Author's Accepted Manuscript

www.elsevier.com/locate/talanta

A colorimetric aptamer biosensor based on cationic polythiophene derivative as peroxidase mimetics for the ultrasensitive detection of thrombin

Mei Liu, Jiao Li, Baoxin Li

PII: S0039-9140(17)30719-1
DOI: http://dx.doi.org/10.1016/j.talanta.2017.07.003
Reference: TAL17702

To appear in: *Talanta*

Received date: 14 April 2017
Revised date: 13 June 2017
Accepted date: 1 July 2017

Cite this article as: Mei Liu, Jiao Li and Baoxin Li, A colorimetric aptame biosensor based on cationic polythiophene derivative as peroxidase mimetics fo the ultrasensitive detection of thrombin, *Talanta* http://dx.doi.org/10.1016/j.talanta.2017.07.003

A colorimetric aptamer biosensor based on cationic polythiophene derivative as peroxidase mimetics for the ultrasensitive detection of thrombin

Mei Liu[a1], Jiao Li[a1], Baoxin Li[b2]

[1]Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, College of Food Engineering and Nutritional Science, Shaanxi normal university, Xi'an 710062, China

[2]Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry & Chemical Engineering, Xi'an 710062, China.

liumei@snnu.edu.cn

libaoxin@snnu.edu.cn

Abstract

A colorimetric assay for the ultrasensitive determination of thrombin was presented, in which the cationic polythiophene derivative was used as catalyst of the 3,3′,5,5′-tetramethylbenzidine (TMB)–H_2O_2 reaction and the thrombin-binding aptamer (TBA) was used as inducing polymer's different conformation elements. It was found the cationic polythiophene derivative, poly[3-(3′-N,N,N-triethylamino-1′-propyloxy)-4-methyl-2,5-t-hiophene hydrochloride] (PMNT), can catalyze the oxidation reaction of TMB in the presence of H_2O_2 to produce a blue color solution. The catalytic activity of PMNT on the TMB–H_2O_2 reaction was closely

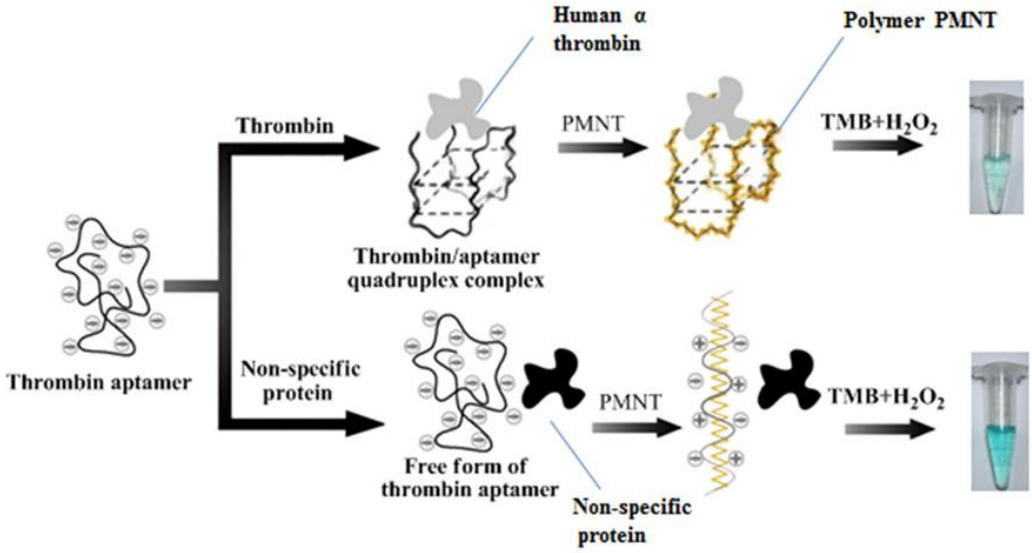
[1] Phone: 86-29-85310517, Fax: 86-29-85310517

[2] Phone: 86-29-81530726, Fax: 86-29-81530727

relevant to the conformation of PMNT. The absorbance of TMB–H_2O_2 was distinctly increased in the presence of TBA. With the addition of thrombin, TBA interacted with thrombin to form a G-quadruplex structure. The conformational change weakened the catalytic activity of PMNT and resulted in a decrease in the absorbance. The colorimetric sensor could detect thrombin down to 4 pM with high selectivity against other interfering proteins. This work is not only of importance for a better understanding of the unique properties of cationic polythiophenes derivative but also have great potential for medical diagnostics and therapy for human health.

