Electronic Supplementary Information

Target-mediated self-assembly of DNA networks for sensitive

detection and intracellular imaging of APE1 in living cells

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Materials and reagents

Apurinic/apyrimidinic endonuclease 1 (APE1), Nt.BbvCI, Exonuclease I (Exo I) and EcoRI were applied by New England Biolabs, Inc. (Beverly, MA, USA). Human 8-oxoguanine DNA glycosylase (hOGG1) was obtained from Trevigen Inc. All oligonucleotides were synthesized by Beijing Hippo Biotechnology Co., Ltd. (Beijing, China), and the sequence information of oligonucleotides were shown in Table S1.

Table S1. Sequences information of the oligonucleotides

name	sequence (5' - 3')
T1	ACAAACAACACAACAACCATTACATTCCTAAGTCTGAAACATT ACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
T2	AAAAAAAAAAAAAAAAAAATTTATCACCAGGCAGTTGACAGT GTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
T3	AAAAAAAAAAAAAAAAAAATTTCAACTGCCTGGTGATAAAAC GACACTACGTGGGAATCTACTATGGCGGCTCTTC
T4	AAAAAAAAAAAAAAAAAAATTTTCAGACTTAGGAATGTGCTT CCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
H1	GGTTGTTGTGTTGTTTGTCTAGGCTAGA/idSp/ATCTCCAGAACT GCTGCGCCGCCGGGAAAATACTGTACGGT/idSp/CTGGAGATGT

CTAGCCTAGCTC

H2	TTTTTTTTTTTTTTTTTTTGAGC/iCy5dT/AGGCTAGACATCTCCAG ACAGATGTCTAGCCT/iBHQ2dT/GAGCGAACGAGT
H3	TTTTTTTTTTTTTTTTTTTCGTTCGCTCAAGGCTAGACATCTGTCT GGAGATGTCTAGCCGACAGATGTCTAGCCTTGA
ssDNA	TGAAGAAGATCACCCTCCTTATTACTCGTTCGC
siPLK	Sense: UGAAGAAGAUCACCCUCCUUAUUACUCGUUCGCUC Antisense: UAAGGAGGGUGAUCUUCUUCATT

In addition, the buffers used in this work were listed as follows:

(1) 1 × TE buffer (containing 10 mM Tris-HCl and 1.0 mM ethylenedia minetetra acetic acid (EDTA), pH 8.0);

(2) Tris-HCl buffer (containing 10 mM Tris-HCl, 1.0 mM EDTA and 100 mM NaCl, pH 8.0);

(3) Tris-Magnesium sulfate buffer (TM buffer, 10 mM Tris-HCl and 50 mM MgCl2, pH 8.0) was used as reaction buffer solution for the tetrahedron DNA walker self-assembly of four single strands of DNA (T1, T2, T3 and T4).

(4) 5 × TBE buffer (including 445 mM Tris base, 445 mM boric acid and 10 mM EDTA, pH 8.0) was used in the native polyacrylamide gel electrophoresis (PAGE)

experiments, which was purchased from Shanghai Sangon Biotech. Co., Ltd. (Shanghai, China).

In addition, the deionized water used in this work (resistivity 18.2 MΩ•cm) was from a water purification system.

Instruments

The fluorescence signal measured by L-7100 spectrometer (Hitachi, Japan). BG-verMIDI standard vertical electrophoresis apparatus (Baygene, China) and a Gel Doc XR+ System (BioRad, California, U.S.A.) were used to native PAGE analysis and imaging. An atomic force microscope (AFM) by BRUKER Company, Ltd. (Karlsruhe, Germany) was employed to characterize DNA nanostructures. Confocal laser scanning microscope (Olympus, Tokyo, Japan) was used for the evaluation of the activity of intracellular of APE1.

Assembly of the functionalized tetrahedral DNA nanostructures

The functionalized tetrahedral DNA nanostructures f-TDNs (f-TDN1 and f-TDN2) were assembled in two steps. First, equal amounts of T1-T4 were dispersed in TM buffer solution and experienced a rapid annealing process to form tetrahedral DNA nanostructures (TDNs). After that, TDNs, hairpin H1 and hairpin H2 were mixed with stoichiometric ratio of 1:1:3 and reacted at 37 °C for 2 hours to form f-TDN1.

