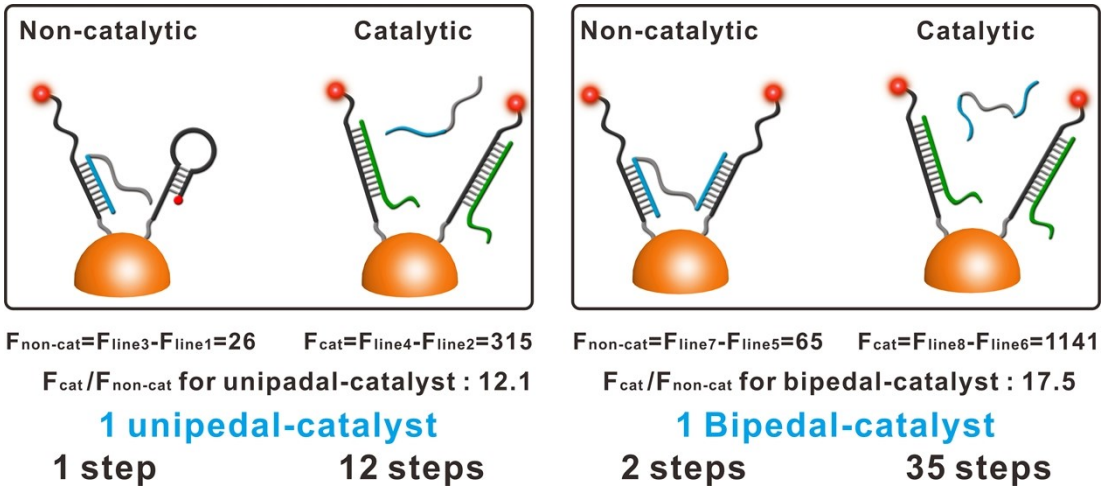


Fig. S4 Gel electrophoresis for control non-abasic H_T versus abasic H0. Lane 1, H1; Lane 2, H2; Lane 3, H_T plus H1 and H2; Lane 4, H_T plus H1, H2 and 20 U/mL APE1; Lane 5, H0 plus H1 and H2; Lane 6, H0 plus H1, H2 and 20 U/mL APE1; M, 25 bp DNA size marker.



Scheme S1. Schematics for unipedal-catalyst reaction on SNAs (a), bipedal-catalyst reaction on SNAs (b), and bipedal-catalyst reaction in solutions (c).



Scheme S2. Schematic illustration of quantitative analysis of the number of steps taken by the unipedal (left) and bipedal DNA walker (right).

Scheme S1. Schematics for unipedal-catalyst reaction on SNAs (a), bipedal-catalyst reaction on SNAs (b), and bipedal-catalyst reaction in solutions (c).

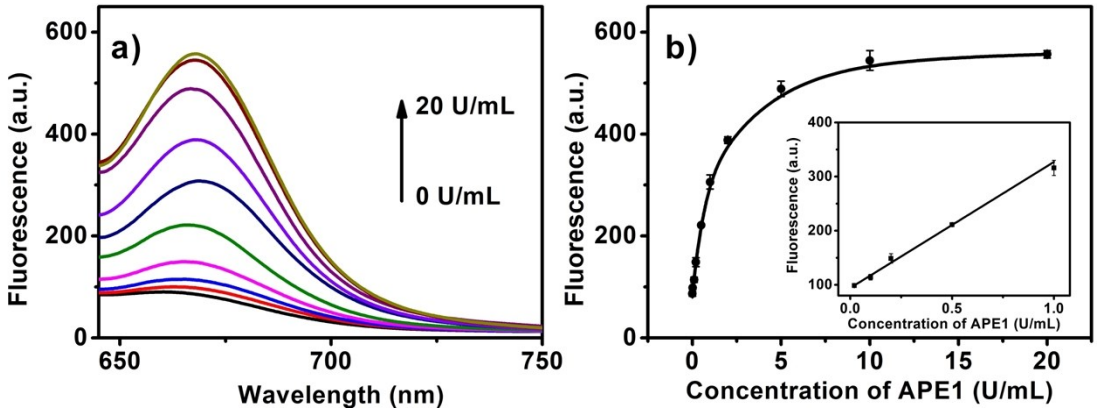


Fig. S5 (a) Fluorescence responses of the DNA walker to different concentrations of APE1 (from bottom to top: 0, 0.02, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 U/mL). (b) Plot of fluorescence peak intensities versus APE1 concentrations. Inset: Linear relationship between the fluorescence intensity and the concentration of APE1. For all the above experiments, error bars are standard deviations of three repetitive experiments.

