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# Ultrasensitive thrombin sensing platform based on three-way junction initiated dual signal amplification

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**Abstract**

The sensitive and precise quantitative determination of thrombin is important for both fundamental research and clinical diagnostics of hypoxic ischemic encephalopathy because it is a key biological molecule in hemostasis and hemolysis. Herein, we depict a sensitive and label-free thrombin detection approach by taking the advantages of aptamer's superior capability to bind with thrombin and the high efficiency of three-way junction initiated dual signal recycle. In this method, a capture probe which is inserted with an aptamer sequence is designed to specifically identify thrombin molecule and facilitate the signal amplification. Based on the DNA polymerase and endonuclease Nb.BbvCI-assisted chain extension, a large amount of single-strand DNA sequences that can fold into G-quadruplex are produced to specifically recognize commercial fluorescent dye thioflavin T for signal generation. Consequently, the approach exhibits a high detection sensitivity with the limit of detection as low as 768 fM, holding a great promise for detection of thrombin and disease diagnosis in the clinic.

**Keywords:** Thrombin, Thioflavin T, G-quadruplex, Hypoxic ischemic encephalopathy, Nb.BbvCI

## Introduction

Thrombin, as a kind of serine proteases belonging to the chymotrypsin family, plays crucial roles in blood coagulation and is involved in a number of pathological processes of paediatric diseases, including hypoxic ischemic encephalopathy, autoimmune thrombocytopenia in children, and apoptosis (Al-Amer 2022; Tripodi 2016; Tuo et al. 2022). The functions of thrombin in arterial and venous thrombosis, wound healing, and a variety of other pathologies, including sepsis, atherosclerosis, and cancer, are increasingly being investigated (Alexander and Gilmour 2022; Cao et al. 2022; Ericksen et al. 2022). The role of thrombin in pathogenesis of various paediatric diseases depends on its concentration. For instance, modest amounts of thrombin have the ability to protect neurons, whereas greater concentrations of thrombin are always accompanied by pathogenic effects in hypoxic ischemic encephalopathy (Shlobin et al. 2021). Exploiting emerging technologies for precise and sensitive thrombin detection is thus highly desired, especially in clinical settings.

Currently, immunoassay is the most established method for measuring thrombin and is regarded as the gold standard in clinical trials (Ito et al. 2022; Wang et al. 2020; Yeh et al. 2014). However, the immunoassay is often criticized for relatively poor sensitivity, high labor intensity and narrow detection range. Aptamers, as small oligonucleotides that can specifically recognize and bind with biomolecules, are widely exploited to construct aptasensors (Wang et al. 2022; Zhang et al. 2022a). Aptasensor can be used in a variety of experimental and clinical settings, including the identification of diseases and the delivery of medications, due to its advantages of low cost, ease of modification, and quick response (Zhao et al. 2020a, b). A variety of aptasensors have been created in recent years for the detection of thrombin. These

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devices work by using an aptamer to transform thrombin signals into nucleic acids signals, which are then amplified using isothermal nucleic signaling techniques (Yu et al. 2021; Zhang et al. 2022b). The aptasensors can be briefly classified based on the transducer as fluorescent, electrochemical (Lin et al. 2017), colorimetric (Yin et al. 2020), and others (Yu and Wu 2019; Zhao and Gao 2015). Among them, the fluorescent biosensors attract considerable attention because they are stable, programmable and easy-to-operate (Musumeci et al. 2017; Swetha et al. 2020; Zhao et al. 2021). For example, Shen et al. (2017) proposed a novel thrombin detection approach by integrating established signal amplification strategy with chiral supramolecular assembly in physiological $K^+$ background. Even though the fluorescent biosensors made remarkable progress to the traditional thrombin detection approaches, two drawbacks are inevitable: (1) DNA polymerase and endonuclease-assisted generation of two sequences with a DNA sequence that contains two nicking site as template may cause fault translation and interfere result calculation; (2) labeling with the FAM moiety can easily suffer from interferences by the experimental conditions, thus causing inaccuracy in results. Therefore, it is significant to develop a high-fidelity signal amplification strategy and to integrate it for accurate and sensitive thrombin detection via a label-free manner.

ThT is a benzothiazole that becomes fluorescent after recognizing G-quadruplexes and has been widely utilized in constructing label-free fluorescent sensors (Renaud de la Faverie et al. 2014). Herein, we describe a novel fluorescent aptasensor for sensitive and accurate thrombin detection in a label-free manner by exploiting the designed three-way junction initiated signal amplification for high-fidelity transcription and specific binding between the fluorescent dye Thioflavin T (ThT) and the nucleic acid G-quadruplexes for label-free signal generation. In the three-way junction initiated signal amplification, thrombin aptamer is integrated in the toehold of the hairpin probe (catch probe); the specifically identification of thrombin by aptamer will lead to the allosterism of the catch probe to induce subsequent signal amplifications. The dual signal amplification endows the method a high sensitivity, showing a promising prospect in clinical practices.

