Electronic Supplementary Material (ESI) for Analyst

Supporting Information

A novel fluorescent assay of Uracil DNA glycosylase activity by 3'-5' exonuclease activity-based endonuclease IV cyclic signal amplification strategy

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Materials and Experimental details

All the oligonucleotides used in this work (Table 1) were synthesized and HPLC-purified by Sangon Biotech. Co. Ltd. (Shanghai, China).

Table S1. Oligonucleotides Used in This Work

Oligonucleotide	Sequence（5'-3'）
MB	Dabcyl-CGCTGTCCAGTCAGTGTCCTCAGCG-FAM
P1	CTA GAG ATT T dU CCA GCT GAG GAA ATG GAA AAT CTC TAG AAA AAA A
P2	CCA GCT GAG GAA ATG GAA AAT CTC TAG AAA AAA A
P3	AGAGATTTTCCACACTGACTTTTTTTT
P4	TAACCACGAGTCAGTGTCCTCAGCG-FAM
P5	TTA ACC ACA AGT CAG TGT CCT CA-FAM
P6	TTA ACC ACA AGT CAG TGT CCT-FAM
P7	TTAACCACAAGTCAGTGTCCTCAGC-FAM

E. coli uracil DNA glycosylase (UDG), E. coli endonuclease IV (Endo IV), E. coli exonuclease III (Exo III), uracil glycosylase inhibitor (UGI), E. coli DNase I, apurinic/apyrimidinic Endonuclease 1 (APE1) and agarose were purchased from New England Biolabs (Ipswich, MA, USA). Bovine serum albumin (BSA), alkaline phosphatase (ALP) and nuclear and cytoplasmic protein extraction kit were purchased from Sangon Biotech. Co. Ltd. (Shanghai, China). The O'Range Ruler 10 bp DNA Ladder (10-150 bp) was purchased from Thermo Fisher Scientific Co. Ltd (MA, USA). Graphene oxide was purchased from XFNANO Company (Nanjing, China). Goldview and ethidium bromide were purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd. (China). All the other chemical reagents were of analytical grade, which were used without further purification. The water used throughout the experiments was obtained from a water purification system with an electric resistance of >18.2 MΩ·cm (Sartorius, Germany).

Fluorescence measurements were performed in a 96-well black microplate on a Varioskan LUX microplate reader with 405nm excitation light (Thermo Scientific,

USA). Fourier Translation Infrared (FT-IR) spectroscopy was measured using a Spectrum Two spectrometer (PerkinElmer, USA). Transmission electron microscope (TEM) image was acquired by TECNAI G^2 20 with an accelerating voltage of 200 kV (FEI, USA). Atomic Force Microscope (AFM) image was acquired by CSPM5500 (Bibby Scientific, China).

