Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Label-free activatable aptamer probe for colorimetric detection of cancer cells based on binding-triggered in situ catalysis of split DNAzyme

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Experimental Section

Materials and reagents. All the DNA probes reported in this article were custom-designed and then synthesized by Sangon Biotech. (Shanghai, China) Co., Ltd. Sequences of the oligos are listed in Table S1. Hemin stocking solution (5 mM) was prepared in DMSO, which was then stored in a freezer and shielded from light. 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) and 2,2'-amino-di(2-ethyl-benzothiazoline sulphonic acid-6) ammonium salt (ABTS) were purchased from Sigma-Aldrich (Shanghai, China). 30% hydrogen peroxide solution (H_2O_2) and other reagents were purchased from Shanghai Chemical Reagent Co., (Shanghai, China). HEPES buffer was prepared by mixing 25 mM HEPES, 20 mM KCl, 200 mM NaCl and 1% v/v DMSO (pH 6.8). Binding buffer was prepared by adding 1 mg/mL BSA and 15% fetal bovine serum (FBS) into the Dulbecco's PBS containing 4.5 g/L glucose and 5 mM $MgCl_2$. All other chemicals were of analytical grade. All buffer solutions were prepared using ultrapure water (18.2 MΩ·cm from Millpore purification system).

Cells. CCRF-CEM cells (T cell line, human acute lymphoblastic leukemia) were obtained from Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences. Ramos cells (B cell line, human Burkitt's lymphoma) were purchased from the Cancer Institute & Hospital (Chinese Academy of Medical Sciences). Cells were cultured in RPMI 1640 medium supplemented with 15% FBS and 100 IU/mL penicillin-streptomycin and incubated at 37 ℃ in a humidified incubator containing 5% wt/vol CO_2. Before cell assays, cells in culture medium were collected through centrifugation at 2,000 rpm for 5 min, and then dispersed with HEPES buffer (pH 6.8). The cell density was determined by hemocytometer counting.

Colorimetric assay. 110 μL solution with or without pre-annealed THAAP (heated at 88 ℃ for 10 min, and slowly cooled down to room temperature for 3 h) was firstly prepared using HEPES buffer. Then, 10 μL hemin (in DMSO) and 50 μL HEPES buffer with or without cells were added to the above solution. After incubation at a designated temperature for a designated time, 15 μL ABTS and 15 μL H_2O_2 were added to initiate the catalytic reaction. For real-time monitoring, absorbance of the above reaction mixture was measured at 418 nm every 2 min since the addition of substrate by using an infinite M1000 multifunctional microplate reader (TECAN Austria GmbH). For endpoint detection, the above reaction mixture was allowed to react for a designated time. Then, after a centrifugation at 2,000 rpm for 5 min, absorbance of the supernatant was measured at 418 nm.

Flow cytometry analysis. Flow cytometry assays were performed to confirm the binding between Sgc8c and target CCRF-CEM cells. Cy5-labeled Sgc8c (Cy5-Sgc8c) was used as the recognition probe, and Cy5-Control, a negative control probe for Cy5-Sgc8c, was constructed with an arbitrary sequence design. Firstly, 12.5 nM Cy5-Sgc8c or Cy5-Control were incubated with 2×10^5 CCRF-CEM cells in 200 μL binding buffer or HEPES buffer at 25℃ for 30 min in the dark. Then, cells were immediately determined with a FACScan cytometer (BD Biosciences) by counting 10,000 events.

Table S1. All of the oligonucleotides used in this work.*

Probe Name	Sequence (5'-3')
Split G-quadruplex	TTTGTGGAGGGTCTAAGCGAAAGGGACGGG
Probe 1	TTTGTGGAGGGTCTAACCGAAAGGGACGGG-Spacer18-ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA
Probe 2	TTTGTGGAGGGTCTAAGCGAAAGGGACGGG-Spacer18-ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA
Probe 3	TTTGTGGAGGGTCTAAACGAAAGGGACGGG-Spacer18-ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA
Cy5-Sgc8c	Cy5-ATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA
Cy5-Control	Cy5-ATACGGTGACTGCGCCGCCGGGAAAATACTGTCTAACCGTA

*A-strand, Spacer, G1-strand, C-strand, and G2-strand are presented in red, yellow, black, blue, and gray, respectively.

