Electronic supplementary information

An acid-responsive DNA hydrogel-mediated cascaded enzymatic nucleic acid amplification system for the sensitive imaging of alkaline phosphatase in living cells

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1. Experimental section

1.1. Chemicals and reagents

All oligonucleotides used in this work were synthesized and purified by Sangon Biotechnology Co. Ltd. (Shanghai, China). Sodium orthovanadate (V) dodecahydrate, and deoxyribonucleotide triphosphates (dNTPs) were purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). Sodium chloride (NaCl) and magnesium chloride ($MgCl_2$) were ordered from Macklin Biochemical Co. Ltd. (Shanghai, China). Bst 3.0 DNA polymerases (Bst DP), nicking endonuclease (Nt.BbvCI), Alkaline phosphatase (ALP), uracil DNA glycosylase (UDG), flap endonuclease 1(FEN1), human alkyladenine DNA glycosylase (hAAG) and bovine serum protein (BSA) were purchased from New England Biolabs (NEB, Ipswich, MA, USA). BCA protein assay kit was purchased from Beyotime Biotechnology (Haimen, China). Alkaline phosphatase (ALP) test kit (Phenylene disodium phosphate colorimetry) was purchased from MREDA (Beijing, China). Dulbecco's modified eagle medium (DMEM), antibiotics penicillin/streptomycin, trypsin, fetal bovine serum (FBS) and 1×phosphate buffered saline (1 × PBS, pH 7.4) were purchased from Biological Industries (Beit-Haemek, Israel). Human serum samples were supplied by the Hospital of Shandong University (Shandong, China). HeLa and MCF-7 cell lines were purchased from Procell Life Science &Technology Co., Ltd. (Wuhan, China). NCM460 cell line was purchased from FuHeng Cell Center (Shanghai, China). All the chemical agents were used without further purification. The ultrapure water with a resistivity of 18.25 MΩ·cm made from Millipore Milli-Q water purification system was used for the preparation of all aqueous solutions.

1.2. Instruments

Transmission electron microscope images were obtained by JSM-6700F transmission electron microscope (JEOL, Japan). The size distribution and was acquired from Zeta Sizer Nano ZS90 (Malvern, UK). The UV-vis spectrum was obtained by UV-2910 spectrometer (Hitachi, Japan). Fluorescence emission spectra were collected using Hitachi F-7000 fluorescence spectrophotometer (Hitachi, Japan). Confocal laser scanning microscopy (CLSM) images of cells were taken using Leica TCS SP8 confocal microscope (Leica, Germany) with a 63 × oil dipping objective less. Circular dichroism (CD) spectra were recorded using a J-810 CD spectrophotometer (JASCO, Japan). The MTT results were obtained by using a microplate reader (Tecan Austria GmbH, Austria). Flow cytometry was performed using the NovoCyte 3130 flow cytometer (ACEA Biosciences Inc, USA).

1.3. Polyacrylamide gel electrophoresis experiment

The feasibility of the amplification reaction for ALP detection was explored by a 12% non-denaturating polyacrylamide gel electrophoresis (PAGE) experiment. The samples were run in 1× TBE buffer (89 mM Tris, 89 mM Boric Acid, 2.0 mM EDTA, pH 8.3) for 1.0 h at a current of 30 mA. Then, the gel was stained with SYBR gold for 40 min. The UV imaging system (Bio-RAD Laboratories Inc., USA) was employed for imaging.

DNA hydrogel was characterized by 6% PAGE. To avoid the structure switching of the i-motif, 1×TAE buffer was adjusted to pH 6.0 in the process of electrophoresis. The gels were run under a current of 30 mA for 2h. Then, the gel was stained with SYBR gold for 40 min. The UV imaging system was employed for imaging.

1.4. Determination encapsulation efficiency of DNA hydrogel for enzyme and DNA probe.

The encapsulation efficiency of DNA hydrogel for enzyme was determined with classical BCA assay. The enzyme concentration was determined following the protocol provided by the supplier. Different concentrations of BSA and the detection solution were mixed and reacted at 37 °C for 30 min. The absorbance of the mixture at 562 nm was determined by UV-vis spectrometer, which was used to fit the standard curve. The enzyme was replaced by BSA for encapsulation experiments. The original BSA concentration (A_1) and the residual BSA concentration after DNA hydrogel encapsulation (A_2) were determined according the above-mentioned method. Compared with the standard curve, the encapsulation efficiency of DNA hydrogel for enzyme was calculated as follows: enzyme encapsulation efficiency = $(A_1\text{-}A_2)/A_1 \times 100\%$.

