Electronic Supplementary Information

Enzyme-free Signal Amplification in the DNAzyme Sensor via Target-Catalyzed Hairpin Assembly

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EXPERIMENTAL SECTION

Reagents. Oligonucleotides designed in this study were synthesized by Shanghai Sangon Biotechnology Co, which were purified by HPLC and confirmed by mass spectrometry. Table 1 shows the sequences of the used Oligonucleotides. Each Oligonucleotide was heated to 90 °C for 5 min, and slowly cooled down to room temperature before use. 2, 2'-azino-bis (3-ethylbenzothiozoline)-6-sulfonic acid (ABTS^{2–}), Hemin and luminol were purchased from Sigma-Aldrich Chemical Co., USA. The hemin stock solution was prepared in dimethyl sulfoxide (DMSO) and stored in the dark. Ultrapure water obtained from a Millipore water purification system (18 MΩ/ cm resistivity) was used in all runs. All other reagents were of analytical grade.

 Table 1. Sequences of the Used Oligonucleotides (in 5' to 3' Direction)

Туре	Sequence
H1	5'- <u>GGG TAG GGC GGG TTG GG</u> AT <i>GAG AAA GGG CTG CCA</i> CA TCC CAA CCC ATA -3'
H2	5'- TAT GGG TTG GGA TGT GGC AGC CAT CCC AAC -3'
T1	5'- TGG CAG CCC TTT CTC -3'
T2	5'- TGG CAG C <u>G</u> C TTT CTC -3'
Т3	5'- TGG CAG <u>GCG</u> TTT CTC -3'
T4	5'- TCT GTC CTG AGA CA -3'

The underlined bold letters of H1 are the sequences of HRP-DNAzyme, and the italic bold letters of H1 are the sequences complementary to the target. T1 is the perfectly complementary target DNA.T2 is a single-base-mismatched target DNA, while T3 is a double-base-mismatched target DNA (the mismatched base is underlined). T4 is a non-complementary target DNA.

Polyacrylamide gel electrophoretic analysis. The experiments were performed in 25 mM HEPES buffer with 100 mM NaCl and 20 mM KCl, pH 7.4. H1 (1 μ M) was analyzed alone or mixed with H2 (1 μ M) and different concentrations of target DNA (0, 10, 100, 500 nM) for 2 h at 37 °C. The final volume of the solution was 20 μ L. The sample was applied to a polyacrylamide (PAGE) gel (20% acrylamide, 19:1 acrylamide/bisacrylamide) to separate the H1-H2 complex from the substrate. The base pairs of DNA ladder are varied from 10 to 300. The electrophoresis was carried in 1×tris-borate-EDTA (TBE) buffer (90 mM Tris, 90 mM boric acid, and 10 mM EDTA, pH 8.0) at 11 mA for 2 h. The gels were stained with ethidium bromide for 10 min, and then destained in distilled water for 10min.

Colorimetric measurements. Hairpins H1 and H2 stock solutions were prepared in 25 mM HEPES buffer with 100 mM NaCl and 20 mM KCl, pH 7.4. H1 (200 nM) and H2 (300 nM) were mixed with different concentrations of target DNA for 2 h at 37 °C. The final concentrations of target in samples varied from 20 pM to 20 nM. Then, hemin (500 nM) was added for another 30 min. The peroxidase-mimicking reaction was started by addition of H_2O_2 (2 mM) and ABTS (2 mM), respectively. The color development was followed at 414 nm with a SH-1000 Lab MICROPLATE READER. The calibration curve was made by using the absorbance intensity at 10 min of each sample as the ordinate and the concentrations of the target DNA as the abscissa.

Chemiluminescence measurements. The different concentrations of target DNA were added to the mixture of H1 (200 nM) and H2 (300 nM), respectively. The samples reacted for 2 h at 37 °C, and then were examined one by one upon the

addition of luminol and H_2O_2 . The final concentrations of target DNA varied from 100 fM to 2 pM. Chemiluminescence experiments were performed using a BPCLWeak Luminescence Analyzer (Institute of Biophysics, Chinese Academy of Science, Beijing, China). Measurements were made in a cuvette that included a buffer solution consisting of 25 mM HEPES buffer (100 mM NaCl and 20 mM KCl, pH 8.0), 500 nM hemin, 0.5 mM luminol, and 30 mM H₂O₂.