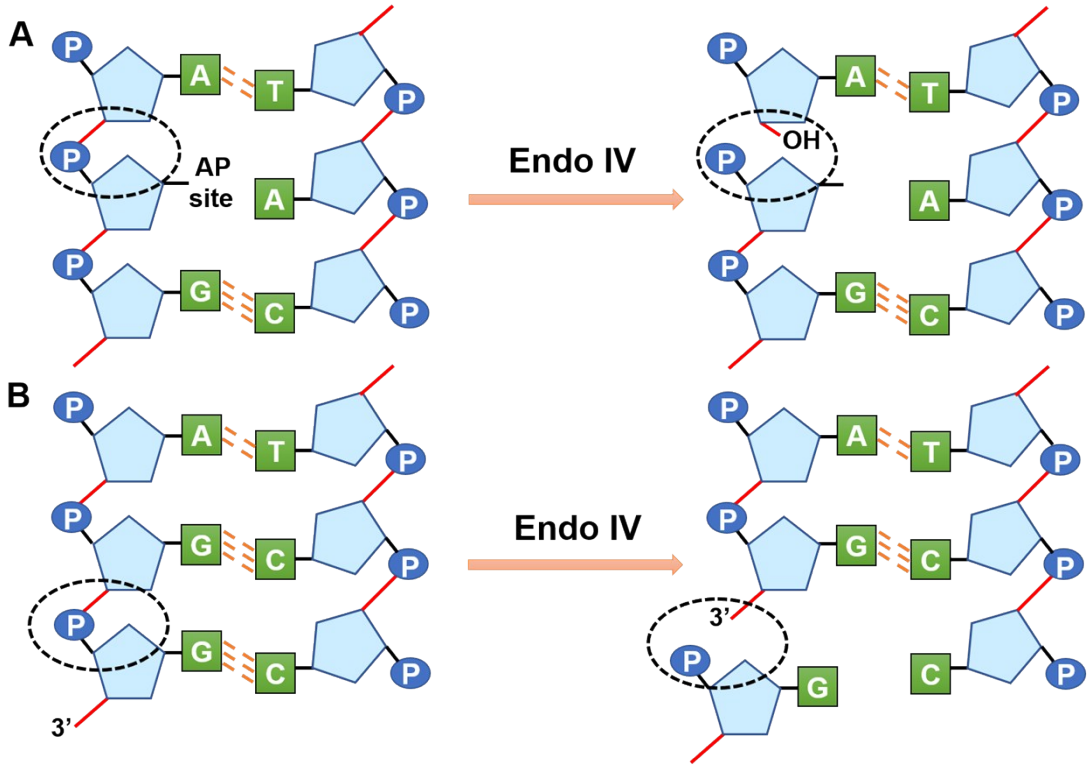

Fig. S1 Chemical structures of EndoIV substrates, including (A) an AP site cleaved by the AP endonuclease activity (B) a 3'-5' exonuclease activity to blunt 3' terminus of dsDNA.

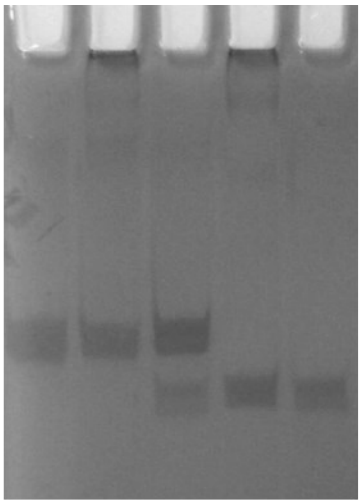

Fig. S2 Denaturing urea PAGE (20%) electrophoresis images of base excising repair enzymes-induced probe P1 cleavage product.

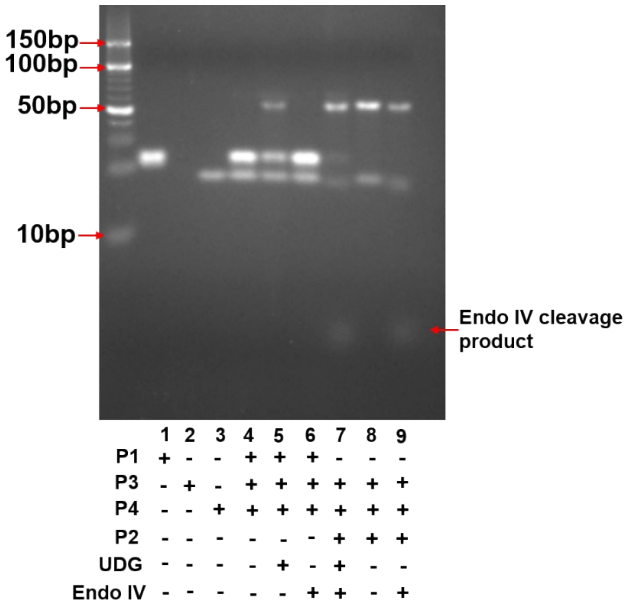

Fig. S3 Agarose gel (4%) electrophoresis images of the feasibility of the EAECSA strategy for UDG activity assay.