Graphical abstracts

Aptamer biosensor for thrombin detection based on cationic polythiophene derivative with different conformation as peroxidase mimetics.



Keywords: Cationic polythiophene derivative, Colorimetry, Thrombin, Aptamer

1. Introduction

Thrombin is a physiological protease that plays an essential role in physiological and pathological coagulation. Meanwhile, it is also one of the biomarkers for cardiovascular disease and a crucial tumor marker for the diagnosis of pulmonary metastasis [1–3]. The normal concentration of thrombin in blood varies from nanomolar to low micromolar levels during the coagulation progress [3]. If the concentration of thrombin in blood was out of this range, it would lead to thrombotic diseases. Therefore, the concentration of thrombin can be applied as a significant index for the early diagnosis and prognosis of some kinds of diseases [4]. A lot of efforts have been done to develop sensitive sensors to detect thrombin. Aptamer-based sensing for thrombin is one of mostly used

strategies because of the excellent specificity of aptamers toward thrombin, such as electrochemistry [6–9], optics [10–16], electrochemiluminescence [17–20] and so on. Compared with those sophisticated methods, colorimetric sensors have attracted special attention due to its short assay time, relatively low cost and no requirement for skillful technicians [21]. However, there are a few reports on colorimetric aptasensor for thrombin detection where the sensitivity and responding time still need to be improved [22–25].

Conjugated polymers (CPs) are characterized by a delocalized electronic structure that exhibits efficient coupling between optoelectronic segments. They draw a lot attention because of their unique structure and excellent electronic properties [26–28]. Amongst the conjugated polymers, polythiophene derivative is the most important for its excellent optoelectronic properties such as advantageous fluorescence property, forster resonance energy transfer, and a tuneable electrical conductivity[29,30]. They are competent to be one of the most useful chemical platforms for the design of biosensors for detection of biological targets [12,31,32]. But for sensor applications where polythiophene acts as a fluorescence probe, some defects would lower the fluorescence quantum yields of polythiophene. For example, the highly hydrophobic backbone leads to close proximity of the optically active units and then aggregate in water, resulting in intractable self-quenching of fluorescence.

It is disadvantageous for improving sensitivity of biosensor due to the defects [33]. Another important property of polythiophenes is showing variational color upon the changes of conformation attributed to the analyte-induced aggregation of the biological macromolecules [34–36]. However, these direct aggregation approach suffer from relatively higher detection limits. Our groups have reported CPs could efficiently catalyze the chemiluminesence reaction of luminol–H_2O_2 and it was found that the catalytic activity of CPs was closely related with the polymer's subsistent conformation, so the detection system based on CPs' different catalytic activity can be extended to detect non-nucleic acid targets (such as small molecules or protein) [37].

In this work, we found poly[3-(3′-N,N,N-triethylamino-1′-propyloxy) -4-methyl-2,5-thiophene hydrochloride] (PMNT), one of the cationic polythiophene derivative, has intrinsic peroxidase-like activity that can catalyze the reaction of peroxidase substrate TMB in the presence of hydrogen peroxide (H_2O_2) to produce a blue color reaction. And this effect is closely related to the conformation of PMNT. The TBA can induce polymer PMNT to be different conformation with or without the presence of thrombin. The absorbance of TMB–H_2O_2 system in the presence of TBA/PMNT was much larger than that in the presence of thrombin/TBA/PMNT. Base on this phenomenon, a selective and sensitive colorimetric determination of thrombin using cationic

polythiophene derivative as peroxidase mimetics is proposed. A schematic diagram of this method is shown in Scheme 1. The obtained results indicated that the proposed aptasensor has quite a good performance for the detection of thrombin and provides a new promising platform for determination of other biomolecules.