The encapsulation efficiency of the DNA hydrogel for DNA probe was investigated by fluorescence. First, the original SP concentration and the residual SP concentration after DNA hydrogels encapsulation hybridized with C-SP to recovery fluorescence signal. Then, the fluorescence intensities of the original SP concentration (F_1) and the residual SP concentration (F_2) were determined. The encapsulation efficiency of the DNA hydrogel for DNA probe was calculated as follows: DNA probe encapsulation efficiency = $(F_1\text{-}F_2)/F_1 \times 100\%$.

Table S1 Oligonucleotides used in this work.

Name	Sequence (5'-3')
YA1	CTG TCC TTG TAA CTC TCA TGA CCG TCT GTC CTA GGG TTA GGG T
YA2	GAC GGT CAT GAG AAC ATA CTA GAT CAT GTC CTA GGG TTA GGG T
YA3	TGA TCT AGT ATG TGT TAC AAG GAC AGT GTC CTA GGG TTA GGG T
YB1	TAG GGT TAG GGT CTG TCC TTG TA A CTC TCA TGA CCG TC
YB2	TAG GGT TAG GGT GAC GGT CAT GAG AAC ATA CTA GAT CA
YB3	TGA TCT AGT ATG TGT TAC AAG GAC AG
LK	CCC TAA CCC TAA CCC TAA CCC TAG GAC
RP	AAA CCC ACT ATT TCG ACC GGC TCG GAG AAG AGA TCG CTG AGG AAC ACA ACA TTT TTT TTT TAA TGT TGT G-P
SP	Cy3-CCA CCA CGA AAT TGA CCC ACT AT /rA/ GGA AGA GAT CTT ACG GGT GGT GG - BHQ2
C-SP	CCA CCA CCC GTA AGA TCT CTT CCT ATA

Table S2 Comparison of this work with some reported work.

Method	Linear range ($U\ mL^{-1}$)	Detection limit ($U\ mL^{-1}$)	Reference
Hemicyanine-based small-molecule fluorescent probes	1.0×10^{-2}-2.0	3.0×10^{-3}	[1]
Graphene oxide integrating with λ exonuclease	3.0×10^{-3}-2.7×10^{-2}	1.9×10^{-4}	[2]
TdT-mediated hemin/G-quadruplex DNAzyme nanowires	1.0×10^{-4}-5.0×10^{-3}	3.0×10^{-5}	[3]
Cascaded polymerization-nicking and DNAzyme amplification system	1.0×10^{-4} -1.0 $\times10^{-2}$	2.0×10^{-5}	This work

Table S3 Results of within-day precision assays for the detection of ALP in vitro.

Concentration ($U\ mL^{-1}$)	Sample 1 ($U\ mL^{-1}$)	Sample 2 ($U\ mL^{-1}$)	Sample 3 ($U\ mL^{-1}$)	RSD (n=3)
5.0×10^{-4}	5.2×10^{-4}	5.3×10^{-4}	5.5×10^{-4}	3.0%
1.0×10^{-3}	1.0×10^{-3}	9.9×10^{-3}	1.0×10^{-3}	1.6%
5.0×10^{-3}	5.3×10^{-3}	5.2×10^{-3}	5.1×10^{-3}	2.4%

Table S4 Results of inter-day precision assays for the detection of ALP in vitro.

Concentration ($U\ mL^{-1}$)	Sample 1 ($U\ mL^{-1}$)	Sample 2 ($U\ mL^{-1}$)	Sample 3 ($U\ mL^{-1}$)	RSD (n=3)
5.0×10^{-4}	5.2×10^{-4}	5.2×10^{-4}	5.1×10^{-4}	1.2%
1.0×10^{-3}	1.0×10^{-3}	9.4×10^{-4}	9.5×10^{-4}	4.0%
5.0×10^{-3}	5.1×10^{-3}	4.8×10^{-3}	4.7×10^{-3}	4.0%

Table S5 Results of the recovery assays for the detection of ALP in human serum.