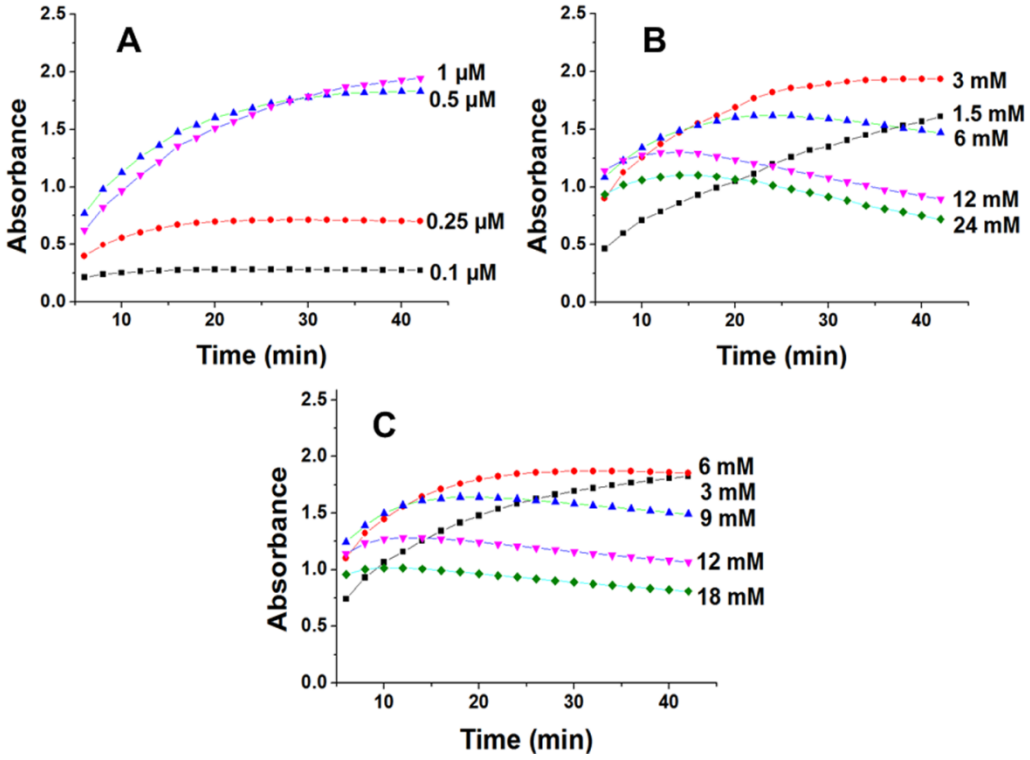

Fig. S1. Time-dependent catalytic curves of split G-quadruplex-hemin DNAzyme for the optimization of reaction conditions. (A) Absorbance of the oxidized $ABTS^{\bullet+}$ with the use of 100 nM split G-quadruplex, different concentrations of hemin, 3 mM H_2O_2 and 6 mM ABTS. (B) Absorbance of the oxidized $ABTS^{\bullet+}$ with the use of 100 nM split G-quadruplex, 0.5 μM hemin, different concentrations of H_2O_2 and 6 mM ABTS. (C) Absorbance of the oxidized $ABTS^{\bullet+}$ with the use of 100 nM split G-quadruplex, 0.5 μM hemin, 3 mM H_2O_2 and different concentrations of ABTS.