2. Experimental

2.1. materials

PMNT was purchased from Changzhou XHK Biochemicals Co., Ltd. (Changzhou, China), and the polymer's concentration was given using its monomer. The thrombin-binding aptamer (TBA) oligonucleotides (5'-GGT TGG TGT GGT TGG-3') were purchased from Beijing Dingguo Biotechnology Company (Beijing, China). 3,3,5,5-tetramethylbenzidine (TMB), thiourea and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Thrombin (1710 Umg^{-1}) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich（St. Louis, MO. USA). 30% H_2O_2 was purchased from Xi'an Reagent Plant (Xi'an, China). Millipore Milli-Q water (18 MΩ cm) was used in all experiments. Unless otherwise indicated, all reagents and solvents were purchased in their highest available purity and used without further purification.

2.2. Apparatus

UV-vis adsorption spectra were recorded on a U-3900H UV-Vis

Spectrophotometer (Hitachi, Japan). Circular dichroism (CD) spectra were measured on a Chirascan CD spectrophotometer (Applied Photophysics, Leatherhead, UK). The electron spin resonance (ESR) measurements were made with a Bruker ESP-300E spectrometer (Bruker, Germany) operating in the X-band at room temperature. The microwave frequency was 9.83 GHz and the modulation amplitude was 2.035 G. The photographs were taken with a Canon 500 digital camera. The pH measurements were carried out on model PB-10 digital ion analyzer (Sartorius Scientific instruments Co., Ltd., China, Beijing).

2.3. Preparation of TBA/PMNT and thrombin/TBA/PMNT complex

TBA/PMNT and thrombin/TBA/PMNT complex was performed as follows: (1) the stock solution of TBA was heated at 80 °C for 10 min and cooled to room temperature. (2) 100 μL of 0.1 μM TBA solution and 100 μL of thrombin with different concentrations in 20 mM Tris–HCl buffer (100 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) were incubated at room temperature for 2h to form TBA/thrombin complex (G-quadruplex complex). (3) 100 μL of 0.1 μM PMNT were respectively added into 0.1 μM TBA solution and TBA/thrombin complex with different concentrations. And the mixed solution was incubated at room temperature for 10 min to successively form TBA/PMNT and thrombin/TBA/PMNT complex.

2.4. Detection of thrombin

Due to the difference of catalytic activities of PMNT with different configuration to TMB–H_2O_2 color reaction, TBA/PMNT complex and thrombin/TBA/PMNT complex was used in the detection system. The detection was performed as follows: firstly, 50 μL of above TBA/PMNT complex and thrombin/TBA/PMNT complex were added respectively into the mixed solution of 100 μL of 5.0 mM TMB and 100 μL of 10 mM H_2O_2. Secondly, 250 μL of 0.25 M HAc-NaAc buffer (pH 3.5) was added into the above reaction solutions, then water bath for 15 min. Finally, the resulting solution monitored absorbance using UV–vis spectra at 652 nm to calculate the change in the value of ΔA ($\Delta A=A_0-A$, A or A_0, respectively, is the absorbance with or without thrombin in system).

Results and Discussion

3.1. The catalytic activities of PMNT on TMB–H_2O_2 reaction

To investigate the peroxidase-like activity of PMNT, the catalytic oxidation of peroxidase substrate TMB in the presence of H_2O_2 was investigated. As shown in Fig.1, blue color reaction produced by oxidation of TMB by H_2O_2 can be noticeable observed with the presence of PMNT, and the color of the product was intense blue in the presence of TBA/PMNT (inset in Fig. 1). UV-Vis absorption spectra were employed to evaluate the peroxidase activity of PMNT. The resulting solutions show a maximum absorbance at 652 nm, which originates from the

oxidation of TMB. Compared with the weak absorption spectrum of the mixture of TMB and H_2O_2 solution (a), obvious absorptions were observed in the presence of PMNT, TMB and H_2O_2 (b), which indicated PMNT could catalyze oxidation of TMB by H_2O_2 to develop a blue color in aqueous solution. The much stronger absorbance can be found in the presence of the mixture of TBA/PMNT, TMB and H_2O_2 solution (c). It indicated that the reaction of TMB with H_2O_2 was greatly accelerated by TBA/PMNT. All of the results confirmed that PMNT exhibited an intrinsic peroxidase-like activity, which was similar to that of horseradish peroxidase. Furthermore, the catalytic activity of TBA/PMNT complex is stronger than PMNT on TMB–H_2O_2 reaction. The reason may be relevant to the conformation change of PMNT before and after the combination of TBA.

