Electronic Supplementary Information

Dual signal amplification-assisted DNAzyme biosensor for ultrasensitive detection of Argonaute 2 activity

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EXPERIMENTAL SECTION

Materials. All oligonucleotides used in this research were HPLC-purified and synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China), and their sequences were listed in Table S1. Recombinant mammalian Ago2 was obtained from Sino Biological Inc. (Beijing, China). Nuclease inhibitor RNasin and ribonuclease A (RNase A) were ordered from Promega. Diethypyrocarbonate (DEPC) was ordered from Amresco (USA). Human hemoglobin, bovine serum albumin (BSA) and α-thrombin from human plasma were purchased from Sigma-Aldrich. The deoxyribonucleoside 5′-triphosphate mixture (dNTP) and phi 29 DNA polymerase were purchased from New England Biolabs (Beverly, MA, USA). The

4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid sodium salt (HEPES) and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich. The SYBR Gold was obtained from Invitrogen (Carlsbad, CA, USA). Human cervical carcinoma cell line (HeLa cells) was purchased from Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). All solutions were prepared using ultrapure water obtained by a Millipore Milli-Q water purification system (Billerica, MA, USA) with an electric resistance of 418.3 MΩ cm. Other chemical reagents were all of analytical grade and used without further purification.

Table S1. Sequences of the Oligonucleotides [α]

note	sequence (5′-3′)
guide RNA	UGG AGU GUG ACA AUG GUG UUU G
substrate RNA	GGC UGC GAU G**CA AAC ACC AUU GUC ACA CUC C**CU AAA AAA AUA UA
circular template	CAA TGG TGT TTG CAT CGC AGC C*CC TAG CAT AGC CTC CCA AAA TAT CCT ATA TTC GGC CCC GAC CTG GTT C*
signal probe	ACT CTT CCT AGC FrAQ GGT TCG ATC AAG A

[α] The boldface regions in substrate RNA indicate the complementary part of guide RNA. The underlined letters indicate the sequences specific to Ago2 cleavage. The italic letters in the circular template indicate the antisense sequence of 8-17 DNAzyme. In signal probe, rA denotes adenosine ribonucleotide at that position while all others are deoxyribonucleotides, F represents FAM, and Q represents BHQl.

Ago2 Assay. The 200 nM guide RNA, 600 nM substrate RNA, 1× Ago2 reaction buffer (30 mM Tris buffer, pH 7.5, 130 mM KCl, 1.1 mM $MgCl_2$, 1 mM DTT, 0.1 mM EDTA), and Ago2 with the indicated concentration in a total volume of 15 μL were incubated at 30 °C for 30 min. Then the products were transferred to the RCA reaction system containing 1× reaction buffer (40 mM Tris–HCl, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 5 mM $(NH_4)_2SO_4$, 4 mM DTT, 250 mM HEPES buffer, 1 M NaCl), 1.2 U of phi29 polymerase, 90 nM circular template, 600 mM dNTP, 1.2 μM signal probes, and 10 μM $Zn(Ac)_2$ in a total volume of 60 μL. The polymerization was carried out at 30 °C for 2 h, followed by incubation at 65 °C for 20 min to deactivate the Phi29 polymerase. The ultimate reaction products were subjected to the fluorescence measurement. All fluorescence spectra were measured using a quartz cuvette on a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan). The excitation wavelength was 492 nm, and the emission spectra were recorded over the wavelength range from 492 to 600 nm with a slit width of 5 nm for both excitation and emission. The fluorescence intensity at 512 nm was recorded for data analysis.

Inhibition Assay. For Ago2 inhibition assay, different-concentration aurintricarboxylic acid (ATA) was added to the Ago2 reaction buffer and the subsequent reactions followed the above steps. The relative activity of Ago2 (RA) was measured according to equation 1:

$$RA(\%) = \frac{F_i\text{-}F_0}{F_t\text{-}F_0} \times 100\% \qquad (1)$$

where F_0 is the fluorescence intensity in the absence of Ago2, F_t is the fluorescence intensity in the presence of 1 μM Ago2, and F_i is the fluorescence intensity in the presence of both Ago2 and ATA. The IC_{50} value was calculated from the curve of RA versus the ATA concentration

Cell Culture and Ago2 Extraction. HeLa cells were cultured in RPMI 1640 medium (Thermo Scientific HyClone) supplemented with 10% heat-inactivated fetal bovine serum and 1%

penicillin-streptomycin. The cells were cultured at 37 ºC in a humidified atmosphere containing 5% CO_2, and were kept in a logarithmic growth phase by routine passage every 2-3 days. Then the cells were collected in EP tube, washed twice with 10 mM PBS (10 mM PB, 0.1 M NaCl, pH 7.4) by centrifuging at 2000 rpm for 3 min at 4 °C. The number of cells was measured by Countstar cell counter. About 1×10^6 HeLa cells were homogenized in ice-cold lysis buffer (pH 7.5, 50 mM Tris, 10 mM sodium phosphate, 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, 10 mM NaF, 5 mM iodoacetate, 1 mM benzamidine, 5 μg/mL leupeptin) and incubated on ice for 30 min. The lysates were centrifuged at 12000 rpm for 20 min to remove the insoluble material, and the resulting supernatant was collected carefully. The lysates were used immediately for Ago2 activity assay.

