Supplementary information

An “off-on” fluorescent sensor based on FRET and magnetic beads for APE1 activity detection in breast cancer cell lysates

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Reagent

All oligonucleotides with different sequences were synthesized and purified by Hippo Biotechnology Co., Ltd. (Zhejiang, China). The sequences of the oligonucleotides used in this study are listed in Table S1. Sodium chloride (NaCl), HCl, and NaOH were sourced from Sigma-Aldrich (St. Louis, MO, USA). Apurinic/apyrimidinic endonuclease 1 (APE1), DNase I, Exonuclease III, T4 DNA Ligase, and bovine serum albumin (BSA) were obtained from Yuanye Biotechnology Co., Ltd. (Shanghai, China). Phosphate-buffered saline (PBS, containing 137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, pH 7.4) were also purchased from Sigma-Aldrich. All reagents used in this study were of analytical grade or higher and were stored at 4 °C for future use.

Instruments

The fluorescence spectra were recorded using a FL-2500 Fluorescent Photometer (Hitachi, Japan), respectively. Magnetic beads (MBs) were characterized using high-resolution transmission electron microscopy (HR-TEM) at an accelerating voltage of 200 kV (JEOL Co., Japan). Particle size analysis of MBs was conducted using a Dynamic Light Scattering (DLS) instrument (NS-90Z Plus, Malvern Panalytical, China). Sample preparation was carried out using a constant temperature oscillating metal bath incubator (MB-102, Hangzhou, China).

DLS experiment

To meet the instrument's detection requirements for scattered light intensity, the sample concentration was controlled within 0.001-1 mg/mL. For optimal analytical results, the liquid level of the sample solution was maintained above 1.5 cm. Sample preparation involved a 100-fold dilution of MBs or MBs complexes with ultrapure water to reach the target concentration and volume. After dilution, the sample was filtered through a 0.22 μm membrane to eliminate impurities and large particles. DLS measurements were performed using an NS-90Z Plus instrument (Malvern Panalytical, China) with a laser wavelength of 633 nm and a scattering angle of 90° to ensure measurement accuracy.

APE1 Detection Procedure

Optimization experiments: For molar ratio optimization, MBs-D1 was incubated with D2 at ratios (1:0.5 to 1:4) and fluorescence intensity was measured. Hybridization time (10-50 min), APE1 activation time (0-20 min), and temperature (25-60 °C) were optimized by monitoring fluorescence changes. Sensitivity and specificity tests: A series of APE1 concentrations (0-5 U/mL) were incubated with MBs-D1-D2 at 37 °C for 10 min; fluorescence intensity was recorded to establish the dose-response curve. For specificity, 1 U/mL APE1 and 1 U/mL interfering enzymes/BSA were tested under the same conditions. Recovery test in FBS: APE1 (0.1, 0.3, 0.5 U/mL) was spiked into 10% FBS, then detected using the OOFN platform; recovery was calculated as (measured concentration/spiked concentration) × 100%.

APE1 Detection in Cell Lysates

Detection of endogenous APE1: 100 μL of cell lysate was incubated with MBs-D1-D2 at 37 °C for 10 min; fluorescence intensity was measured, and APE1 levels were quantified using the calibration curve (correlating $\log_{10}$ (cell number) with fluorescence intensity).

Table S1 DNA sequences (5′-3′) utilized in this work. (X represent AP site).

Strand	Sequence (5′-3′)
D1	Biotin-CAGTCACTCGATCCAATCTACAGC (A-Fam)
D2	BHQ-TTGGATCGAXTGACTG

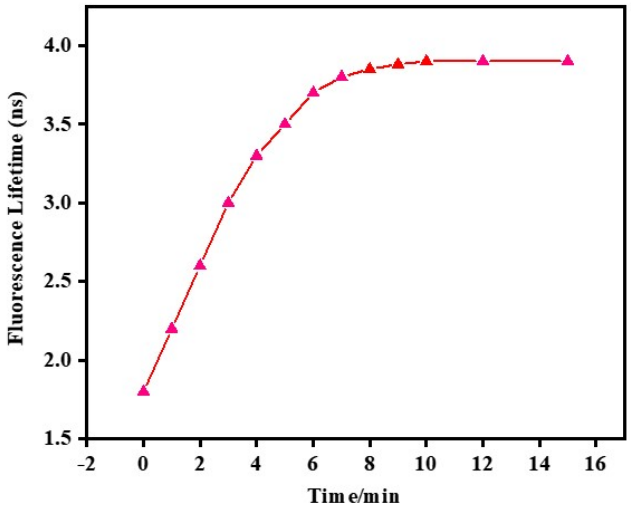

Figure S1. Fluorescence lifetime curve of OOFN system.