## Results and discussion

### The principle of the three-way junction initiated dual signal amplification approach for thrombin detection

The working mechanism of the established approach is shown in Fig. 1. The capture probe is designed with hairpin structure, containing four functional parts, to combine the functions of specifically identifying thrombin and triggering three-way junction initiated dual signal amplification. The ***a*** part in the capture probe is thrombin aptamer; ***b*** and ***c*** part assist the proximity ligation to form three-way junction; and ***d*** part is complementary to endonuclease Nb.BbvCI nicking site. The hairpin structure of capture probe blocks the possible hybridization with assistance probe. Only when thrombin is present in the sensing system, aptamer specifically binds with thrombin and causes the allosterism of capture probe, consequently deconstructing the hairpin structure to linear state and exposing ***b*** and ***c*** parts. The exposed ***b*** and ***c*** parts can bind with the complementary sequences (***b′*** and ***c′*** parts) and mediate the hybridization between assistance probe and capture probe–thrombin complex. The formation of three-way junction makes it possible for chain extension with ***b*** and ***c′*** as primers. Under the assistance of DNA polymerase, a sequence that is complementary with linear capture probe is formed in which process the thrombin is disassociated with aptamer. The released thrombin binds with a next capture probe to induce the first signal recycle. Afterward, two nicking sites have been generated by Nb.BbvCI. Under the cooperation of Nb.BbvCI and DNA polymerase, a large collection of ***g′*** sequence and ***a′*** sequences have been produced. The ***a′*** sequence can bind with ***a*** part in capture probe and unfold capture probe to induce a next signal assembly, constituting the second signal amplification. The ***g′*** sequence can fold into G-quadruplex, which can be specifically recognized by commercial fluorescent dye thioflavin T(ThT), generating fluorescence signals which is correlated with the concentration of thrombin in the system in a label-free way.

### Feasibility of the established approach

To test the feasibility of the proposed approach for thrombin detection, the assembly of capture probe and its capability to bind with thrombin was firstly verified by a fluorescence assay as illustrated in Additional file 1: Fig. S1. In brief, the two terminals of the capture probe were labeled with FAM moiety and BHQ, respectively. In the linear state of the probe, FAM signal was not quenched by BHQ and maintained a high level of 7894 *a.u.*. On the contrary, the FAM signal was significantly decreased when capture probe was assembled to hairpin structure due to Foerster resonance energy transfer between FAM (carboxyfluorescein) and BHQ (black hole quencher) moiety. In the experiment testing the thrombin recognition capability of capture probe, the FAM signal recovered to 6481 *a.u.* (Fig. 2a). The phenomenon could be explained by the fact that the successful binding of the aptamer with thrombin caused the transformation of capture probe from a hairpin structure a linear state. The result that no significant increment of FAM signal was obtained when detecting other protein molecule (CEA,