Similarly, f-TDN2 was formed from TDN, hairpin H1 and hairpin H3 in a 1:1:3 ratio under the same conditions. All hairpins H1, H2 and H3 were heated at 95 °C for 10 min and progressively cooled to 8 °C to form hairpin nanostructures before use, and the 3′-end of H2 was blocked by siRNA via base complementary pairing.

Kinetic comparison of conventional CHA and f-TDNs CHA

Real-time fluorescence experiment was adopted to compare the kinetics of conventional CHA and f-TDNs CHA reactions. Firstly, f-TDN1, f-TDN2, hairpins H1, H2-ssDNA (ssDNA was used instead of siRNA), and H3 were prepared before use. Then, different reaction systems of f-TDNs CHA reaction (containing 125 nM f-TND1, 125 nM f-TDN2, $1 \times$ NEBuffer 4 and 1×10^{-1} U/μL of APE1) and conventional CHA reaction (containing 250 nM H1, 375 nM H2-ssDNA, 375 nM H3, $1 \times$ NEBuffer 4 and 1×10^{-1} U/μL of APE1) were separately recorded on the excitation wavelength of 640 nm and emission wavelength of 664 nm.

Activity assay of APE1 in vitro

Firstly, 50 μL of sample solutions containing 125 nM f-TND1, 125 nM f-TDN2, $1 \times$ NEBuffer 4 and different concentrations of APE1 (0, 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-5}, 1×10^{-4}, 1×10^{-3}, 1×10^{-2}, and 1×10^{-1} U/μL) were incubated at 37 °C for 2.5 h.

After that, corresponding emission spectras of 600 to 750 nm of the sample solutions were recorded at the excitation wavelength of 640 nm, respectively.

Intracellular APE1 activity imaging

Firstly, MCF-10A, MCF-7, HeLa and MAD-MB-231 cells were incubated in confocal dishes to reach an 80% in a humid atmosphere with 5% CO_2. Then, all the cells were washed with phosphate buffered saline (PBS, pH 7.2) and incubated with fluorescence probes in fresh medium at 37 °C for 4 h. Subsequently, all cells were stained with Hoechst 33342 solution for 10 min and washed with PBS for three times before confocal fluorescence imaging.

MTT cytotoxicity assay

MTT assay was used to evaluate the apoptosis of tumor cells by the proposed strategy. Firstly, HeLa and MAD-MB-231 cells were introduced in 96-well plates to achieve approximately 80% confluence, respectively. Subsequently, different concentrations of TDN, siRNA and f-TDNs were added and incubated for 24 hours, respectively. After that, 10 µL of MTT solution (5 mg/mL) was added in each wells and incubated at 37 °C for 4 h. After removing the MTT solution, 200 µL of DMSO solution was added and shaked for 10 min to dissolve the precipitate. Finally, the absorbance value of each well at 490 nm was recorded with RT6000 microplate reader.
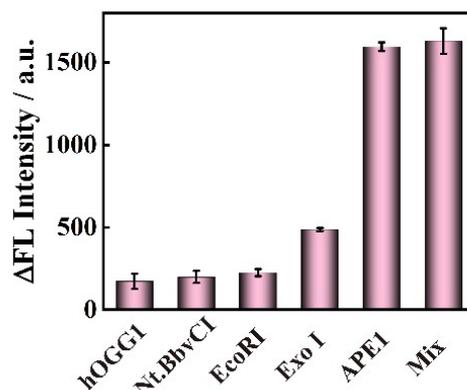
Specificity of detection of APE1

Fig. S1 Specificity of the detection for APE1 against other nucleases by the f-TDNs CHA system (APE1: 1×10^{-2} U/μL; hOGG1: 1×10^{-1} U/μL; Nt.BbvcI: 1×10^{-1} U/μL; EcoRI: 1×10^{-1} U/μL; Exo I: 1×10^{-1} U/μL).