3.2. Mechanism of enhancement effect of PMNT on TMB–H_2O_2 CL

Based on our previous report that polythiophene derivative, poly[2-(3-thienyl) ethyloxy-4-butylsulfonate] (PTEBS), could catalyze the reaction of TMB and H_2O_2, a possible mechanism was proposed [38]. The generated ·OH radical by H_2O_2 might be accelerated by PTEBS via the partial electron exchange interaction, which may contribute to the catalytic ability of PTEBS. In this system, the mechanism is also applicable to the cationic polythiophene derivative PMNT. The

absorption spectra of PMNT before and after the oxidation reaction with H_2O_2 were measured. There was no obvious change on the absorption spectra of PMNT (Fig. S1), which indicated that PMNT itself did not change during the oxidation reaction with H_2O_2.

At the same time, the nature of peroxidase-like activities of PMNT may originate from their catalytic ability to decompose H_2O_2 into ·OH radicals, being confirmed with the ESR spin-trapping technique (Fig. S2). The ESR spectra in the presence of PMNT displayed a characteristic peak of the typical DMPO–·OH adduct. It suggested that ·OH radicals have produced in PMNT–H_2O_2 system. Furthermore, a scavenger of ·OH (thiourea) was added to TMB–H_2O_2–PMNT reaction system. It was shown in Fig. S3 that relative catalytic activities of PMNT were reduced 64.7%. These results further confirmed that ·OH was the key intermediate product in the system.

3.3. Effects of PMNT's conformation on the catalyst activity of PMNT

It has been proven that the catalytic properties of PMNT in the luminol–H_2O_2 system can be influenced by its conformation [37]. So, we speculated that the catalytic activity of PMNT in TMB–H_2O_2 was closely related to PMNT's conformation. The PMNT itself takes a random-coil conformation in water solution, and it can form an inter polyelectrolyte complex with TBA through electrostatic interactions, in which PMNT

takes a highly conjugated and planar conformation. In the presence of thrombin, TBA folds into a compact quadruplex conformation, and the cationic PMNT wraps this quadruplex structure, which seems to partially hinder the aggregation and planarization. Three different conformations can be characterized by circular dichroism (CD) spectroscopy (see the supporting information, Figure S4). The PMNT random-coil form did not reveal any optical activity in the CD spectra, PMNT/TBA duplex form showed a positive peak at about 570 nm. There is appreciable change in their CD patterns in the presence of thrombin. The positive peak at 565 nm disappeared, but a positive peak at 265nm and a negative peak at 295 nm appeared, which is typical of an antiparallel quadruplex conformation. Thus, it was confirmed that the presence of TBA and thrombin can induce the change of PMNT's conformation.

We compared with the catalytic activities of PMNT with three different conformations in TMB–H_2O_2. As shown in Figure 2, PMNT/TBA duplex form showed the strongest catalytic activity, and thrombin/TBA/PMNT complex form had almost the same as the catalytic activity of PMNT in its random coil. Therefore, it was concluded that the catalytic activity of PMNT in TMB–H_2O_2 was closely related to PMNT's conformation. The highly conjugated and planar conformation is propitious to the catalytic activity of PMNT, possibly due to electron

transfer.

3.4. Optimization of experimental conditions

It is well known that enzymatic activity is dependent upon the reaction conditions. Therefore, the catalytic activity of the PMNT may be affected by pH and temperature. As shown in Fig. S5, the difference of absorbance intensity ΔA ($\Delta A = A_{(signal)} - A_{(blank)}$) at 652 nm increased with pH in the range of 3.0-3.5, and then declined with increasing. The absorbance intensity increased with temperature range from 25 to 35 °C, and then declined. Hence, the catalytic activity was best in pH 3.5 HAc-NaAc buffer at 35 °C.

