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Supporting Information

Label-free and amplified colorimetric assay of ribonuclease H activity and inhibition based on an enzyme-responsive DNAzyme cascade

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Experimental Section

Materials and Methods

Materials: All oligonucleotides were purchased from the Life Tech. Co. (Shanghai, China) and purified by HPLC. The sequences of the involved oligonucleotides were as follow: the RNA-DNA hybrid hairpin probe: 5'-TACCCAACAGCGATCACCCATGTA CCCATCCTTTUGAUCGCUGUUGGGUA-3'; The G-riched DNA probe: 5'-ACACAG GGTTGGGCGGGATGGGTTrAGGTTGGGTAGGGCGGGTTGGGAATT-3'; and the blocker DNA: 5'-CCTGTGTAATTCCC-3'. *E. coli* RNase H with an enzyme activity of 6×10^4 U/mL was purchased from Takara Biotechnology (Dalian, China). EcoRI, DpnII, EcoRV and BamHI endonucleases were obtained from New England Biolabs. 2,2'-Azino-bis(3-ethylbenzothiazoline)-6-sulfonate disodium salt (ABTS), H_2O_2, and [tris(hydroxymethy-l)aminomethane] (Tris) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical grade. Tris buffer (20 mM Tris, 100 mM NaCl, 25 mM KCl, 10 mM $MgCl_2$, pH 7.4) was used for the RNase H assays. The water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA), and used throughout the work.

Absorbance measurements: Absorbance measurements were performed using a TU-1901 UV-visible spectrophotometer (Beijing Purkinje General Instrument Co, Ltd., China) with a 0.5 cm path length quartz cuvette. The absorption spectra of the sample solution were measured in the wavelength range from 900 nm to 400 nm at a fixed time interval of 5 min. The rate of the horseradish peroxidase (HRP)-mimicking DNAzyme reaction was monitored at 418 nm.

RNase H activity assay: The procedure for *E. coli* RNase H assay was as follows. First, the G-riched DNA probe (25 μL, 5 μM) and blocker DNA (25 μL, 6 μM) were mixed and incubated at room temperature for 1 h. Second, 10 μL of 20 mM Tris buffer containing 10 μM of the RNA-DNA hybrid probe and various concentrations of *E. coli* RNase H or other endonucleases was added the above mixture, and incubated for another 2 h at 37 °C. After the solution was diluted with 300 μL Tris buffer, hemin (20 μL) was added to obtain the mixture and give the final concentration of 0.5 μM hemin, and incubated for 1 h at room temperature to form the hemin/G-quadruplex structures. Finally, the ABTS (60 μL) and H_2O_2 (60 μL) substrates were added to the above mixture to give the final concentrations of 2 mM ABTS and 2 mM H_2O_2, respectively. The resulting samples were tested with a UV-vis spectrometer. All experiments were repeated seven times.

RNase H activity inhibition evaluation: For *E. coli* RNase H inhibition experiments, the procedure was similar to detection of its activity mentioned above, except that different concentrations of the inhibitor were added together with *E. coli* RNase H.

Gel electrophoresis: Gel electrophoresis was used to confirm the cleavage of RNA-DNA hybrid hairpin by RNase H and the activated Mg^{2+}-dependent DNAzyme-catalyzed cleavage reaction. Samples for gel electrophoresis assays were prepared as follows: (1) the RNA-DNA hybrid hairpin probe (2.0 μM) was used as sample one; (2) the G-riched DNA probe (2 μM) was used as sample two; (3) the blocker DNA (2 μM) was used as sample three; (4) the mixture of the G-riched DNA probe (2 μM) and blocker DNA (2 μM) was incubated at room temperature for 1 h, and used as sample four; (5) sample was

prepared by incubating the mixture of G-riched DNA probe (2 μM) and blocker DNA (2 μM) at room temperature for 1 h, and then added RNA-DNA hybrid hairpin probe (2.0 μM) to the mixture; (6) the mixture of RNA-DNA hybrid hairpin probe (2.0 μM) and *E. coli* RNase H (40 units) was incubated for 1 h at 37 ℃, and used as sample six; (7) sample was prepared by incubating the mixture of G-riched DNA probe (2 μM) and blocker DNA (2 μM) at room temperature for 1 h, and then added RNA-DNA hybrid hairpin probe (2.0 μM) and *E. coli* RNase H (40 units) to the mixture for incubating another 1 h at 37 ℃. The gel electrophoresis assays were carried out by using a DYCP-31A Electrophoresis Cell equipped with DYY-5 Electrophoresis Power Supply (Beijing LiuYi Instrument Factory, China). Each prepared sample (8 μL) was put on 5% agarose gels to separate the related substances, and the separation was carried in 0.5×TBE buffer (pH 7.9) at 120 V constant voltage for 1 h. After EB staining, the gels were scanned using the Omega 16ic Gel imaging system (ULTRA-LUM, USA).

