

Electronic Supplementary Information

Nicking Enzyme Based Homogenous Aptasensor for Amplification Detection of Protein

Ai-Xian Zheng, Jin-Ru Wang, Juan-Li, Xiao-Rong Song, Guo-Nan Chen, and

Huang-Hao Yang*

The Key Lab of Analysis and Detection Technology for Food Safety of the MOE, Fujian Provincial

Key Laboratory of Analysis and Detection Technology for Food Safety

College of Chemistry and Chemical Engineering

Fuzhou University, Fuzhou 350108, P. R. China

Email: hhyang@fio.org.cn

EXPERIMENTAL SECTION

Apparatus. Fluorescence experiments were all performed using a Hitachi F-4600 fluorometer (Hitachi Co. Ltd., Japan). The optical path length of a quartz fluorescence cell was 1.0 cm. The excitation was made at 480 nm with a recording emission range of 500-600 nm. The fluorescence intensity at 518 nm was used to evaluate the performance of the proposed aptasensor. The fluorescence spectra were recorded under room temperature unless otherwise indicated.

Reagents. Oligonucleotides designed in this study were synthesized by Shanghai Sangon Biotechnology Co, which were purified by HPLC and confirmed by mass spectrometry. Each Oligonucleotide was heated to 90 °C for 5 min, and slowly cooled down to room temperature before use. All proteins were purchased from Sigma-Aldrich Chemical Co., USA. Nb.BbvCI and 10× NEB buffer 2 (500 mM NaCl, 100 mM Tris-HCl, 100 mM MgCl₂, and 10 mM dithiothreitol, pH 7.9) were purchased from the New England Biolabs, Inc. 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.

1:1 binding strategy. Human thrombin stock solution was prepared by first dissolving in glycerol (about 50% of the final volume) and then further diluting with ultrapure water to obtain the desired concentration. Hairpins H1 and H2 stock solutions were prepared in 1× NEB buffer 2. H1 (100 nM) and H2 (100 nM) were mixed with different concentrations of human thrombin for 1 hour at 37 °C. The final concentrations of human thrombin in samples varied from 2 nM to 50 nM. Then, the

fluorescence intensities were detected.

Signal Amplification Strategy. H1 (100 nM) and H2 (20 nM) were mixed with different concentrations of human thrombin varying from 5 pM to 50 nM. Successively, Nb.BbvCI (5 U) was then added and allowed to incubate for 1 hour at 37 °C. Finally, the fluorescence intensities were detected. The calibration curve was made by using the fluorescence intensities of each sample as the ordinate and the concentrations of the human thrombin as the abscissa.

Selectivity of the Aptasensor. H1 (100 nM) and H2 (20 nM) were mixed with human thrombin or other non-specific protein (human IgG, human serum albumin (HSA), bovine serum albumin (BSA), or bovine thrombin, each at a concentration of 5 nM.) respectively. Successively, Nb.BbvCI (5 U) was then added and allowed to incubate for 1 hour at 37 °C. Then, the fluorescence intensities were detected.

Human Thrombin Detection in the cell lysate Samples. H1 (100 nM) and H2 (20 nM) were mixed with different concentrations of human thrombin varying from 20 pM to 10 nM. Successively, Nb.BbvCI (5 U) and HeLa cell lysate (125µl) were then added and allowed to incubate for 1 hour at 37 °C. The final volume of the solution is 250µl. Then, the fluorescence intensities were detected.

Optimization of Probe H1

To optimize this aptasensor, the structure of hairpin H1 had to be optimized. Table 1 shows three different hairpin sequences for hairpin H1 (H1-a, H1-b, and H1-c) that include 9, 8, and 7 base pairs in the stem region, with hairpin H2 as the readout substrate. Based on the fluorescence change of the systems with or without 5 nM

human thrombin, we found that H1-b gave the best signal-to-noise level (Figure S1). The H1-a probe has the most stable structure and therefore showed a rather low ability of interaction with target. Thus, the fluorescence intensity of the system somewhat increased upon the addition of human thrombin. The H1-b probe was the most effective probe in the group and had a significant fluorescence enhancement. Further decrease of the stem sequence length in the H1-c increased the background signal, hindering the sensitivity of the aptasensor. Thus, the H1-b probe was chosen as the optimum probe in the experiments due to its more effective than other probes.