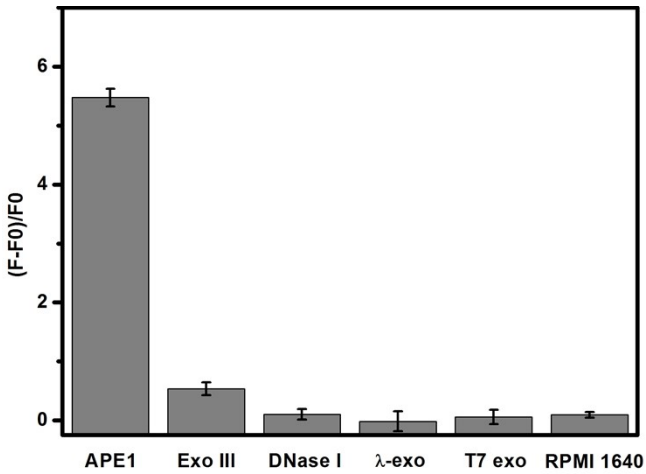


Fig. S6 Specificity of DNA walker for APE1 (2.0 U/mL) against other nucleases (Exo III: 4.0 U/mL; DNase I: 5.0 U/mL; λ-exo: 66.7 U/mL; T7: 50U/mL) and RPMI 1640 cell growth medium. F means fluorescence intensity, F0 means the original fluorescence intensity.

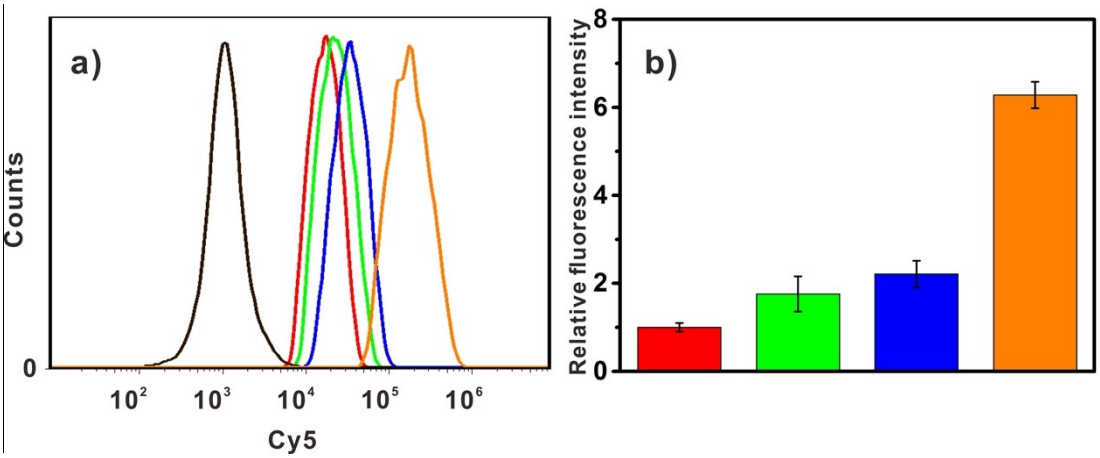


Fig. S7 (a) Intracellular verification of DNA walker by flow cytometry. HeLa cells were incubated with H_T plus GNP-H1 and H2 (red); H0 plus GNP-H1 (green); H0 plus GNP-H1 and H2 (orange); Hs plus GNP-H1 and H2 (blue); Control (brown). (b) The histogram of relative fluorescence intensity of flow cytometry.

