Electronic Supplementary Information

Highly Sensitive Detection of Epidermal Growth Factor Receptor in Lung Cancer Cells by Aptamer-Based Target-/Probe-Mediated Cyclic Signal Amplification

Dandan Zhang,[‡,1] Fei Ma,[‡,1] Qianyi Zhang,[‡,2] and Chun-yang Zhang[*,1]

[1]College of Chemistry, Chemical Engineering and Materials Science, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging in Universities of Shandong, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Provincial Key Laboratory of Clean Production of Fine Chemicals, Shandong Normal University, Jinan 250014, China.

[2]Nantou High School Shenzhen, Shenzhen, 518052, China

‡These authors contributed equally.

*To whom correspondence should be addressed. E-mail: cyzhang@sdnu.edu.cn; Tel.: +86 0531-86186033; Fax: +86 0531-82615258.

MATERIALS AND METHODS

Materials. Oligonucleotides (Table S1) were synthesized and purified by Sangon Biotechnology Co. Ltd. (Shanghai, China). The EGFR and vascular endothelial growth factor (VEGF) were purchased from R&D Systems (Minneapolis, Minnesota, USA). Fetal bovine serum (FBS) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The deoxynucleotide triphosphates (dNTPs) were obtained from TaKaRa Biotechnology Co. Ltd. (Dalian, China). Klenow fragment polymerase (3'→5' exonuclease$^-$, KF polymerase), the nicking

endonuclease Nt.BbvCI and the T7 exonuclease were purchased from New England Biolabs Ltd. (Hitchin, UK). Ultrapure water was prepared by a Millipore filtration system (Millipore Corp., Bedford, Mass.).

Table S1 Sequences of oligonucleotides used in this work[a]

note	sequence (5'-3')
template 1	G*G*A* CCC **TGC CGT TTC TTC TCT TTC GCT TTT TTT GCT TTT GAG CAT G**GG ACC ACC GCG T
template 2	TTT TTT TTT ACG C**I**G TG**I** TCC CCT CAG CAC G**I**C AG**I** GTC C
primer 1	G*G*A* CCC TGC CGT
primer 2	TTT TTT TTT ACG CGG TGG TCC CCT CAG CAC GCG GTG GTC C
Taqman probe	FAM-TTT TTT TTT ACG CGG TGG TCC -BHQ

[a]The boldface regions in template 1 indicate the sequence of EGFR aptamer. The underlined regions symbolize the recognition sequences of Nt. BbvCI nickase. The underlined bold regions symbolize the inosine (I) substitutions. The asterisk indicates the phosphorothioate modification.

EGFR-Induced Circular SDA Reaction. The reaction was performed at 37 °C for 50 min in a total volume of 20 μL containing 200 nM template 1 / 200 nM template 2, 250 nM primer 1 and 250 nM primer 2, 1 nM EGFR, 2 μL of 10× NEB 2 buffer (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, pH 7.9), 0.5 μL of dNTPs (200 mM each), 1.2 unit KF polymerase and 10 unit Nt.BbvcI nicking enzyme. The reaction was terminated by heating at 85 °C for 10 min.

Preparation of Cell Extracts. The human lung adenocarcinoma cells (A549 cells) and normal human breast cells (MDA-MB-435 cells) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C in a humidified chamber containing 5% CO_2. In the exponential phase of growth, the cells were collected for extraction with a membrane protein extraction kit (Sangon, Shanghai, China). The obtained membrane protein was aliquoted and stored at −20 °C. The protein concentration was quantified by a modified BCA protein assay kit with BSA as the standard (Sangon, Shanghai, China)

Measurement of Fluorescence Intensity. The 25 μL of solution containing 20 μL of SDA reaction products and 600 nM Taqman probe, 2 μL of 10× NEB 4 buffer (20 mM Tris-Ac, 50 mM KAc, 10 mM $Mg(Ac)_2$, 1 mM DTT, pH 7.9) and 10 unit T7 exonuclease were diluted to a final volume of 60 μL with ultrapure water. All fluorescence spectra were measured by a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan). The excitation wavelength was 490 nm, and the emission spectra were recorded over the wavelength range from 490 to 600 nm with a slit width of 5 nm for both excitation and emission.