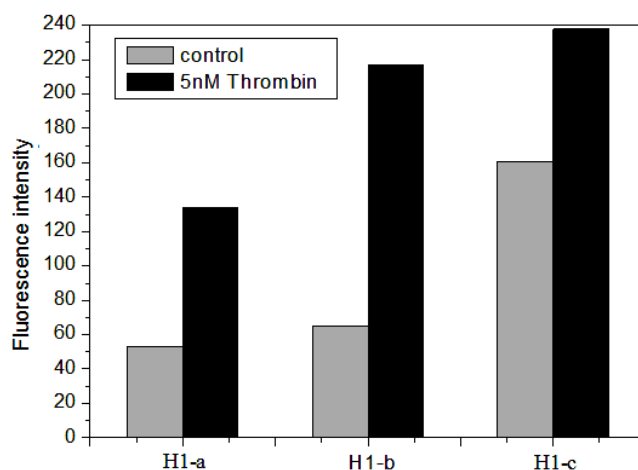


Figure S1 Signaling profile of the aptasensor using the following hairpins: H1-a, H1-b, and H1-c in the absence of human thrombin (gray bars) or in the presence of human thrombin (5nM) (black bars), respectively.

Optimization of the Concentration of Probe H1-b

The concentration of probe plays an important role in the sensing process. As shown in Figure S2, the fluorescence intensity of the aptasensor and the background increased with increasing the concentration of H1-b. The results indicated that the optimum concentration of H1-b used in this aptasensor was 20 nM due to its best signal-to-noise level.

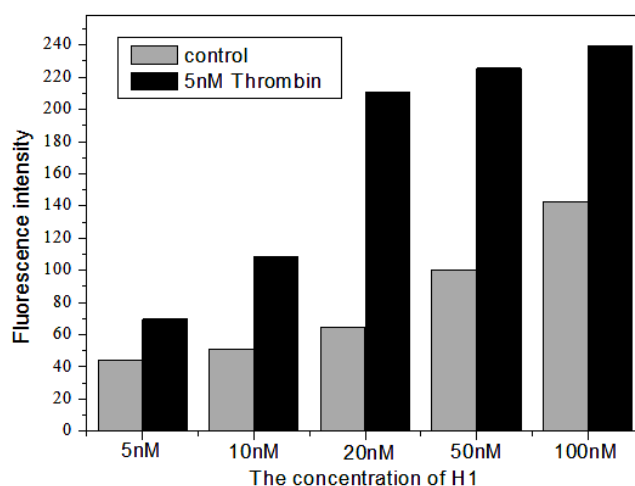


Figure S2 Effect of the H1-b concentration on the signal-to-noise level of the detection system. The bars represent the changes of fluorescence signal in the presence of human thrombin (5 nM). The concentration of H2 was 100 nM. And the concentration of H1-b varied from 5 nM to 100 nM.

Optimization of the incubation time

The process of signal amplification was influenced by the incubation time of the nicking of nicking enzyme. To optimize the incubation time, we recorded the fluorescence intensities before and after the addition of 500pM human thrombin into the system. As shown in Figure S3, the optimum incubation time was 60 min according to the best signal-to-noise level.

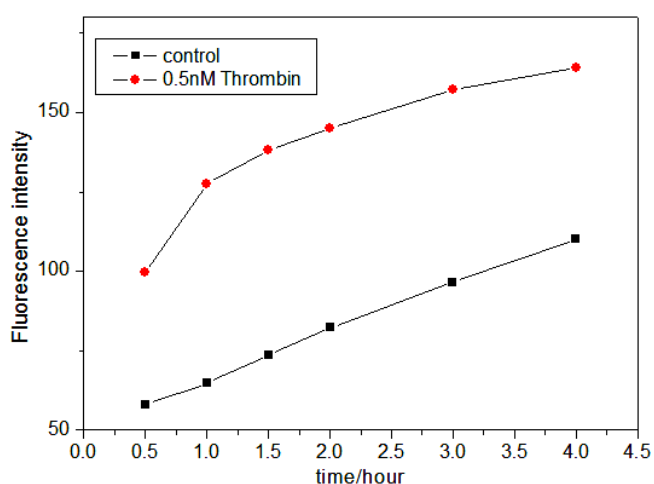


Figure S3. Effect of the incubation time on the signal-to-noise level of the detection system. Time course of fluorescence intensities recorded before (a) and after (b) the addition of 500pM human thrombin into the system.

Analytical Performance of the Aptasensor

Figure S4 shows the fluorescence-emission spectra of the mixture of H1-b and H2 upon the addition of nicking enzyme and different concentrations of human thrombin. The fluorescence intensity increased significantly as the target binding was increased.