Circular dichroism experiments: Two samples for circular dichroism (CD) assays were were prepared as follows: (1) The mixture of G-riched DNA probe (1 μM) and blocker DNA (1 μM) was incubated at room temperature for 1 h, and then added RNA-DNA hybrid hairpin probe (1.0 μM) to the mixture. The obtained mixture was used as sample one; (2) sample two was prepared by first incubating the mixture of G-riched DNA probe (1 μM) and blocker DNA (1 μM) at room temperature for 1 h, and then added RNA-DNA hybrid hairpin probe (1.0 μM) and *E. coli* RNase H (100 units) to the mixture for incubating another 1 h at 37 °C. Each prepared sample (500 μL) were measured by using J-810 Circular Dichroism Spectrophotometer (JASCO, Japan) with a quartz cell with 1

cm path length. CD spectra were collected from 230 to 350 nm with a canning speed of 200 nm/min. The bandwidth was 2 nm, the data pitch was 1 nm, and the response time was 2 s.

Results and Discussion

CD Characterization: Evidence for the cleavage of the quasi-circular DNA, and the formation of G-quadruplex was provided by CD analysis. As shown in Fig.S2, the CD spectrum of the mixture of G-riched DNA probe/blocker DNA complex and RNA-DNA hybrid hairpin probe had a negative peak around 240 nm, a small positive peak around 260 nm and a positive peak at 280 nm (curve a). It had been reported that the peaks at 240 and 280 nm are characteristic from the DNA duplexes,[1] and a negative peak at 240 nm along with a small positive peak at 260 nm are characteristic for a parallel G-quadruplex system.[2,3] However, upon incubation of the mixture of G-riched DNA probe/blocker DNA complex and RNA-DNA hybrid hairpin probe with *E. coli* RNase H, the intensity of the peak around 260 nm increased, and the intensity of the peak at 240 nm decreased (curve b). This was because *E. coli* RNase H was able to cleave the RNA strand within the RNA-DNA hybrid hairpins, and the activated Mg^{2+}-dependent DNAzymes were generated. The activated Mg^{2+}-dependent DNAzymes could cleave the quasi-circular DNA and thus and thus generating more parallel G-quadruplex.

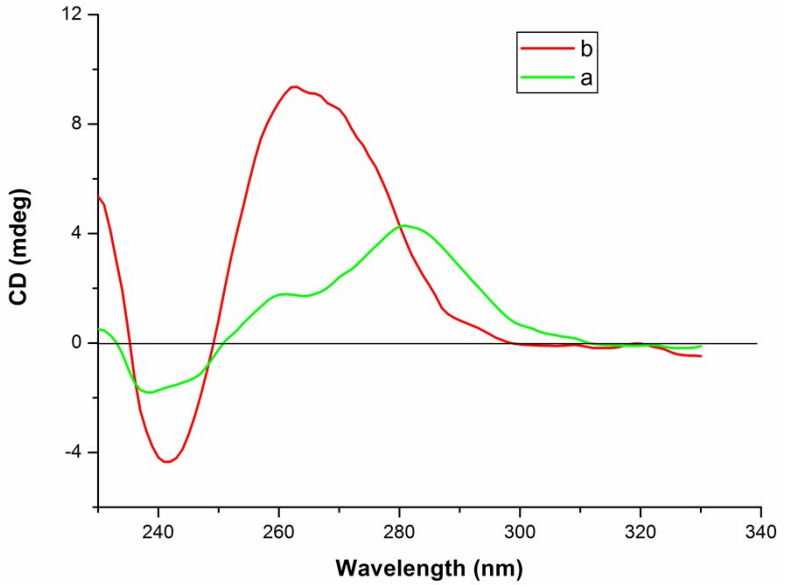

Fig. S1 CD spectra of different samples: (a) the mixture of G-riched DNA probe/blocker DNA complex/RNA-DNA hybrid hairpin probe; (b) the mixture of G-riched DNA probe/blocker DNA complex, RNA-DNA hybrid hairpin probe and *E. coli* RNase H.