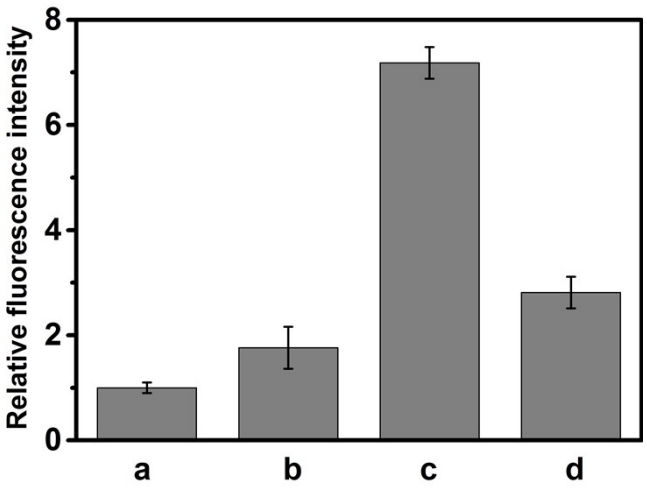


Fig. S8 The relative fluorescence intensity of intracellular verification of DNA walker by further analysis of confocal fluorescence image. HeLa cells were incubated with H_T plus GNP-H1 and H2 (a); H0 plus GNP-H1 (b); H0 plus GNP-H1 and H2 (c); Hs plus GNP-H1 and H2 (d). The corresponding fluorescence intensity (FL) were obtained for the cytosolic fluorescence in the cells in Fig. 3.

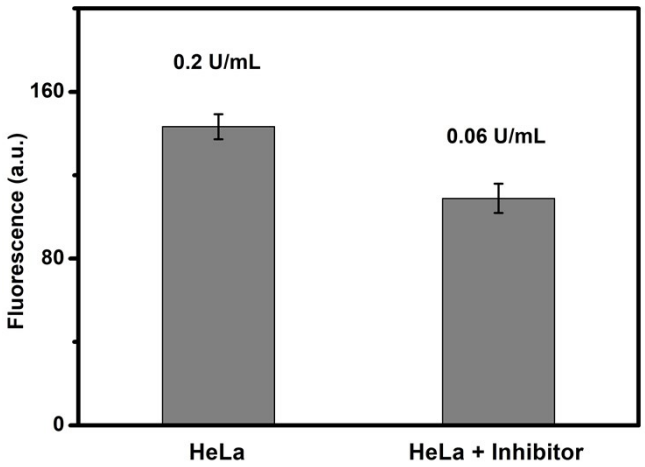


Fig. S9 Fluorescence intensity of different APE1 activity in HeLa cell extracts. The numbers of normal and inhibitor (2 μM NCA) treated HeLa cells were both 100. Error bars represent standard deviations from three replicates.

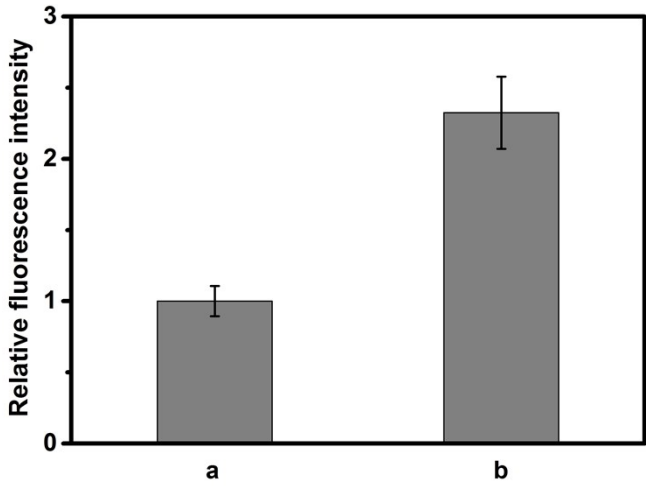


Fig. S10 The relative fluorescence intensity of reduced intracellular APE1 activity by further analysis of confocal fluorescence image. Cells were pretreated with 2μM NCA and then incubated with Hs, GNP-H1 and H2 (a); H0, GNP-H1 and H2 (b). The corresponding fluorescence intensity (FL) were obtained for the cytosolic fluorescence in the cells in Fig. 4.