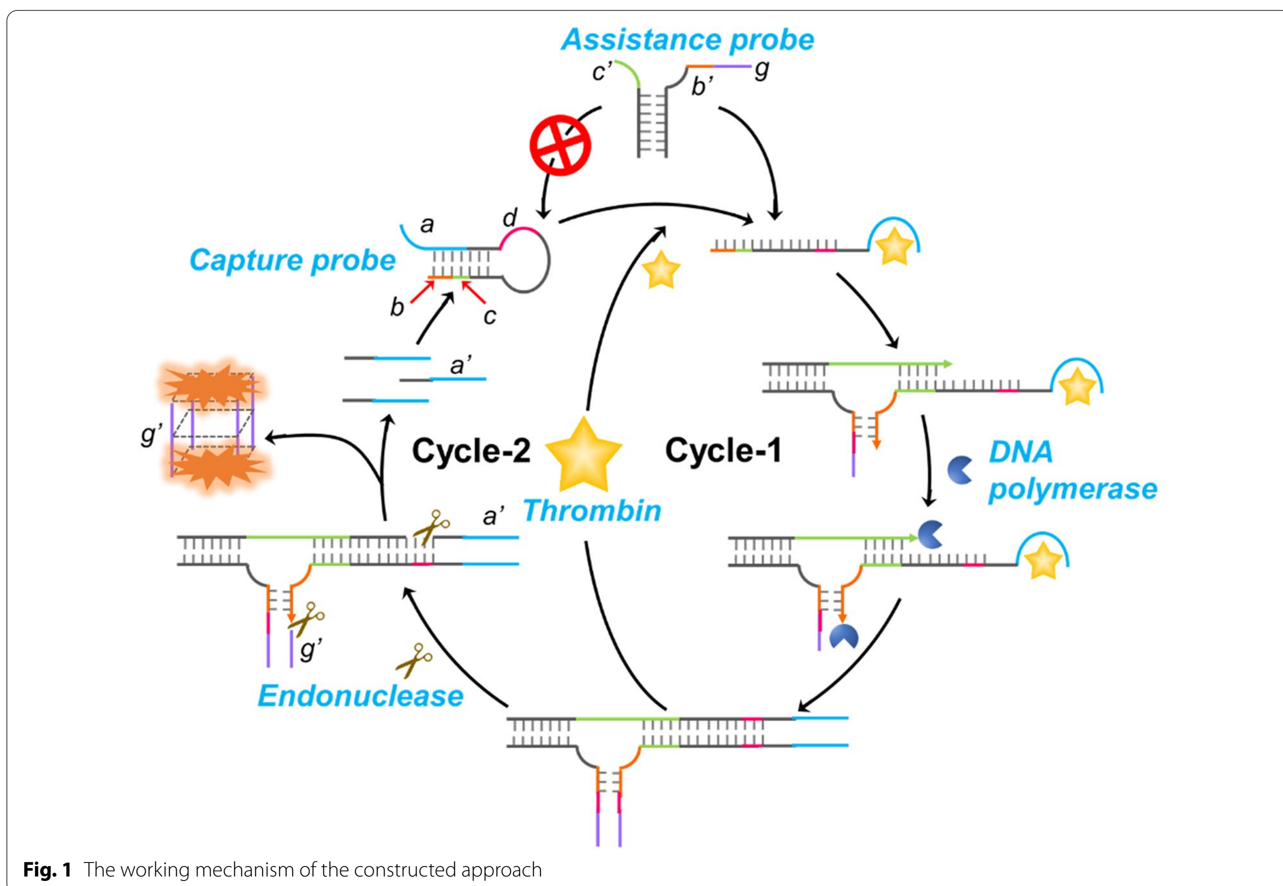


**Fig. 1** The working mechanism of the constructed approach

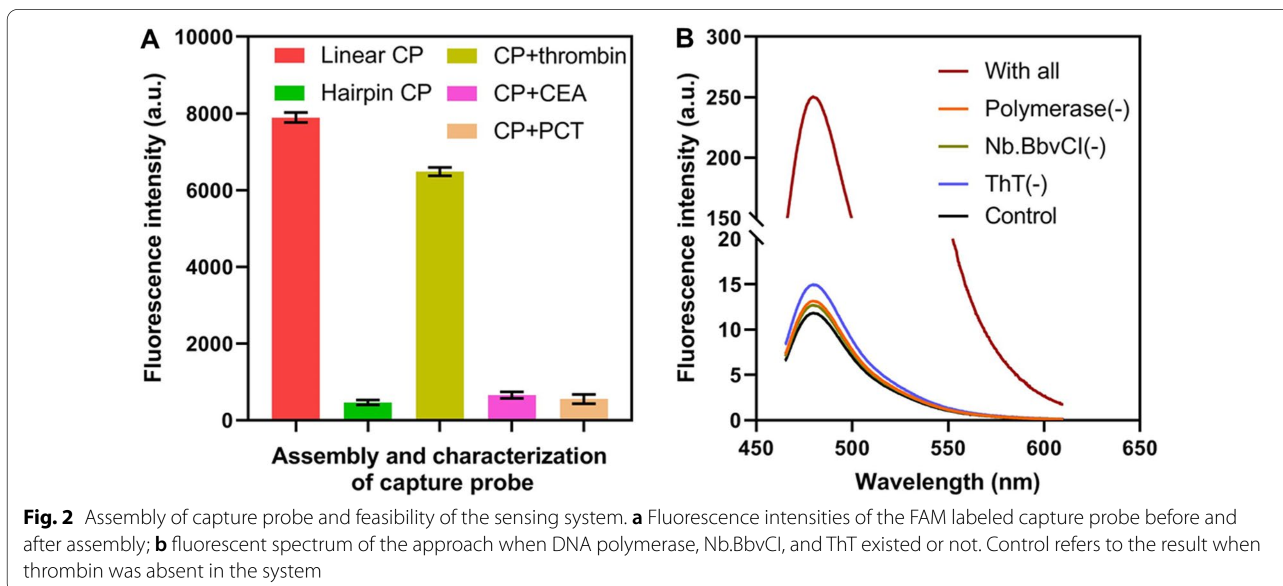


**Fig. 2** Assembly of capture probe and feasibility of the sensing system. **a** Fluorescence intensities of the FAM labeled capture probe before and after assembly; **b** fluorescent spectrum of the approach when DNA polymerase, Nb.BbvCI, and ThT existed or not. Control refers to the result when thrombin was absent in the system

carcinoma embryonic antigen; PCT, procalcitonin) demonstrated a high specificity of the designed capture probe (Fig. 2a). We then investigated role of essential experimental components, including DNA polymerase, Nb.BbvCI, and ThT. The result in Fig. 2b showed the ThT can be efficiently activated to emit a significant fluorescent signal only by the combination of DNA polymerase, Nb.BbvCI, and ThT. In the absence of either DNA polymerase, Nb.BbvCI, or ThT, only negligible fluorescence intensities could be recorded, indicating the essential roles of these experimental components in constructing the approach.