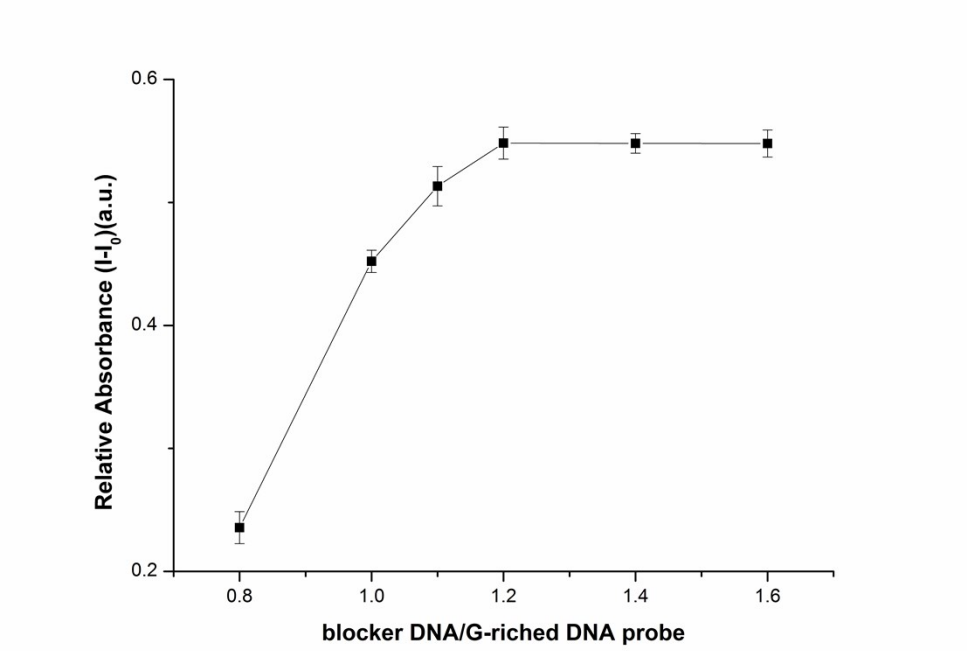

Fig. S2 The effects of different ratio of the blocker DNA and G-riched DNA probe on the absorption intensity in the sensing system. The concentration of *E. coli* RNase H was 0.05 U/mL. Error bars were derived from N=7 experiments.

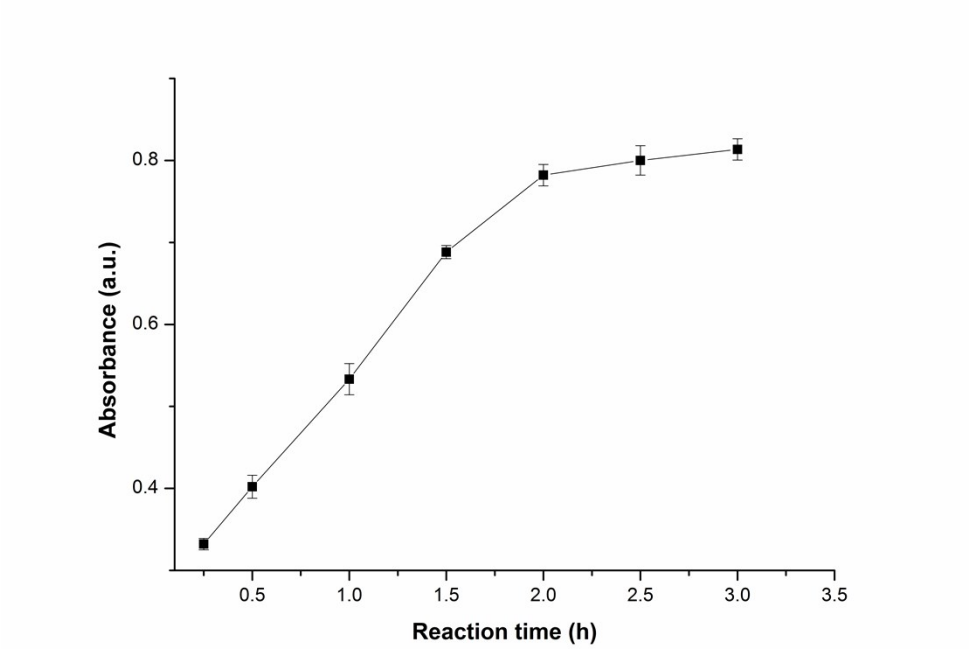

Fig. S3 The effects of enzymatic cleavage reaction time on the absorption intensity in the sensing system. The concentration of *E. coli* RNase H was 0.05 U/mL. Error bars were derived from N=7 experiments.

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