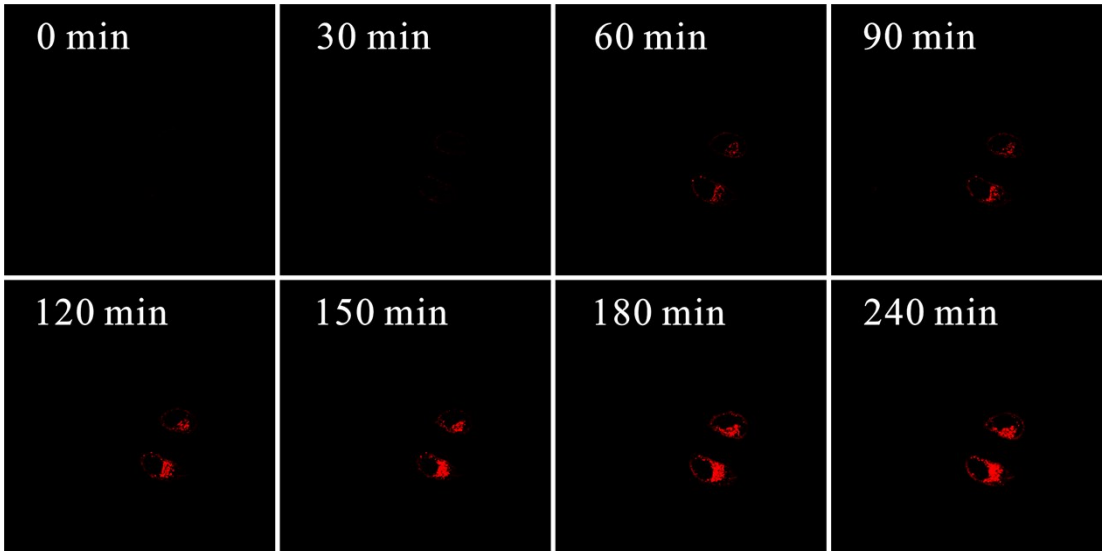


Fig. S11 Real time monitoring for the performance of DNA walker in living cells. HeLa cells were incubated with H0, GNP-H1 and H2 and the fluorescence image were captured by an interval of 0.5 h.