Sample Number	Added ALP ($U\ mL^{-1}$)	Detected ALP ($U\ mL^{-1}$)	Recovery (%)	RSD (n=3)
1	5.0×10^{-4}	5.3×10^{-4}	106	5.0%
2	1.0×10^{-3}	9.5×10^{-4}	95	5.4%
3	5.0×10^{-3}	4.9×10^{-3}	98	4.6%

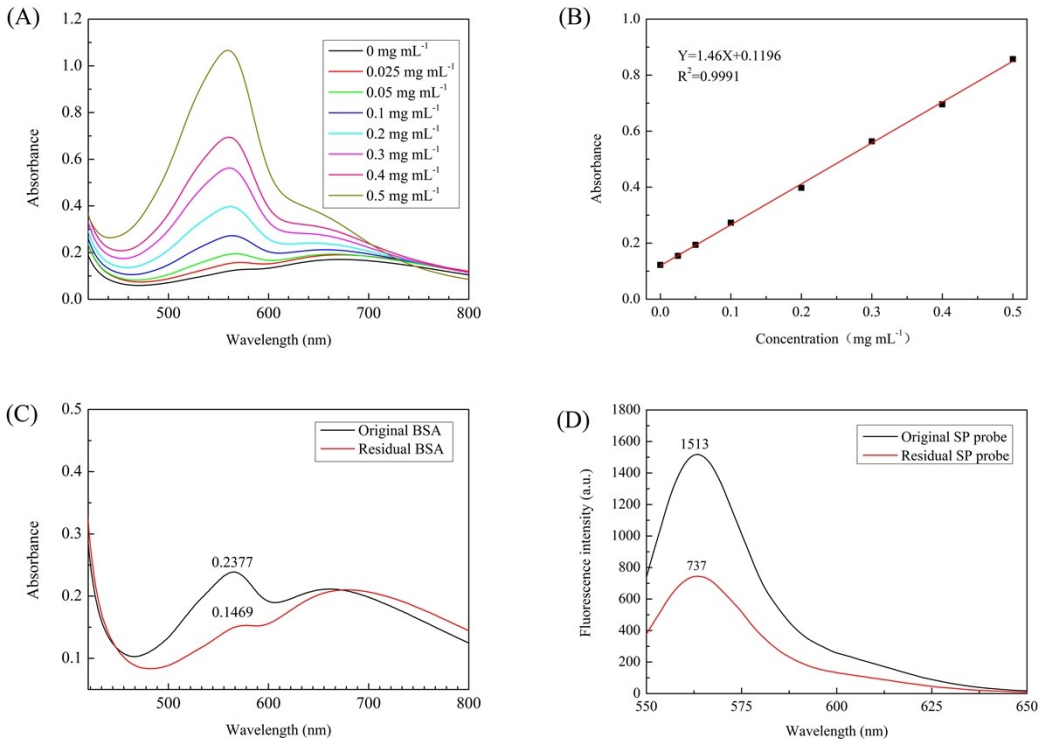

Fig. S1 (A) UV-vis absorbance spectra of standard protein BSA with different concentrations. (B) Linear relationships between the protein concentration and the UV-vis absorbance at 562nm. (C) UV-vis absorbance spectra of standard protein BSA before and after hydrogel entrapment. (D) Fluorescence spectra of SP before and after hydrogel entrapment.