Table S2. Comparison of the OOFN platform with reported methods for APE1 detection

Method	Signal Mechanism	Detection Limit	Cell Detection Capability	Operational Complexity	Validation in Biological Samples	Key Advantages / Limitations
Radioactive assay (Esqueda et al., 2012) [S1]	^{32}P-labeled oligonucleotide, gel electrophoresis	Not specified (semi-quantitative)	Requires large cell numbers	High – multiple steps, radioactive handling, electrophoresis	Limited	Limitation: Hazardous, time-consuming, not suitable for routine lab use
DNA walker (Liu et al., 2024) [S2]	3D bipedal DNA walker, fluorescence recovery	0.03 U/mL	~100 cells (estimated)	Moderate – DNA walker assembly, multiple incubation steps	Serum samples	Advantage: High sensitivity; Limitation: Requires careful probe design and nanoparticle functionalization
Oligonucleotide-based assay (Gupta et al., 2022) [S3]	Radioactive or fluorescent oligonucleotides, gel-based separation	Not specified (qualitative/quantitative)	Typically, >10^4 cells	High-gel electrophoresis, fluorescence/radioactivity detection	Not validated in complex samples	Limitation: Labor-intensive, requires specialized equipment
HCR amplification (Zhang et al.,	Hybridization chain reaction + G-	1.0 × 10^{-8} U/mL	Not demonstrated	High-multiple hybridization steps, long assay time	Not validated	Advantage: Ultrahigh sensitivity;

2025) [S4]	quadruplex/ThT, fluorescence					Limitation: Complex procedure, not yet applied to cell lysates
Live-cell imaging probe (Acero et al., 2025) [S5]	Chimeric d/l-DNA molecular beacon, real-time imaging	Not quantitative	Single-cell imaging	Moderate-transfection required, live-cell imaging setup	Live cells	Advantage: Enables spatiotemporal tracking; Limitation: Not designed for quantitative activity measurement in lysates
OOFN (this work)	FRET-based "off-on" switching + magnetic bead enrichment	0.03 U/mL	~50 cells (55 for MCF-7, 62 for MDA-MB-231)	Low-single-step cleavage, magnetic separation	Validated in serum (>96% recovery) and cell lysates (normal vs. cancer)	Advantages: Simple, rapid, sensitive, low background, applicable to crude samples, quantitative

Reference

[S1] Esqueda A, Mohammed M Z, Madhusudan S, et al. Purification and specific assays for measuring APE-1 endonuclease activity[M]//Rational Drug Design: Methods and Protocols. Totowa, NJ: Humana Press, 2012: 161-174.

[S2] Liu Q, Zhang Q, Zhang Y, et al. A recognition-induced three-dimensional bipedal DNA walker for highly sensitive detection of APE1. Analytical Methods, 2024, 16(36): 6220-6228.

[S3] Gupta K B, Kaur S, Dhiman M, et al. Methods to assess oxidative DNA base damage repair of apurinic/apyrimidinic (AP) sites using radioactive and nonradioactive oligonucleotide-based assays[M]//Cancer Biomarkers: Methods and Protocols. New York, NY: Springer US, 2022: 155-163.

[S4] Zhang Y, Ma H, Gao Z, et al. Label-free and ultrasensitive APE1 detection based on hybridization chain reaction combined with G-quadruplex. Biomolecules, 2025, 15(9): 1275.

[S5] Acero R E P, Deckard III C E, Sczepanski J T. Real-time, light-activated, and multiplexed monitoring of base excision repair in living cells using chimeric d/l-DNA molecular beacons. ACS sensors, 2025, 10(8): 5655-5663.