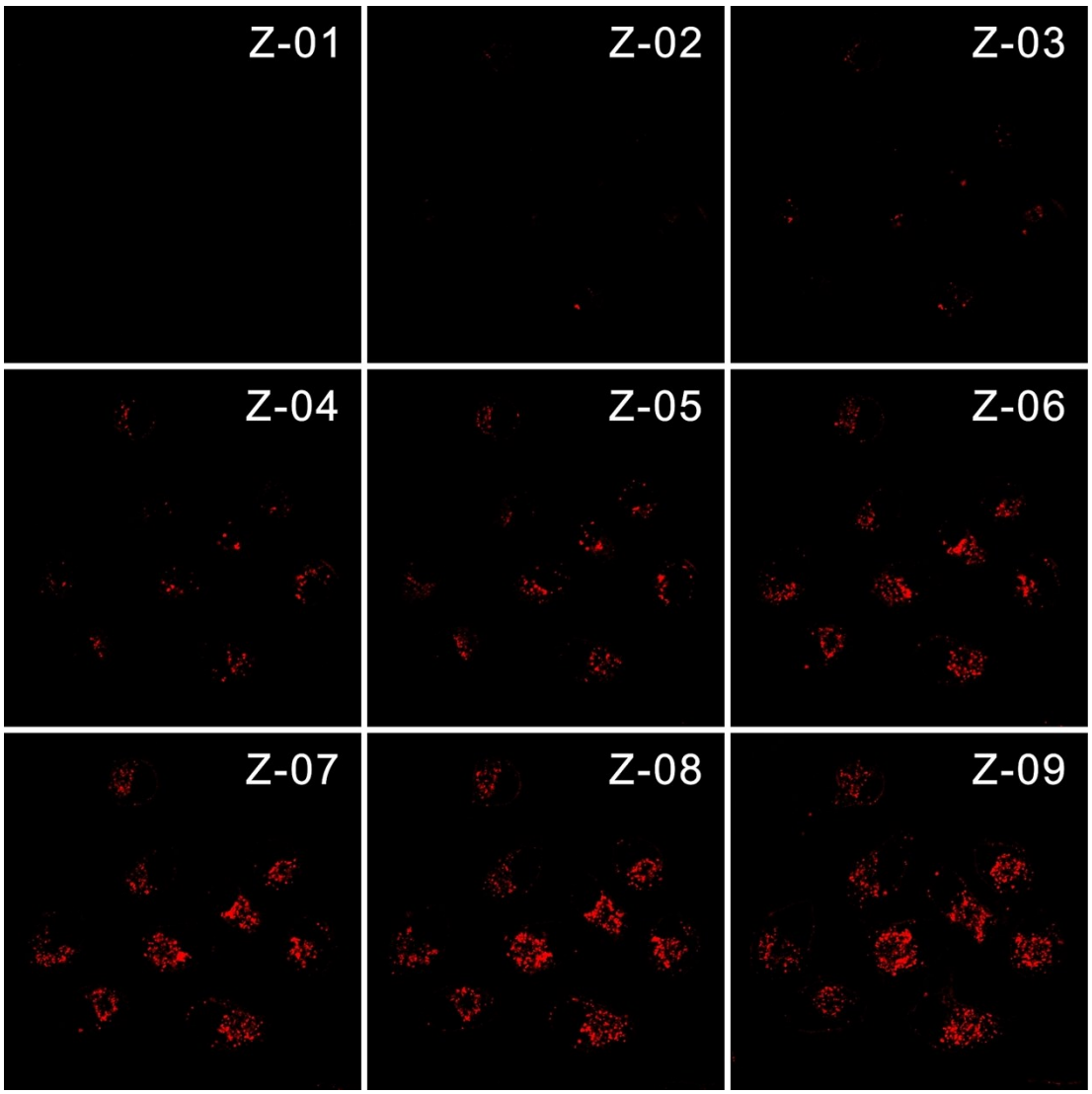


Fig. S12 Z-stacks analysis of APE1 activity in HeLa cells by using DNA walker. (1.0 μm per step)

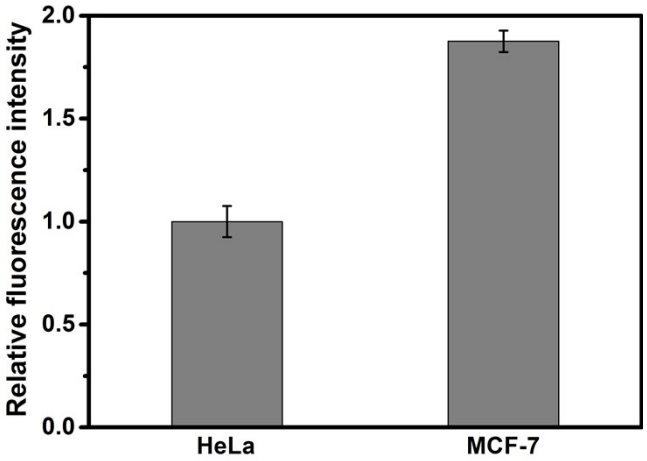


Fig. S13 The relative fluorescence intensity of APE1 activity in different cells by further analysis of confocal fluorescence image. The corresponding fluorescence intensity (FL) were obtained for the cytosolic fluorescence in the cells in Fig. 5.

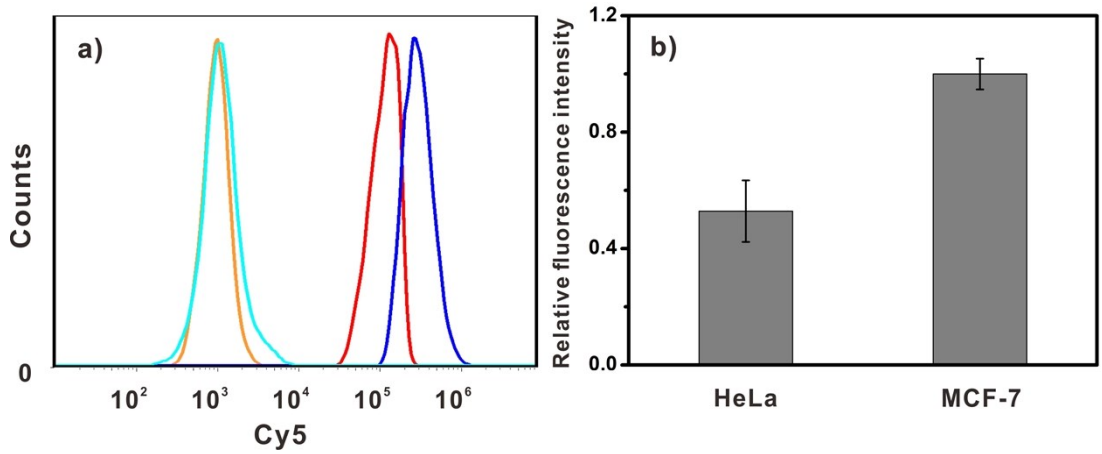


Fig. S14 (a) Flow cytometry analysis of HeLa (Orange for control and red for DNA walker) and MCF-7 (Cyan for control and blue for DNA walker). (b) The normalized histogram of fluorescence intensity from flow cytometry.