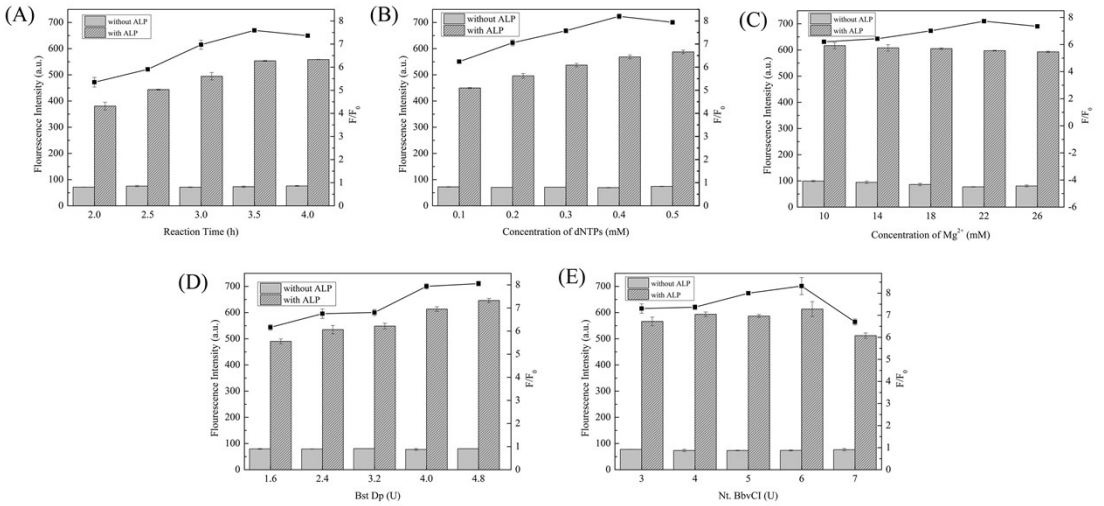

Fig. S2 Effect of the operation conditions on the cascaded polymerization-nicking and DNAzyme amplification system. (A) Reaction time. (B) Concentration of dNTP. (C) Concentration of Mg^{2+}. (D) Bst DP activity. (E) Nt. BbvCI activity. The error bar shows the standard deviation of three parallel tests.

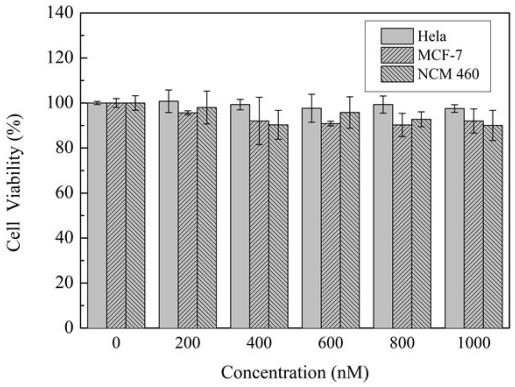

Fig. S3 Cytotoxicity of HeLa, MCF-7 and NCM 460 cells after being treated with different concentrations of DHE for 24h. The error bar shows the standard deviation of three parallel tests.

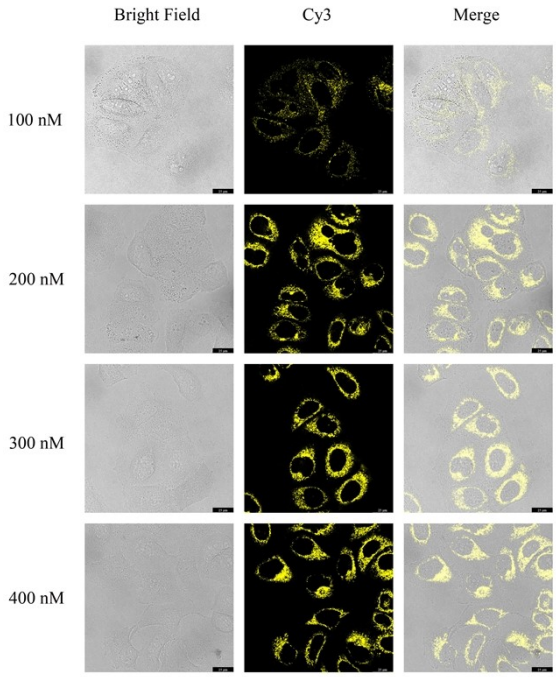

Fig. S4 CLSM images of ALP in HeLa cells after incubation with different concentrations of DHE. The scale bars were 25 μm.

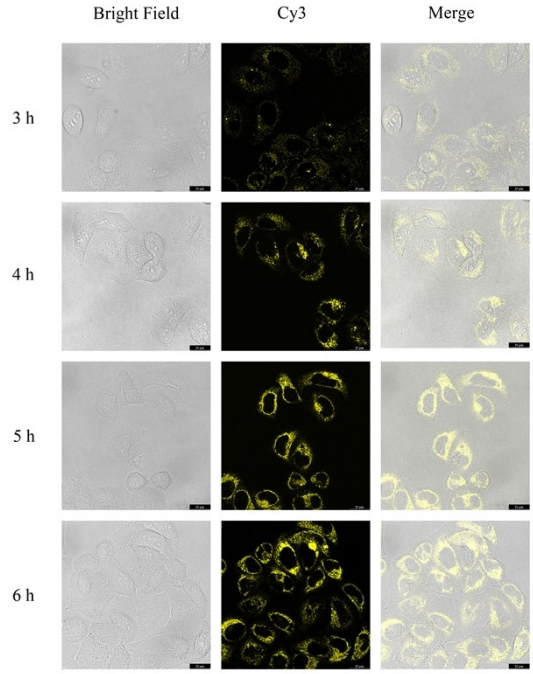

Fig. S5 CLSM imaging of ALP in HeLa cells after incubation with DHE for different time. The scale bars were 25 μm.