### Optimizations of experimental parameters

Several experimental parameters have been investigated for an optimal detection performance. The fluorescence ratio (F/F0) of the recorded ThT signal when thrombin was present or not was exploited to evaluate the detection performance. The length of ***b*** and ***c*** part in capture probe determines the formation and stability of three-way junction. If the length of ***b*** part is too short, it is difficult to bind with ***b′*** sequence. Nevertheless, if the ***b*** part is too long, it will cause the non-target dependent chain assembly. Therefore, we firstly investigated the length of b part in capture probe. The trend variation of F/F0 contained a significant elevation when the length ranged from 2 to 5 bp and a decreased F/F0 when the length was more than 5 bp (Fig. 3a). Meanwhile, the F/F0 elevated when the length of *c* ranged from 2 to 6 bp, while no

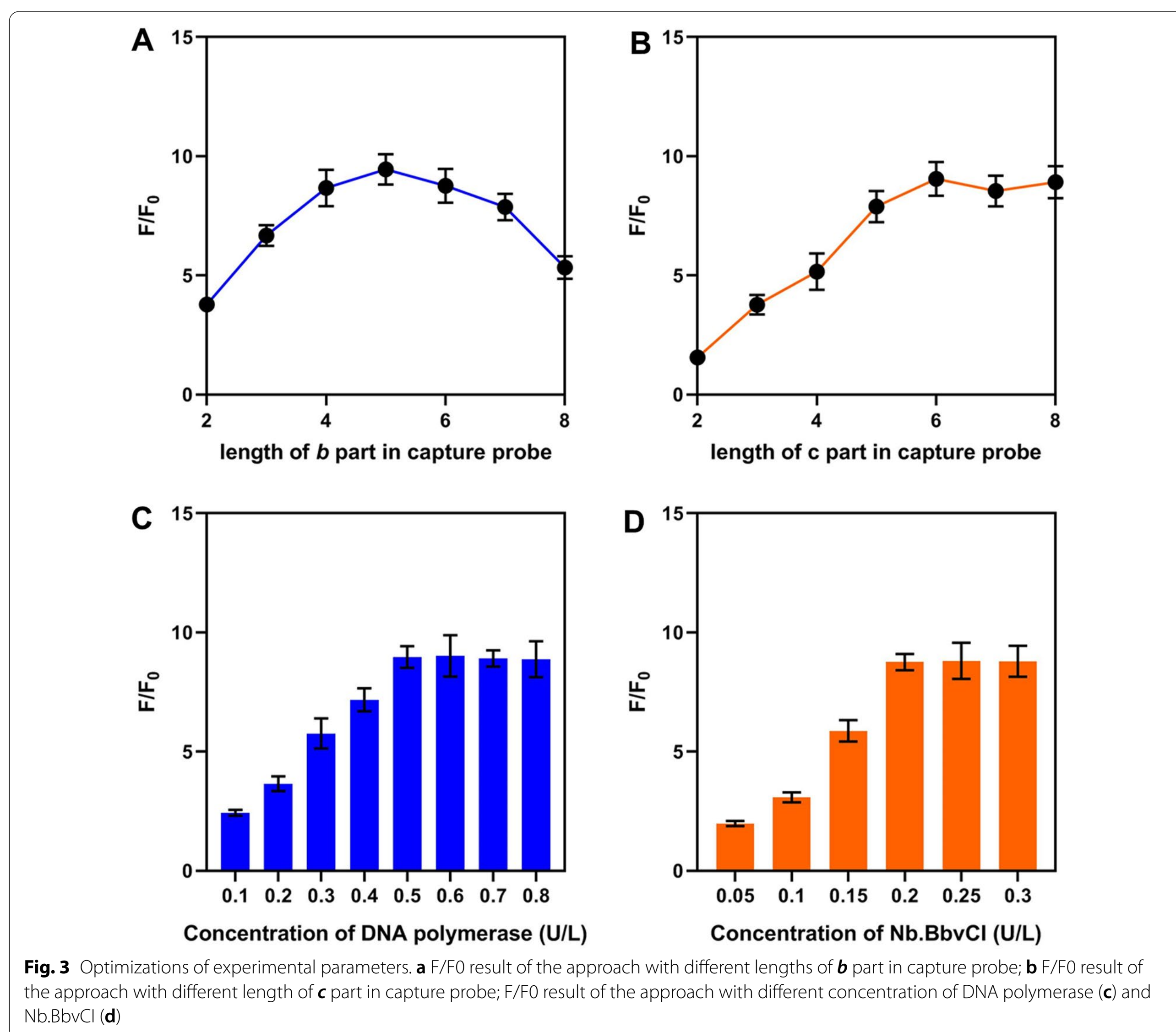


**Fig. 3** Optimizations of experimental parameters. **a** F/F0 result of the approach with different lengths of ***b*** part in capture probe; **b** F/F0 result of the approach with different length of ***c*** part in capture probe; F/F0 result of the approach with different concentration of DNA polymerase (**c**) and Nb.BbvCI (**d**)

more increment could be observed when the length was over 6 bp (Fig. 3b). The chain extension reaction could be affected by the concentration of DNA polymerase. As shown in Fig. 3c, the F/F0 elevated when the concentration of DNA polymerase exceeded 0.5 U/L, which was selected as the optimized concentration and applied for the subsequent experiments. With the constant concentration of DNA polymerase, the concentration of Nb.BbvCI was studied. The result in Fig. 3d showed a maximum signal-to-noise ratio at 0.2 U/L.