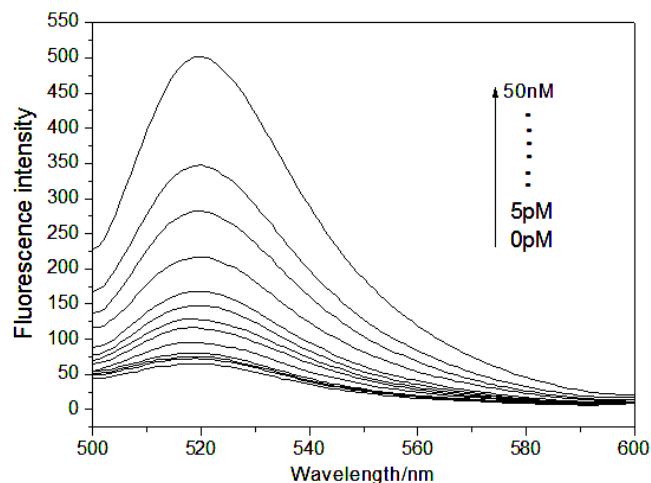


Figure S4. Fluorescence-emission spectra of the mixture of H1-b (20 nM) and H2 (100 nM) upon the addition of nicking enzyme (5 U) and human thrombin at different concentrations.

The result of 1:1 Binding Strategy

As shown in Figure S5 the fluorescence intensity gradually increased as the concentration of human thrombin was increased. This assay allowed for the detection of human thrombin at concentration as low as 2 nM.

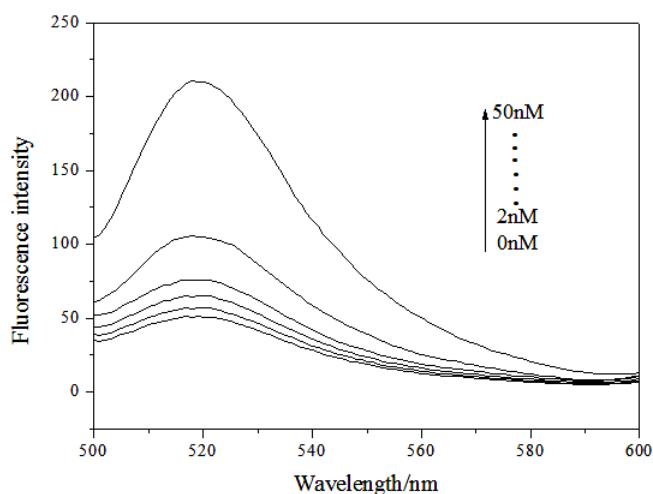


Figure S5. Fluorescence spectra of the mixture of H1 (100nM) and H2 (100nM) in the presence of different concentrations of human thrombin (0, 2, 5, 10, 20, 50nM).

Application of the Aptasensor to Target Detection in Real Samples

We applied this aptasensor to detect the human thrombin in 1:1 dilution of HeLa cell lysate. As shown in Figure S6, the fluorescence intensity gradually increased as the concentration of human thrombin was increased. This assay allowed for the detection of human thrombin at concentration as low as 20 pM.

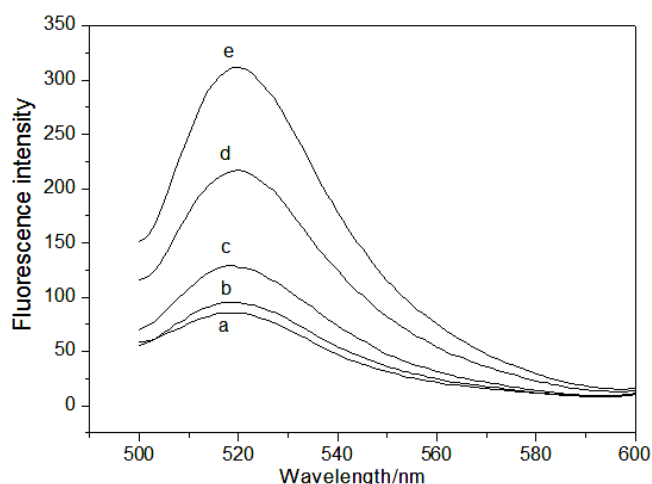


Figure S6. Fluorescence-emission spectra of the mixture of H1-b (20 nM) and H2 (100 nM) in 1:1 dilution of HeLa cell lysate upon the addition of nicking enzyme (5 U) and human thrombin at different concentrations (a, 0 pM; b, 20 pM; c, 100 pM; d, 1 nM and e, 10 nM).