At the same time, we tested the effect of different heating time and the concentration of TMB, H_2O_2. The ΔA is no obvious change after 15 min heating time (Fig S5.), the optional concentration of TMB, H_2O_2 was 5 mM and 20 mM respectively.

3.5. Application of TMB–H_2O_2–PMNT system to thrombin detection

TBA is a single stranded guanine-rich nucleic acid and is widely used as a sensing element for constructing thrombin aptasensor [39]. This aptamer demonstrates a loose random coil structure in the absence thrombin. Upon addition of thrombin, TBA folds into a compact quadruplex. On the other hand, the cationic polymer PMNT was found to

have high catalytic activity to the TMB–H_2O_2 reaction, and the catalytic activity was closely related with the conformation of PMNT. The conformation of PMNT in the presence of TBA is different form that in the presence of thrombin. So, the TMB–H_2O_2–PMNT system could be used to detection of thrombin. A schematic diagram of this method is shown in Scheme 1. In the absence of thrombin, TBA could bind PMNT through electrostatic interaction to form PMNT/TBA complex with duplex conformation, and PMNT/TBA duplex form promoted TMB–H_2O_2 reaction to intense blue color. With the addition of thrombin, PMNT wraps this quadruplex structure to form thrombin/TBA/PMNT complex which decreases TMB–H_2O_2 reaction in intensity. Fig. 3A showed the absorption spectra of TMB–H_2O_2 mixed solution in the presence of different concentrations of thrombin. As is shown in Fig. 3B, the change in the value of ΔA at 652 nm increased gradually as the concentrations of thrombin varied from 0.01 nm to 0.10 nm. The relative standard deviation (R.S.D.) for 1.0×10^{-10} M thrombin measurement is 3.6% (n=11). The detection limit that is taken to be three times the standard derivation in the blank solution was found to be 4 pM. In this study, Table S1 summarized the reported colorimetric aptamer biosensors for the determination of thrombin along with the proposed one. As can be seen, the sensitivity and respond time of our method was superior to that of most reported methods [22–25].

The specificity of the proposed assay for thrombin was evaluated by testing the response of the assay to other common proteins (bovine serum albumin, human IgG and lysozyme) at a concentration of 0.1 nM. The experimental results (Fig. 4) showed that significant change in absorbance value is only observed for thrombin and not for other proteins, indicating that the TBA–PMNT complex specifically responds to thrombin. The results reveal that this colorimetric method for thrombin detection is sensitive and effective.

Conclusions

To sum up, we have found that cationic polythiophene derivative PMNT possesses catalytic activity to TMB–H_2O_2 reaction which was closely related with it's subsistent conformation. Combined with the conformation change of PMNT toward TBA and thrombin/TBA, we introduced a novel method for the colorimetric detection of thrombin. Significantly, the proposed colorimetric method requires no any complicated probe labeling step and provides a potential capability of practical application in clinical chemistry and medical diagnostics.

Acknowledgements

This work was supported by the National Natural Science

Foundation of China (No. 21405101, 21475083).

References

[1] M.L. Nierodzik, S. Karpatkin, Thrombin induces tumor growth, metastasis, and angiogenesis: Evidence for a thrombin-regulated dormant tumor phenotype, Cancer Cell. 10 (2006) 355–362.

[2] B. Sinha, T.S. Ramulu, K.W. Kim, R. Venu, J.J. Lee, C.G. Kim, Planar Hall magnetoresistive aptasensor for thrombin detection, Biosens. Bioelectron. 59 (2014) 140–144.

[3] J. Yoon, N. Choi, J. Ko, K. Kim, S. Lee, J. Choo, Highly sensitive detection of thrombin using SERS-based magnetic aptasensors, Biosens. Bioelectron. 47 (2013) 62–67.

[4] M.A. Shuman, P.W. Majerus, The measurement of thrombin in clotting blood by radioimmunoassay, J. Clin. Invest. 58 (1976) 1249–1258.

[5] G.S. Bang, S. Cho, B.G. Kim, A novel electrochemical detection method for aptamer biosensors, Biosens. Bioelectron. 21 (2005) 863–870.