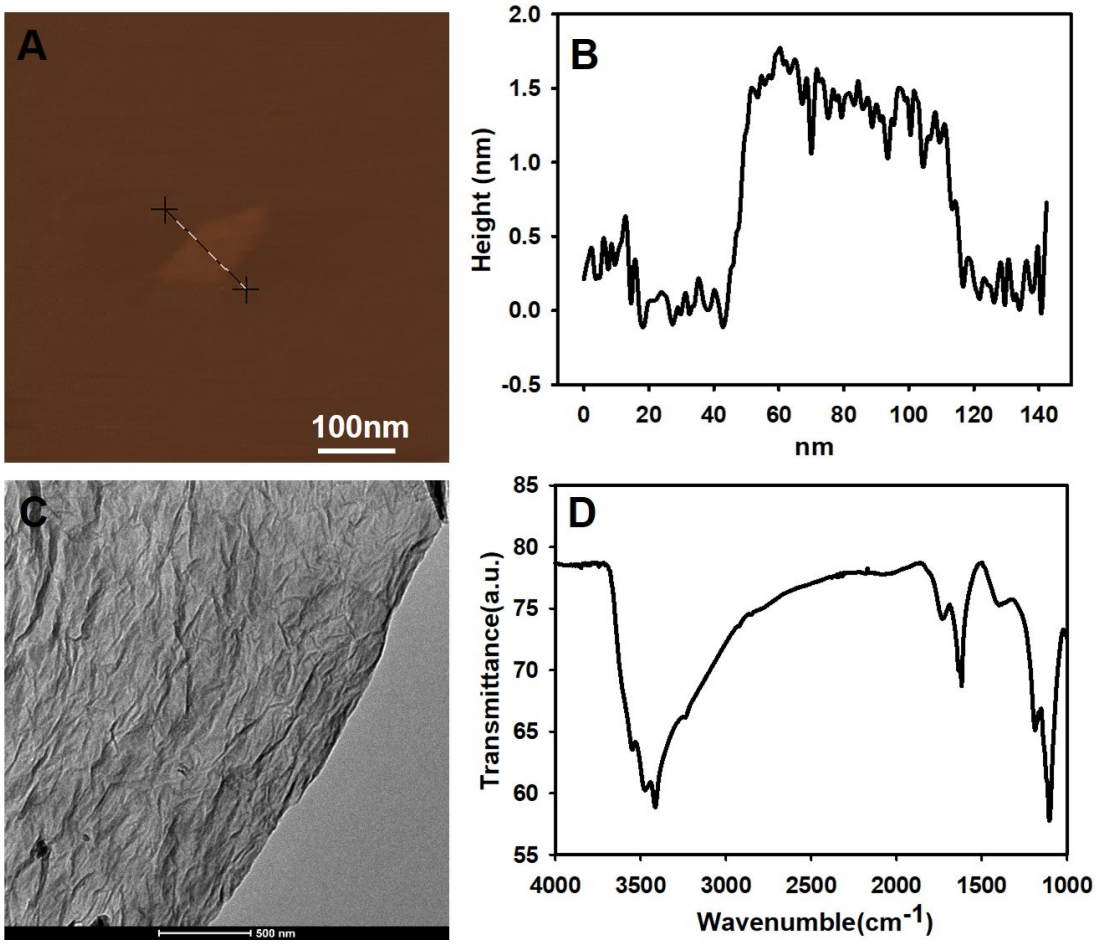

Fig. S4 Characterization of GO. (A) AFM image (B) Height profiles (C)TEM image (D) FTIR.

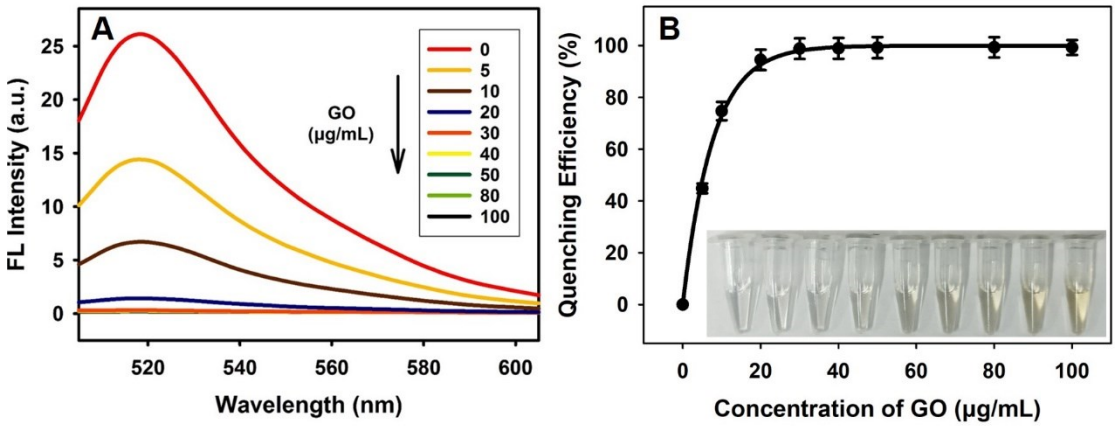

Fig. S5 (A) Fluorescence spectra of the sensing system after incubation with various concentrations of GO. (B) Plot of fluorescence quenching efficiency versus GO concentration. (Inset: photographs of the sensing aqueous solution with a series of different GO concentrations.