Target Detection in 1:5 dilution of saliva Samples. The saliva samples were obtained from laboratory personnel, which were centrifuged before use. H1 (200 nM), H2 (300 nM) and the treated saliva (50 μ L) were mixed with different concentrations of target for 2 h at 37 °C. Then, hemin (500 nM) was added for another 30min. Finally, the absorbance intensity was monitored by the addition of ABTS (2 mM) and H₂O₂ (2 mM) for 10 min. The final volume of the solution was 250 μ L.

Optimization of the Concentration of H2

To optimize this amplified DNAzyme sensor, the concentration of H2 had to be optimized, which plays an important role in the sensing process. To investigate the effect of H2 concentration, H1 (200 nM) and target DNA (10 nM) were mixed with different concentrations of H2 ranged from 50 nM to 500 nM. As shown in Fig. S1, the absorbance intensity of the system with or without target DNA was monitored by the addition of ABTS and H_2O_2 for 10 min. It can be observed that the absorbance intensity of the system and the background increased with increasing the concentration of H2. The results indicated that the optimum concentration of H2 used in this system was 300 nM due to its best signal-to-noise level.



Fig. S1 Effect of the H2 concentration on the signal-to-noise level of the detection system. The bars represent the intensity of absorbance at 414 nm with or without T1 (10 nM). The concentration of H1 was 200 nM. And the concentration of H2 varied from 50 nM to 500 nM.

Optimization of the Incubation Time of Target-catalyzed Hairpin Assembly

The process of signal amplification was strongly influenced by the assembly time. To optimize the assembly time, we recorded the absorbance intensity with and without the addition of target DNA into the system. As shown in Fig. S2, the absorbance intensity of the system and the background maintained its increase with assembly time. Meanwhile, the absorbance reached equilibrium after assembly for about 4 hours in the proposed system. However, the optimum assembly time was 2 h according to the best signal-to-noise level.



Fig. S2 Effect of the assembly time on the signal-to-noise level of the detection system. The bars represent the intensity of absorbance at 414 nm in the absence of T1 (gray bars) or in the presence of T1 (10 nM) (black bars), respectively.

Amplified DNAzyme Sensor Using Colorimetric Detection

Fig. S3 shows the time-dependent absorbance changes upon analyzing different concentrations of T1 under the optimization conditions using target-catalyzed hairpin assembly. A dramatic increase in the absorbance intensity was observed as the concentration of target DNA was increased from 20 pM to 20 nM.



Fig. S3 The time-dependent absorbance changes upon analyzing different concentrations of T1 at the fixed concentrations of H1 (200 nM) and H2 (300nM), a-k: 0, 20, 50, 100, 200, 500, 1000, 2000, 5000, 10000 and 20000 pM, respectively.

1:1 Binding DNAzyme Sensor Using Colorimetric Detection

Fig. S4 shows the time-dependent absorbance changes of H1 in the presence of different concentrations of target. The absorbance intensity gradually increased as the concentration of T1 was increased. The detection limit of this 1:1 binding strategy using colorimetric detection was about 5 nM, which is consistent with the reported DNAzyme sensors.



Fig. S4 The time-dependent absorbance changes upon analyzing different concentrations of T1 at the fixed concentration of H1 (200 nM) without H2, a-f: 0, 5, 10, 20, 50 and 100 nM, respectively.

Application of the Amplified DNAzyme sensor in Real Samples

We detected the target DNA in real sample (1:5 dilution of saliva), which is one of the most challenging media containing a variety of proteins and other serious interference. The diluted saliva were analyzed alone or spiked with target DNA, and the results were compared with that from buffer analysis. As shown in Fig. S5, comparable responses were obtained for the detection of target DNA in both buffer and real samples. As shown in Fig. S6, the absorbance intensity gradually increased as the concentration of target DNA was increased. This assay allowed for the detection of target DNA at concentration as low as 20 pM.



Fig. S5 Target DNA detected by proposed DNAzyme sensor in buffer and in 1:5 dilution of saliva. The bars represent the intensity of absorbance at 414 nm with or without Target (T1).



Fig. S6 The time-dependent absorbance changes upon analyzing different concentrations of T1 in 1:5 dilution of saliva. a-e: 0, 20 pM, 200 pM, 2 nM and 20 nM, respectively.