#### Sensitivity of the approach

Under the optimized experimental parameters, the sensitivity of the proposed approach was tested by quantifying different concentrations of thrombin. As shown in Fig. 4a, the F/F0 increased proportionally with the amounts of the thrombin. (F/F0 was calculated according to the result in Additional file 1: Fig. S2. F/F0 is the ratio of fluorescence intensity at 520 nm when detecting thrombin and blank sample.) There is a clear relationship between the F/F0 and the logarithmic concentration of thrombin in the scope of 1 pM to 100 nM. The correlation equation was determined $Y = 1.467*lgC + 11.38$ with the correlation coefficient of 0.9935 (Fig. 4b). The limit of detection (LOD) of the established approach was calculated to be 768 fM according to the intensity of the blank tests plus 3 times the standard deviation, which is superior to most of the reported thrombin detection methods (Additional file 1: Table S2). In addition to the high sensitivity, the simple experimental procedures and the label-free producing signal mode endowed the approach a promising applicable scenes.

#### Specificity and repeatability investigation

The specificity of the approach was investigated though detecting interferents, including CEA, PCT, urease, and pepsin at the same concentration (10 nM). From the result in Fig. 5a, the F/F0 results of interferents detection were approximate to the F/F0 of the control group. Nevertheless, a greatly enhanced fluorescence signal was obtained when detecting thrombin, indicating that the proposed approach possesses a high selectivity to the thrombin detection. The repeatability of the proposed approach was evaluated though measuring the 1 nM and 100 pM thrombin for five times, respectively. The relative standard deviations (RSDs) of thrombin detection were both less than 5%, demonstrating an acceptable reproducibility of the approach (Fig. 5b). To determine whether the established approach could be potentially applied in actual sample analysis, serum samples were prepared. Due to the resource limitation, we were not able to collect enough clinical samples, and alternatively, we verified the analytical performance of the method through detecting thrombin in constructed clinical samples. The constructed clinical samples were prepared though adding different concentrations of thrombin in artificial serum samples which were brought from ExCell Bio Co., Ltd. (Shanghai, China). The result in Fig. 5c showed a high correlation between the calculated thrombin concentration and the added concentration ($R^2 = 0.9972$), indicating that the method could also provide an reliable quantitative result even in complicated biological conditions.

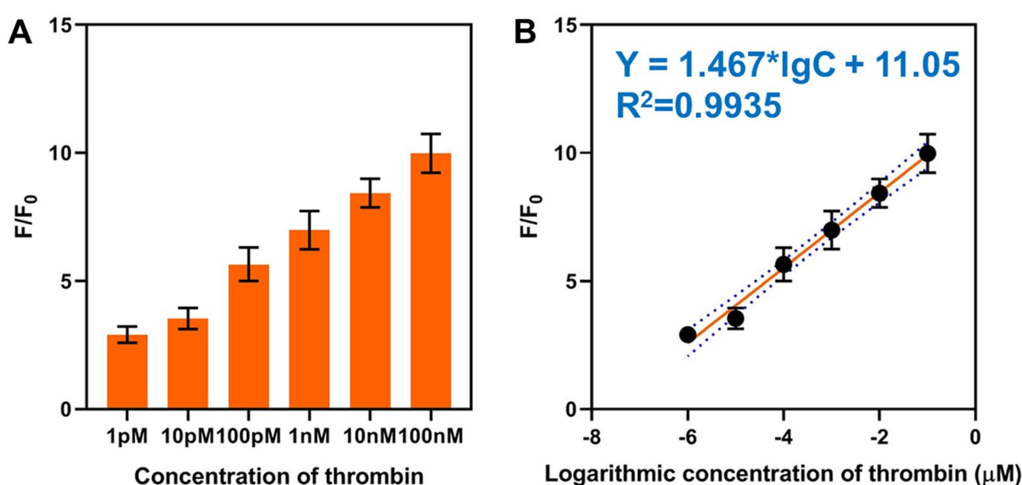


**Fig. 4** Sensitivity of the established approach. **a** F/F0 result of the approach when detecting different concentrations of thrombin; **b** correlation equation between the F/F0 result and the concentration of thrombin