[6] P. He, L. Shen, Y. Cao, D. Li, Ultrasensitive electrochemical detection of proteins by amplification of aptamer-nanoparticle bio bar codes, Anal. Chem. 79 (2007) 8024–8029.

[7] Y. Xiao, A.A. Lubin, A.J. Heeger, K.W. Plaxco, Label-free electronic detection of thrombin in blood serum by using an aptamer-based sensor, Angew. Chemie -Int. Ed. 44 (2005) 5456–5459.

[8] J. Zhao, G. Chen, L. Zhu, G. Li, Graphene quantum dots-based platform for the

fabrication of electrochemical biosensors, Electrochem. Commun. 13 (2011) 31–33.

[9] Y. Wang, Y. Zhang, T. Yan, D. Fan, B. Du, H. Ma, Q. Wei, Ultrasensitive electrochemical aptasensor for the detection of thrombin based on dual signal amplification strategy of Au@GS and DNA-CoPd NPs conjugates, Biosens. Bioelectron. 80 (2016) 640–646.

[10] J.T. Cao, J.J. Zhang, Y. Gong, X.J. Ruan, Y.M. Liu, Y.H. Chen, S.W. Ren, A competitive photoelectrochemical aptasensor for thrombin detection based on the use of TiO_2 electrode and glucose oxidase label, J. Electroanal. Chem. 759 (2015) 46–50.

[11] F. Le Floch, H.A. Ho, M. Leclerc, Label-free electrochemical detection of protein based on a ferrocene-bearing cationic polythiophene and aptamer, Anal. Chem. 78 (2006) 4727–4731.

[12] H.A. Ho, M. Leclerc, Optical Sensors Based on Hybrid Aptamer/Conjugated Polymer Complexes, J. Am. Chem. Soc. 126 (2004) 1384–1387.

[13] Z. Chen, Y. Tan, C. Zhang, L. Yin, H. Ma, N. Ye, H. Qiang, Y. Lin, A colorimetric aptamer biosensor based on cationic polymer and gold nanoparticles for the ultrasensitive detection of thrombin, Biosens. Bioelectron. 56 (2014) 46–50.

[14] H. Chang, L. Tang, Y. Wang, J. Jiang, J. Li, Graphene fluorescence resonance energy transfer aptasensor for the thrombin detection, Anal. Chem. 82 (2010) 2341–2346.

[15] Y. Zhang, W. Ren, H.Q. Luo, N.B. Li, Label-free cascade amplification

strategy for sensitive visual detection of thrombin based on target-triggered hybridization chain reaction-mediated in situ generation of DNAzymes and Pt nanochains, Biosens. Bioelectron. 80 (2016) 463–470.

[16] S. Yan, R. Huang, Y. Zhou, M. Zhang, M. Deng, X. Wang, X. Weng, X. Zhou, Aptamer-based turn-on fluorescent four-branched quaternary ammonium pyrazine probe for selective thrombin detection, Chem. Commun. 47 (2011) 1273–1275.

[17] J. Wang, Y. Shan, W.W. Zhao, J.J. Xu, H.Y. Chen, Gold nanoparticle enhanced electrochemiluminescence of CdS thin films for ultrasensitive thrombin detection, Anal. Chem. 83 (2011) 4004–4011.

[18] H. Huang, J.J. Zhu, DNA aptamer-based QDs electrochemiluminescence biosensor for the detection of thrombin, Biosens. Bioelectron. 25 (2009) 927–930.

[19] L. Fang, Z. Lü, H. Wei, E. Wang, A electrochemiluminescence aptasensor for detection of thrombin incorporating the capture aptamer labeled with gold nanoparticles immobilized onto the thio-silanized ITO electrode, Anal. Chim. Acta. 628 (2008) 80–86.

[20] F. Li, H. Cui, A label-free electrochemiluminescence aptasensor for thrombin based on novel assembly strategy of oligonucleotide and luminol functionalized gold nanoparticles, Biosens. Bioelectron. 39 (2013) 261–267.