Theoretical calculation of local concentration of hairpins

The local concentration of hairpins for free system and f-TDNs system was analyzed by collision theory ($V = 1/cN$),[1, 2] where V is the local sphere volume, c is the concentration of probes, and N is the Avogadro constant. And f-TDN1 was selected as the model for the calculation. In a homogeneous solution containing 100 nM H1 and 100 nM H2, the volume of a sphere containing both H1 and H2 molecules was calculated to be 1.66×10^{-17} L with a radius of 158 nm. In f-TDN1 nanostructure, the distance between H1 and H2 was about 20 nm including the anchoring segment and linkage segments of H1 and H2 (60 base pairs). Confined within a sphere of 20 nm in radius, the local concentrations of H1 and H2 were calculated as 49.55 μM and

148.50 μM in f-TDN1, respectively. The local concentrations of H1 and H2 in f-TDN1 increased by 495.5-fold as compared with the situation in homogeneous solution.
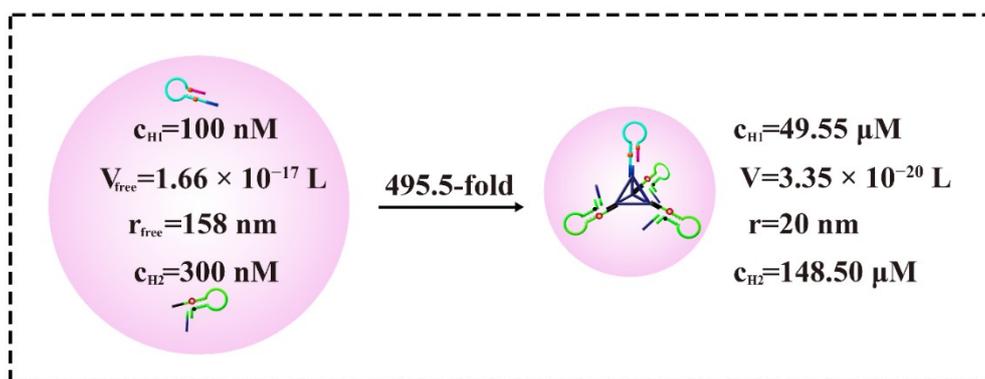

Fig. S2 Comparison of the local concentrations of hairpins for free system and f-TDNs system.

Optimizations of the reaction time of APE1 triggered f-TDNs CHA

The reaction time of f-TDNs CHA triggered by APE1 was an extremely momentous factor for the detection efficiency and imaging performance of the proposed strategy. Thus, the different incubation time of f-TDNs CHA was investigated as shown in Figure S1. The experiment results indicated that with the increase of the incubation time, the FL intensity heightened gradually and tended to be stable under the incubation time of 2.5 h. Consequently, 2.5 h was chosen as the optimal incubation time of the f-TDNs CHA reaction.
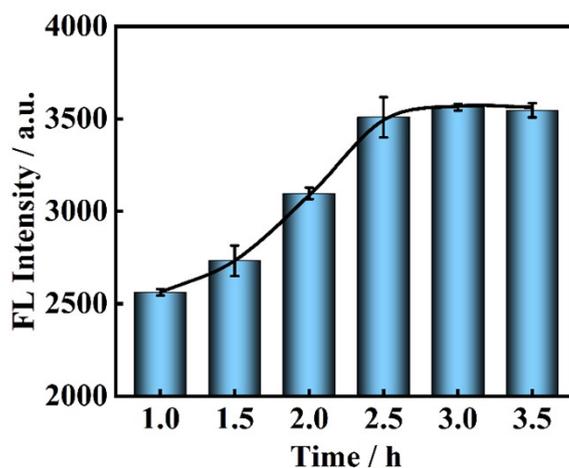
Fig. S3 The incubation time of APE1 to trigger CHA on f-TDNs. Error bars: SD, *n = 3*.

Comparison of different methods for APE1 detection

Table S2. Comparison of different methods for APE1 detection

Method	Linear range /(U/µL)	Detection limit /(U/µL)	Reference
Fluorescence	$1 \times 10^{-5} \sim 1 \times 10^{-4}$	1×10^{-5}	3
Fluorescence	$2 \times 10^{-5} \sim 1 \times 10^{-3}$	1×10^{-5}	4
Fluorescence	$1 \times 10^{-5} \sim 1 \times 10^{-1}$	5.54×10^{-6}	5
Fluorescence	$1 \times 10^{-5} \sim 1 \times 10^{-3}$	1×10^{-5}	6
Fluorescence	$1 \times 10^{-6} \sim 1 \times 10^{-2}$	5×10^{-7}	7
Fluorescence	$1 \times 10^{-7} \sim 1 \times 10^{-1}$	3.34×10^{-8}	**This work**

Reference

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