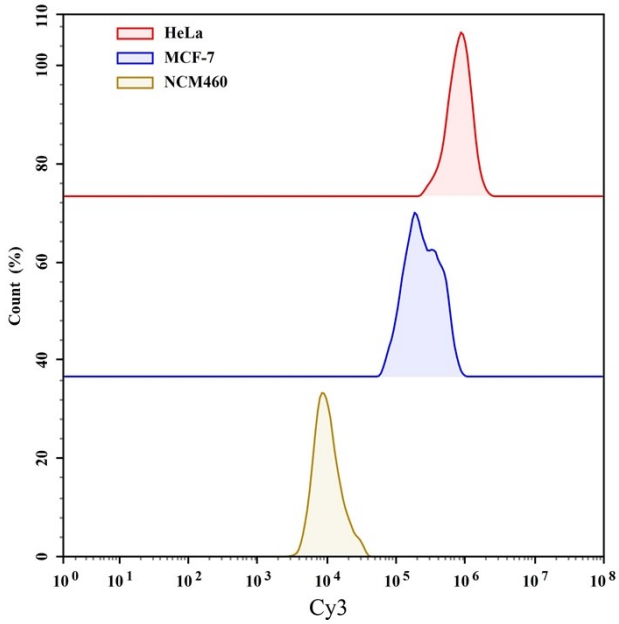

Fig. S6 Flow cytometry analysis of ALP in HeLa, MCF-7 and NCM460 cells after incubation with DHE.

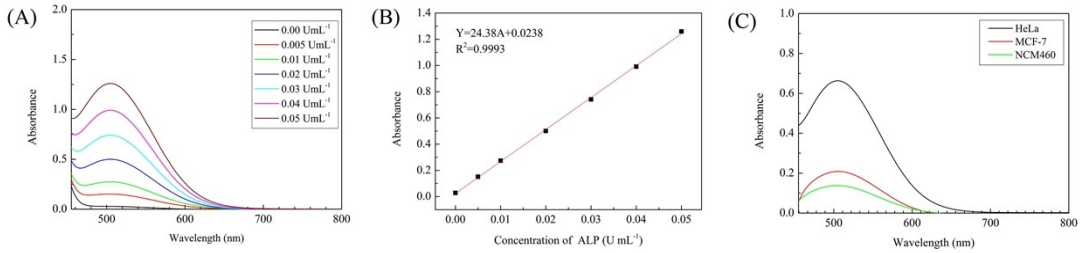

Fig. S7 Determination of ALP activity in cell lysates by an alkaline phosphatase detection kit (Phenylene disodium phosphate colorimetry). (A) UV-vis absorbance spectra of ALP with different activities. (B) Linear relationships between the ALP activity and the UV-vis absorbance intensity at 505 nm. (C) UV-vis absorbance spectra of different cell lysates.

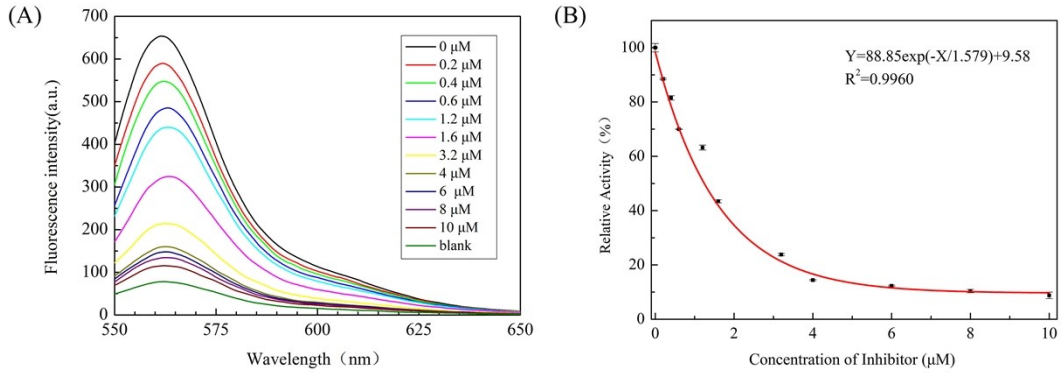

Fig. S8 (A) Fluorescence spectra of ALP treated with inhibitor Na_3VO_4 of different concentrations. (B) Regression curve for the measured activity of ALP and the concentration of inhibitor Na_3VO_4. The error bar shows the standard deviation of three parallel tests.

Reference

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