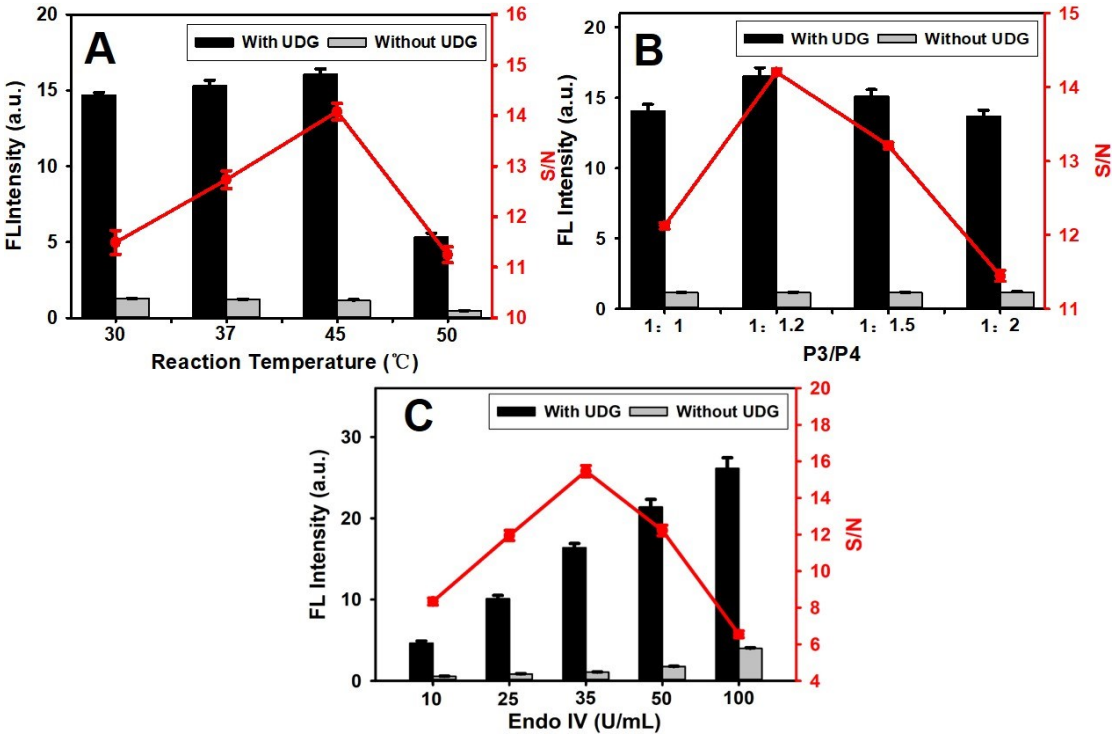

Fig. S6 Optimization of experimental conditions. Variance of the fluorescence intensity with (A)Endo IV reaction temperature, (B) hybridization ratio of probe P3 and probe P4, (C) the concentration of Endo IV.

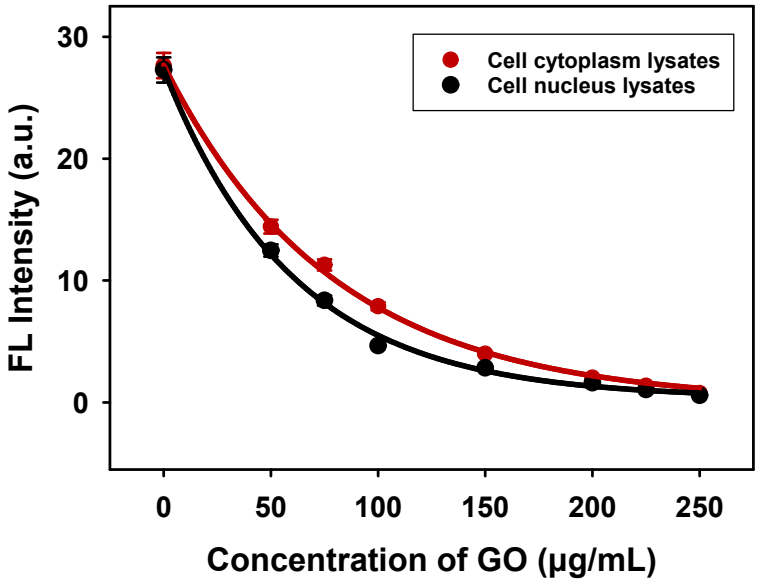

Fig. S7 Performance about different concentrations of GO Hela cell cytoplasm lysates (red) and cell nucleus lysates (black).

Table S2. Comparison of the proposed assay with other previously reported methods.

Methods	Detection time	LOD (U mL^{-1})	Reference
Fluorescence/ Polymerase, nicking enzyme and RNase H-mediated Bicyclic Cascade Signal Amplification strategy	1 h 50 min	1.0×10^{-4}	[1]
Fluorescence/ BssHII and Endo Ⅳ mediated exponential amplification strategy	> 6 h	3.0×10^{-3}	[2]
Fluorescence/ Pyridinium luminescent switch-on molecular probe	> 2 h	5.0×10^{-3}	[3]
colorimetric and smartphone readable method/ G-quadruplex structure with hemin to catalyze H2O2-mediated oxidation reaction	1 h 15 min	8.0×10^{-3}	[4]
Electrochemical/ porphyrin-based covalent-linked nanomaterial assisted signal amplication	>72 h	6.97×10^{-4}	[5]
Fluorescence/target induced the removal of uracil in DNA and enhanced the fluorescence fluorophore	10 min	8.0×10^{-4}	[6]
SERS/ Exonuclease I and Endo IV assisted plasmonic coupling reaction	>96 h	4.29×10^{-4}	[7]
Fluorescence/	>15 h	1.5×10^{-4}	[8]
Fluorescence/3’-5’exonuclease activity-based Endo IV cyclic signal amplification strategy	1 h 25 min	7.0×10^{-4}	This work

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