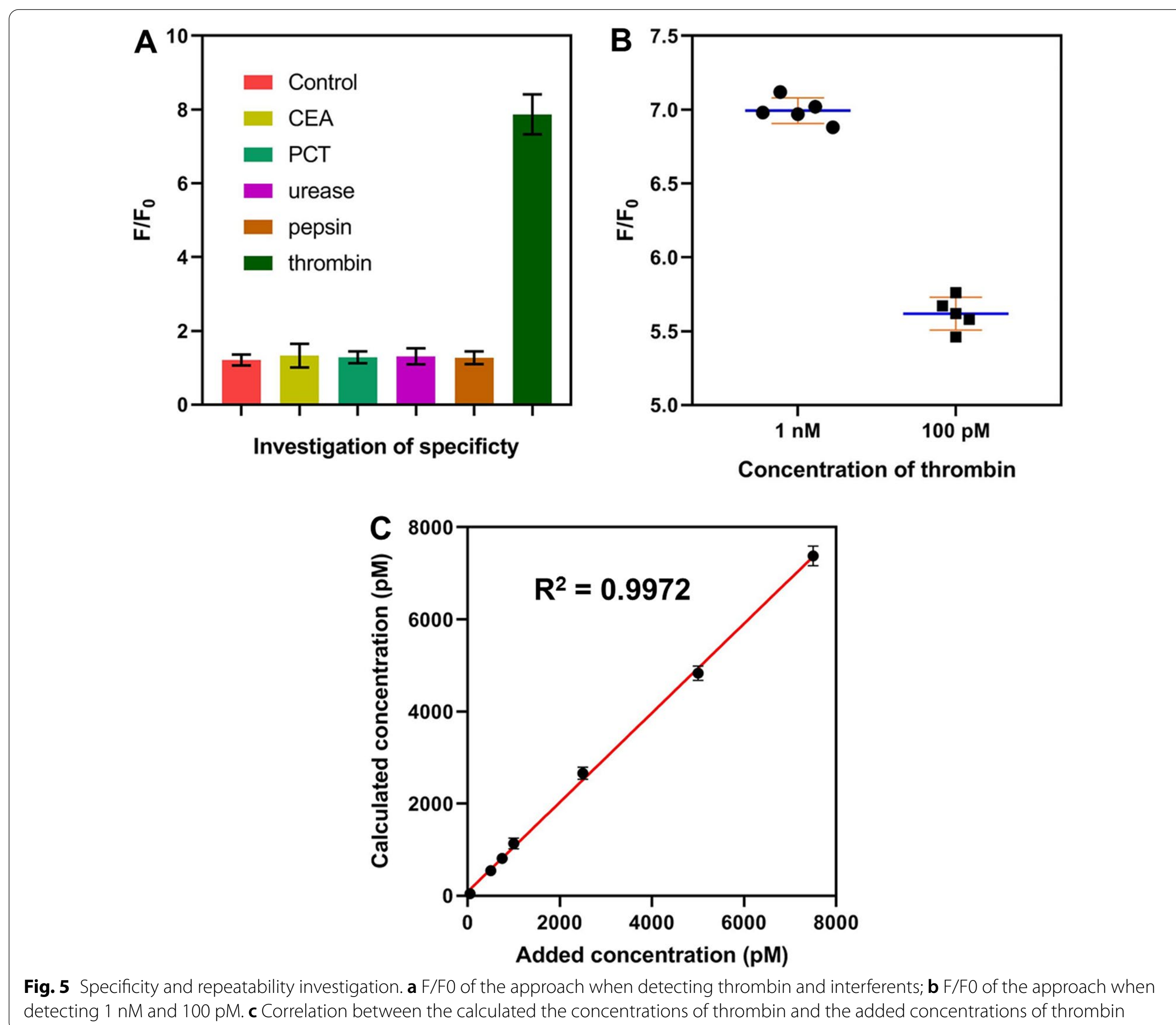


**Fig. 5** Specificity and repeatability investigation. **a** F/F0 of the approach when detecting thrombin and interferents; **b** F/F0 of the approach when detecting 1 nM and 100 pM. **c** Correlation between the calculated the concentrations of thrombin and the added concentrations of thrombin

## Conclusion

The crucial of thrombin in regulating the blood coagulation made it a promising biomarker for the analysis of hypoxic ischemic encephalopathy. In summary, a sensitive and label-free thrombin detection aptasensor has been constructed in this research by exploiting the three-junction initiated dual signal amplification. The proposed thrombin detection possesses the capability to detect thrombin in pM level with a wide detection range from 1 pM to 100 nM. In addition, the approach also exhibits a high selectivity to the thrombin detection and a high repeatability. The excellent detection performance can be attributed to the integration of aptamer-based specific target recognition and dual signal amplification. It is known that the salt composition ($Na^+$ and $K^+$ concentrations) can affect the assembly of G-quadruplexes, and that the salt composition of real biological sample may affect the detection performance of the established approach. From our point of view, the deviation brought by salt composition can be reduced by the calibration curve. Taken together, the capability of the developed approach to sensitive and specific detection of thrombin offers a potential alternative platform for thrombin analysis in disease diagnosis, such as hypoxic ischemic encephalopathy.

## Materials and methods

### Materials and reagents

The DNA oligonucleotides used are listed in Additional file 1: Table S1. The sequences were synthesized

by Sangon Biotechnology Co., Ltd. (Shanghai, China), and diluted to certain concentration by DEPC water. The dNTP mixture solution was also purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). The KF exo$^-$ and Nb.BbvCI were brought from New England Biolabs (Ipswich, MA, USA). The CEA, PCT, urease, and pepsin were supplied by Takara Biotech. Inc. (Dalian, China). All chemical reagents were of analytical grade, and RNase-free water was used throughout this study. Artificial serum samples which were brought from ExCell Bio Co., Ltd. (Shanghai, China).