SUPPLEMENTARY RESULTS

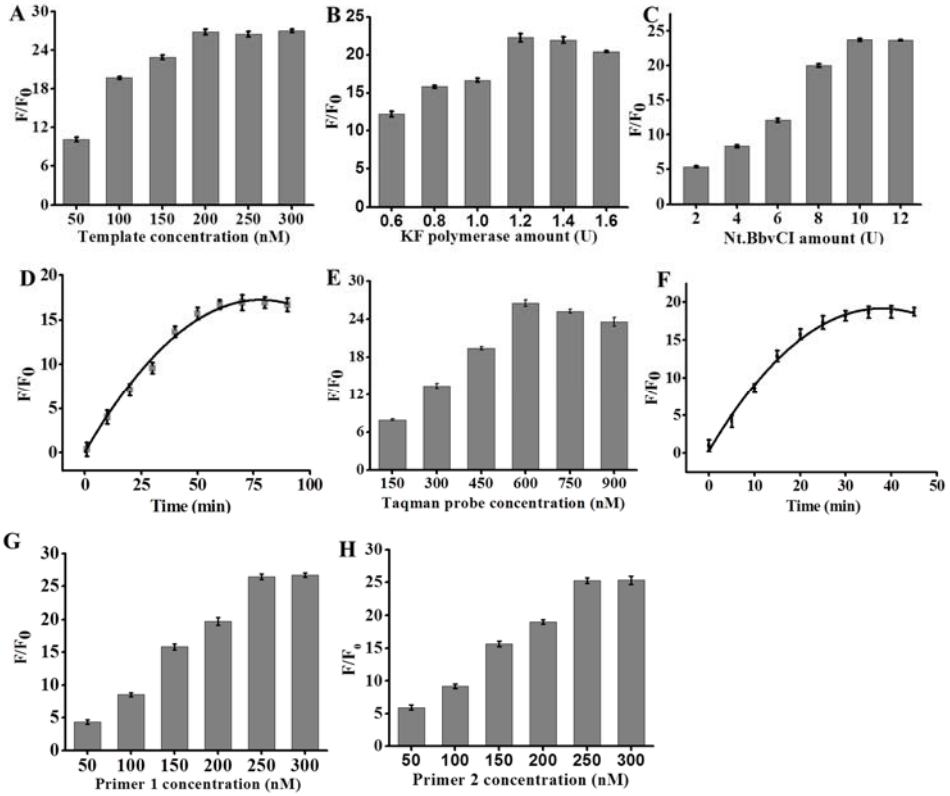

Fig. S1 (A) Variance of F/F_0 value with the concentration of template. (B) Variance of F/F_0 value with the amount of KF polymerase. (C) Variance of F/F_0 value with the amount of Nt.BbvCI. (D) Variance of F/F_0 value with the reaction time of nucleic acid amplification. (E) Variance of F/F_0 value with the concentration of Taqman probe. (F) Variance of F/F_0 value with the reaction time of T7 exonuclease-assisted cleavage. (G) Variance of F/F_0 value with the concentration of primer 1. (H) Variance of F/F_0 value with the concentration of primer 2. F and F_0 are the fluorescence intensity in the presence of 1 nM EGFR and in the absence of EGFR, respectively. Error bars show the standard deviations of three experiments.

Optimization of Experimental Conditions. In order to obtain the best performance of the proposed EGFR assay, we optimized the experimental conditions including the concentrations of

template, Taqman probe and primers 1 and 2, the amounts of polymerase, nicking endonuclease and T7 exonuclease, and the reaction time of nucleic acid amplification and T7 exonuclease-assisted cleavage (Fig. S1). We firstly optimize the template concentration. As shown in Fig. S1A, the F/F_0 value improves with the increasing concentration of template from 50 nM to 200 nM (F and F_0 represent the fluorescence intensity in the presence and in the absence of EGFR, respectively), and then remains unchanged beyond the concentration of 200 nM probably due to the consumption of all available templates. Therefore, 200 nM template is used in the following experiments.

The close cooperation of polymerase and nicking enzyme are crucial for efficient nucleic acid amplification.[1] Although the precise mechanism remains unclear, the reaction can be modulated by changing the amounts of polymerase and nicking endonuclease.[2] We first investigated the effect of polymerase amount on the F/F_0 value at a fixed amount of Nt.BbvCI nickase (5 U) (Fig. S1B). The F/F_0 value enhances with the increasing amount of KF polymerase from 0.6 to 1.2 U, and then levels off beyond the amount of 1.2 U. Therefore, 1.2 U KF polymerase is used in the subsequent experiments. We further investigate the influence of Nt.BbvCI amount upon the F/F_0 value at a fixed amount of KF polymerase (1.2 U) (Fig. S1C). The F/F_0 value improves with the increasing amount of Nt.BbvCI from 2 to 10 U, followed by remaining unchanged beyond the amount of 10 U. Therefore, 10 U Nt.BbvCI is used in the subsequent experiments.

We further optimized the reaction time of SDA (Fig. S1D). The fluorescence intensity increases rapidly and reaches a plateau within 50 min. This may be explained by either the complete losing of activities of polymerase and nicking enzyme or the consumption of all available templates. Thus, 50 min is selected as the optimal reaction time of SDA.

In theory, the higher the Taqman probe concentration, the more the Taqman probes being hydrolyzed by T7 exonuclease, and the higher the fluorescence signal obtained. However, the high-concentration Taqman probes may result in a high background signal due to the incomplete quenching of Taqman probes, and thus the concentration of Taqman probe should be optimized. As shown in Fig. S1E, the F/F_0 value enhances with the increasing concentration of Taqman probe from 150 to 600 nM, followed by the decrease beyond the concentration of 600 nM. Therefore, 600 nM Taqman probe is used in the subsequent research.

Additionally, we optimized the reaction time of T7 exonuclease-assisted cleavage. As shown in Fig. S1F, the fluorescence intensity improves dramatically with the reaction time of T7 exonuclease-assisted cleavage, and reaches a plateau within 30 min due to the complete consumption of available Taqman probes. Thus, 30 min is selected as the optimal reaction time of T7 exonuclease-assisted cleavage.

We further optimized the concentrations of primer 1 and primer 2. The F/F_0 value improves with the increasing concentrations of primer 1 (Fig. S1G) and primer 2 (Fig. S1H) from 50 to 250 nM, and then levels off beyond the concentration of 250 nM probably due to the consumption of all available primers. Therefore, 250 nM primer 1 and 250 nM primer 2 are used in the subsequent research.

References

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