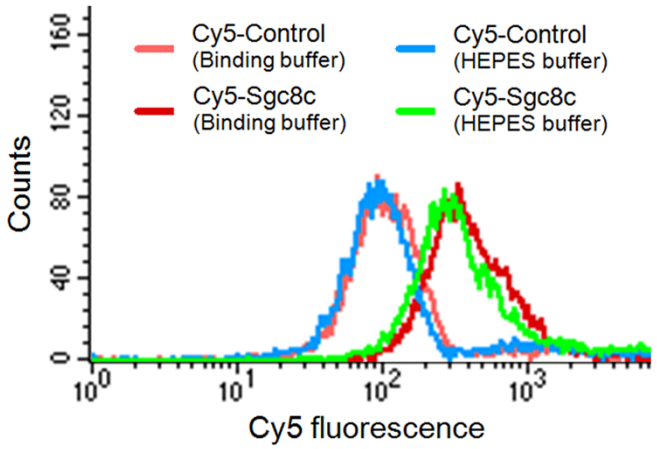

Fig. S2. Flow cytometry analysis of CCRF-CEM cells in different buffer. CCRF-CEM cells were incubated with Cy5-Sgc8c or Cy5-Control in binding buffer or HEPES buffer, and then detected on a flow cytometer.

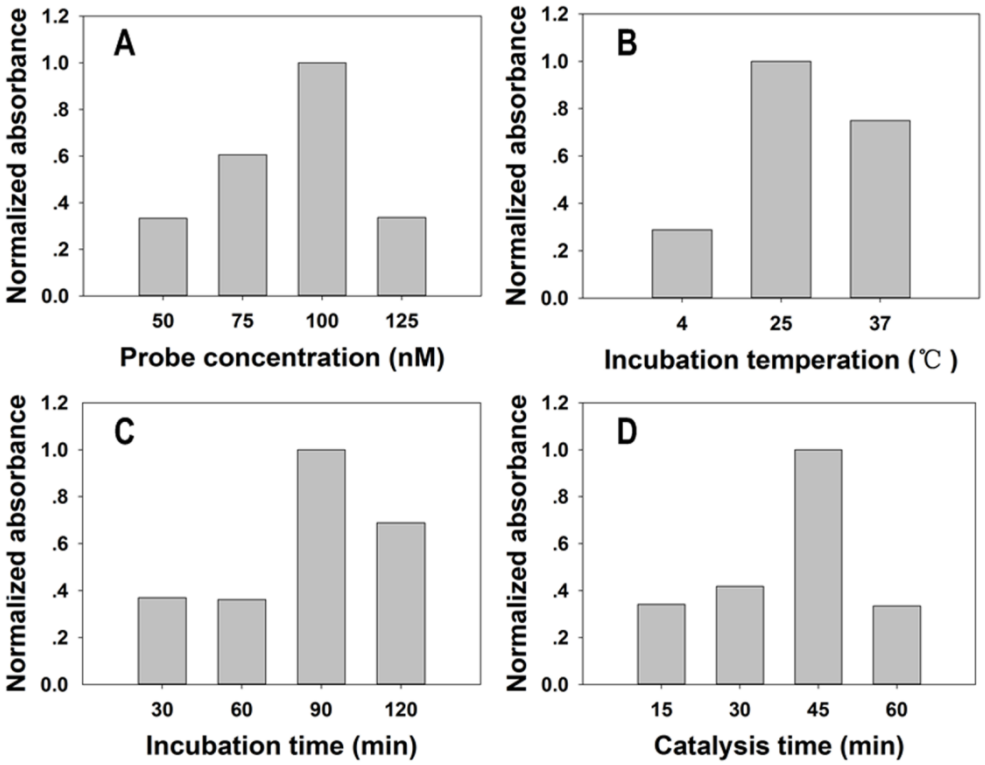

Fig. S3. Optimization of experimental conditions in the THAAP-based strategy for colorimetric detection of CCRF-CEM cells. (A) Concentration of Probe 2. (B) Temperature for incubation of Probe 2 with cells. (C) Time for incubation of Probe 2 with cells. (D) Time for *in situ* catalytic reaction. (The Y-axis represents the normalized background-subtracted absorbance.)