### Assembly of capture probe and assistance probe

For the assembly of capture probe, the obtained solution containing 10 μM capture probe was heated to 90 °C and cooled to room temperature. The assembled capture probe (10 μL) was mixed with 2 μL thrombin for 20 min to study the target recognition capability. The assembly of assistance follows the same procedures.

### Thrombin detection procedures

#### *Sensitivity*

Typically, different concentrations of thrombin (2 μL) were mixed with 6 μL capture probe (2 μM) in a tube containing 2 μL Tris–HCl (0.5 M) and the mixtures were incubated at 37 °C for 20 min. 8 μL assistance probe (2 μM) was then added in the mixture and incubated for 10 min. 2.5 μL KF exo$^-$ w, 1.5 μL Nb.BbvCI, and 8 μL NEBuffer 3.0 (0.5 M Tris–HCl, 1 M NaCl, 0.1 mM KCl, 0.1 M $MgCl_2$, pH 7.9) were added in the mixture and incubated at 37 °C for 30 min. After the mixture was denatured for 5 min at 95 °C, 5 μM of ThT (5 μL) and 15 μL DEPC water were added in the mixture and incubated at 37 °C for 10 min (total volume: 50 μL). The fluorescence signal was recorded using a Shimadzu RF-5301PC fluorescence spectrometer.

#### *Selectivity*

2 μL thrombin (10 nM), 2 μL CEA (10 nM), 2 μL PCT (10 nM), 2 μL urease (10 nM), and 2 μL pepsin (10 nM) were mixed with 6 μL capture probe (2 μM) in a tube containing 2 μL Tris–HCl (0.5 M), respectively. The mixtures were incubated at 37 °C for 20 min. 8 μL assistance probe (2 μM) was then added in the mixture and incubated for 10 min. 2.5 μL KF exo$^-$ w, 1.5 μL Nb.BbvCI, and 8 μL NEBuffer 3.0 (0.5 M Tris–HCl, 1 M NaCl, 0.1 mM KCl, 0.1 M $MgCl_2$, pH 7.9) were added in the mixture and incubated at 37 °C for 30 min. After the mixture was denatured for 5 min at 95 °C, 5 μM of ThT (5 μL) and 15 μL DEPC water were added in the mixture and incubated at 37 °C for 10 min (total volume: 50 μL). The fluorescence signals of the approach when detecting different targets were recorded using a Shimadzu RF-5301PC fluorescence spectrometer.

#### *Actual sample test*

The artificial clinical samples with different concentrations of thrombin were prepared through adding thrombin in 8 μL artificial serum samples. Afterward, 2 μL clinical sample was mixed with 6 μL capture probe (2 μM) in a tube containing 2 μL Tris–HCl (0.5 M) and the mixtures were incubated at 37 °C for 20 min. 8 μL assistance probe (2 μM) was then added in the mixture and incubated for 10 min. Afterward, 2.5 μL KF exo$^-$ w, 1.5 μL Nb.BbvCI, and 8 μL NEBuffer 3.0 (0.5 M Tris–HCl, 1 M NaCl, 0.1 mM KCl, 0.1 M $MgCl_2$, pH 7.9) were added in the mixture and incubated at 37 °C for 30 min. The mixture was denatured for 5 min at 95 °C. 5 μM of ThT (5 μL) and 15 μL DEPC water were added in the mixture and incubated at 37 °C for 10 min (total volume: 50 μL). The fluorescence signal was recorded using a Shimadzu RF-5301PC fluorescence spectrometer.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-022-00362-1.

**Additional file 1: Table S1.** The details of the oligonucleotides sequences. **Table S2.** A brief comparison of the established approach with former ones. **Fig. S1.** Illustration of the fluorescence assay to study the assembly of capture probe. **Fig. S2.** Fluorescence spectrum of the approach when detecting different concentrations of thrombin.

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#### Author contributions

HY and LL designed this study; HY carried out the experiments; HY, LH, CL, and WL contributed to analytical works; HY wrote the manuscript with assistance from all authors; and LL revised the manuscript and supervised the research. All authors read and approved the final manuscript.

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#### Availability of data and materials

Almost all details of experimental data are presented in the article or additional file.

## Declarations

#### Ethics approval and consent to participate

The manuscript does not contain clinical or trial studies on patients, humans, or animals.

#### Competing interests

The authors declare that they have no competing interests.