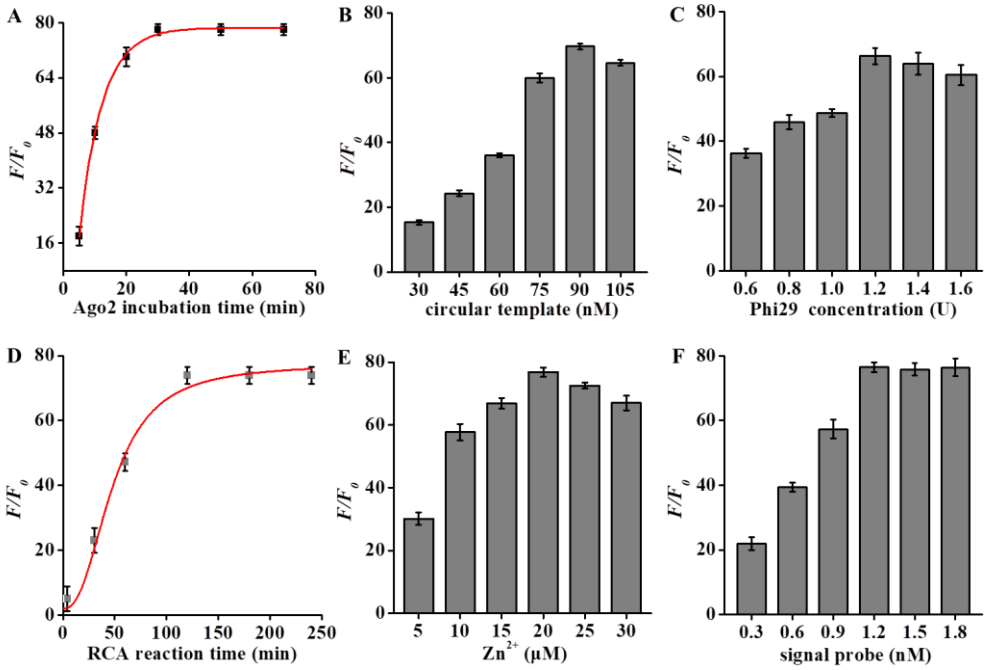

Fig. S1 (A) Variance of the value of F/F_0 with the incubation time of Ago2. (B) Variance of the value of F/F_0 with the amount of circular template. (C) Variance of the value of F/F_0 with the concentration of Phi29 Polymerase. (D) Variance of the value of F/F_0 with the reaction time of RCA. (E) Variance of the value of F/F_0 with the concentration of Zn^{2+}. (F) Variance of the value of F/F_0 with the concentration of signal probe. F and F_0 are the fluorescence intensities in the presence and absence of 1 μM Ago2, respectively. Error bars show the standard deviations of three experiments.

Optimization of Experimental Conditions. To improve amplification efficiency of the Ago2-mediated 8-17 DNAzyme-based RCA, some critical parameters such as the incubation time of Ago2, the concentration of circular template, the amount of phi29 DNA polymerase, the reaction time of RCA, the concentration of Zn^{2+}, and the concentration of signal probe were optimized. The ratio of F/F_0 is used for assessing the assay performance, where F is the fluorescence intensity in the presence of 1 μM Ago2 and F_0 is the fluorescence intensity in the

absence of Ago2. We investigated the effect of Ago2 incubation time upon the assay performance. As shown in Fig. S1A, the F/F_0 value enhances with the incubation time from 5 to 30 min, and levels off beyond 30 min. This can be explained by either the complete loss of Ago2 activity after 30 min incubation or the consumption of all available substrates. Therefore, 30 min is used as the Ago2 incubation time in the following experiments.

We further investigated the effect of circular template concentration upon the amplification efficiency of RCA reaction. Although the high amplification efficiency can be obtained at high-concentration circular template, the background signal might increase correspondingly.[1] As shown in Fig. S1B, the F/F_0 value enhances with the increasing concentration of circular template from 30 to 90 nM, followed by the decrease beyond the concentration of 90 nM. Therefore, 90 nM circular template is used in the subsequent research.

The phi29 also influences the RCA amplification efficiency. As shown in Fig. S1C, the F/F_0 value enhances with the polymerase concentration from 0.6 to 1.2 U, and slightly decreases when polymerase amount is further increased. Therefore, 1.2 U of phi29 DNA polymerase is used in the subsequent researches.

The effect of RCA reaction time upon assay performance was investigated as well. As shown in Fig. S1D, the F/F_0 value enhances with the incubation time from 5 to 120 min, and levels off beyond 120 min. This can be explained by either the complete loss of phi29 DNA polymerase activity after 120 min incubation or the consumption of all available primers. Therefore, 120 min is used as the RCA reaction time in the following experiments.

Zn^{2+} acts as a cofactor of 8-17 DNAzyme and has a significant effect on the 8-17 DNAzyme–mediated signal amplification.[2] As shown in Fig. S1E, the maximum F/F_0 value is obtained when

20 μM Zn^{2+} is added. Therefore, 20 μM Zn^{2+} was used in the subsequent research.

We also investigated the concentration of signal probe upon the assay performance.[2] As shown in Fig. S1F, the F/F_0 value increases gradually in the range from 0.3 μM to 1.2 μM, and reaches the highest value at 1.2 μM. Thus, 1.2 μM signal probe was used in the subsequent experiments.

REFERENCES

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