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**References**

Al-Amer OM. The role of thrombin in haemostasis. Blood Coagul Fibrinolysis. 2022;33(3):145–8.
Alexander ET, Gilmour SK. Immunomodulatory role of thrombin in cancer progression. Mol Carcinog. 2022;61(6):527–36.
Cao H, Seto SW, Bhuyan DJ, Chan HH, Song W. Effects of thrombin on the neurovascular unit in cerebral ischemia. Cell Mol Neurobiol. 2022;42(4):973–84.
Ericksen WL, Levy JH, Kim ES, Nie L, Senzel LB, Bennett-Guerrero E. Thrombin generation in cardiac versus noncardiac surgical cohorts. Anesth Analg. 2022;134(3):606–14.
Ito K, Inoue KY, Ino K, Shiku H. Highly sensitive electrochemical immunoassay using signal amplification of the coagulation cascade. Anal Chem. 2022;94(36):12427–34.
Lin KC, Jagannath B, Muthukumar S, Prasad S. Sub-picomolar label-free detection of thrombin using electrochemical impedance spectroscopy of aptamer-functionalized MoS2. Analyst. 2017;142(15):2770–80.
Musumeci D, Platella C, Riccardi C, Moccia F, Montesarchio D. Fluorescence sensing using DNA aptamers in cancer research and clinical diagnostics. Cancers (basel). 2017;9(12):174.
Renaud de la Faverie A, Guedin A, Bedrat A, Yatsunyk LA, Mergny JL. Thioflavin T as a fluorescence light-up probe for G4 formation. Nucleic Acids Res. 2014;42(8):e65.
Shen G, Zhang H, Yang C, Yang Q, Tang Y. Thrombin ultrasensitive detection based on chiral supramolecular assembly signal-amplified strategy induced by thrombin-binding aptamer. Anal Chem. 2017;89(1):548–51.
Shlobin NA, Har-Even M, Itsekson-Hayosh Z, Harnof S, Pick CG. Role of thrombin in central nervous system injury and disease. Biomolecules. 2021;11(4):562.
Swetha P, Fan Z, Wang F, Jiang JH. Genetically encoded light-up RNA aptamers and their applications for imaging and biosensing. J Mater Chem B. 2020;8(16):3382–92.
Tripodi A. Thrombin generation assay and its application in the clinical laboratory. Clin Chem. 2016;62(5):699–707.
Tuo QZ, Liu Y, Xiang Z, Yan HF, Zou T, Shu Y, Ding XL, Zou JJ, Xu S, Tang F, Gong YQ, Li XL, Guo YJ, Zheng ZY, Deng AP, Yang ZZ, Li WJ, Zhang ST, Ayton S, Bush AI, Xu H, Dai L, Dong B, Lei P. Thrombin induces ACSL4-dependent ferroptosis during cerebral ischemia/reperfusion. Signal Transduct Target Ther. 2022;7(1):59.
Wang C, Sun Y, Zhao Q. A sensitive thrombin-linked sandwich immunoassay for protein targets using high affinity phosphorodithioate modified aptamer for thrombin labeling. Talanta. 2020;207:120280.
Wang Z, Yang X, Lee NZ, Cao X. Multivalent aptamer approach: designs, strategies, and applications. Micromachines (basel). 2022;13(3):436.
Yeh FY, Liu TY, Tseng IH, Yang CW, Lu LC, Lin CS. Gold nanoparticles conjugates-amplified aptamer immunosensing screen-printed carbon electrode strips for thrombin detection. Biosens Bioelectron. 2014;61:336–43.
Yin C, Jiang D, Xiao D, Zhou C. An enzyme-free and label-free visual sensing strategy for the detection of thrombin using a plasmonic nanoplatform. Analyst. 2020;145(6):2219–25.
Yu N, Wu J. Rapid and reagentless detection of thrombin in clinic samples via microfluidic aptasensors with multiple target-binding sites. Biosens Bioelectron. 2019;146:111726.
Yu H, Zhao Z, Xiao B, Deng M, Wang Z, Li Z, Zhang H, Zhang L, Qian J, Li J. Aptamer-based solution-gated graphene transistors for highly sensitive and real-time detection of thrombin molecules. Anal Chem. 2021;93(40):13673–9.
Zhang J, Huang Y, Sun M, Wan S, Yang C, Song Y. Recent advances in aptamer-based liquid biopsy. ACS Appl Bio Mater. 2022a;5(5):1954–79.
Zhang W, Hu H, Ruan G, Huang Y, Du F, Chen Z. Aptamer functionalized and reduced graphene oxide hybridized porous polymers SPE coupled with LC-MS for adsorption and detection of human alpha-thrombin. Anal Bioanal Chem. 2022b;414(4):1553–61.
Zhao Q, Gao J. Sensitive and selective detection of thrombin by using a cyclic peptide as affinity ligand. Biosens Bioelectron. 2015;63:21–5.
Zhao X, Luo C, Mei Q, Zhang H, Zhang W, Su D, Fu W, Luo Y. Aptamer-cholesterol-mediated proximity ligation assay for accurate identification of exosomes. Anal Chem. 2020a;92(7):5411–8.
Zhao X, Zeng L, Mei Q, Luo Y. Allosteric probe-initiated wash-free method for sensitive extracellular vesicle detection through dual cycle-assisted CRISPR-Cas12a. ACS Sens. 2020b;5(7):2239–46.
Zhao X, Dai X, Zhao S, Cui X, Gong T, Song Z, Meng H, Zhang X, Yu B. Aptamer-based fluorescent sensors for the detection of cancer biomarkers. Spectrochim Acta A Mol Biomol Spectrosc. 2021;247:119038.

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