[21] M. Su Han, D.H. Kim, Naked-Eye Detection of Phosphate Ions in Water at Physiological pH: A Remarkably Selective and Easy-To-Assemble

Colorimetric Phosphate-Sensing Probe, Angewandte. Chemie. 41 (2002) 3963–3965.

[22] Z. Zhang, Z. Wang, X. Wang, Magnetic nanoparticle-linked colorimetric aptasensor for the detection of thrombin, Sensors & Actuators B Chemical. 147(2010) 428–433.

[23] X.Y. Rao, J.J. Zhang, C. Jing, Au Nanoparticle-DNAzyme Dual Catalyst System for Sensitively Colorimetric Detection of Thrombin, Chemical Research in Chinese Universities. 29(2013) 868–873.

[24] Z. Chen, L. Tan, L. Hu, Real Colorimetric Thrombin Aptasensor by Masking Surfaces of Catalytically Active Gold Nanoparticles, ACS Applied Materials & Interfaces. 8(2016) 102–108.

[25] Y. Zhao, X. Liu, L. Jie, Microfluidic chip-based silver nanoparticles aptasensor for colorimetric detection of thrombin, Talanta. 150(2016) 81–87.

[26] W.J. Feast, J. Tsibouklis, K.L. Pouwer, Synthesis, processing and material properties of conjugated polymers, Polymer, 37 (1996) 5017–5047.

[27] R.D. McCullough, The Chemistry of Conducting Polythiophenes, Adv. Mater. 10 (1998) 93–116.

[28] A. Grill, Electrical and Optical Properties of, Thin Solid Films. 355 (1999) 189–193.

[29] D. Tyler McQuade, A.E. Pullen, T.M. Swager, Conjugated polymer-based chemical sensors, Chem. Rev. 100 (2000) 2537–2574.

[30] P.S. Heeger, a J. Heeger, Making sense of polymer-based biosensors., Proc.

Natl. Acad. Sci. U. S. A. 96 (1999) 12219–12221.

[31] K.P.R. Nilsson, O. Inganäs, Chip and solution detection of DNA hybridization using a luminescent zwitterionic polythiophene derivative., Nat. Mater. 2 (2003) 419–424.

[32] H.-A. Ho, M. Boissinot, M.G. Bergeron, G. Corbeil, K. Doré, D. Boudreau, M. Leclerc, Colorimetric and fluorometric detection of nucleic acids using cationic polythiophene derivatives., Angew. Chem. Int. Ed. 41 (2002) 1548–1551.

[33] T.-Q. Nguyen, V. Doan, B.J. Schwartz, Conjugated polymer aggregates in solution: Control of interchain interactions, J. Chem. Phys. 110 (1999) 4068.

[34] H.A. Ho, A. Najari, M. Leclerc, Optical detection of DNA and proteins moth cationic polythiophenes, Acc. Chem. Res. 41 (2008) 168–178.

[35] Z. Yao, C. Li, G. Shi, Optically active supramolecular complexes of water-soluble achiral polythiophenes and folic acid: Spectroscopic studies and sensing applications, Langmuir. 24 (2008) 12829–12835.

[36] Z. Yao, H. Bai, C. Li, G. Shi, Analyte-induced aggregation of conjugated polyelectrolytes: role of the charged moieties and its sensing application., Chem. Commun. (Camb). 46 (2010) 5094–5096.

[37] M. Liu, B. Li, X. Cui, Conjugated polyelectrolytes-initiated chemiluminescence: A biosensing platform for label-free and homogeneous DNA detection, Biosens. Bioelectron. 47 (2013) 26–31.

[38] M. Liu, B. Li, X. Cui, Anionic polythiophene derivative as peroxidase mimetics and their application for detection of hydrogen peroxide and glucose, Talanta.

115 (2013) 837–841.

[39] T.Li, E.Wang, S.Dong, G-quadruplex-based DNAzyme for facile colorimetric detection of thrombin, Chem. Commun., (2008) 3654–3656.

Scheme 1. Schematic representation of colorimetric determination of

human α-thrombin based on the reaction of TMB–H_2O_2 catalyzed by conjugated polythiophene derivative with different conformation.

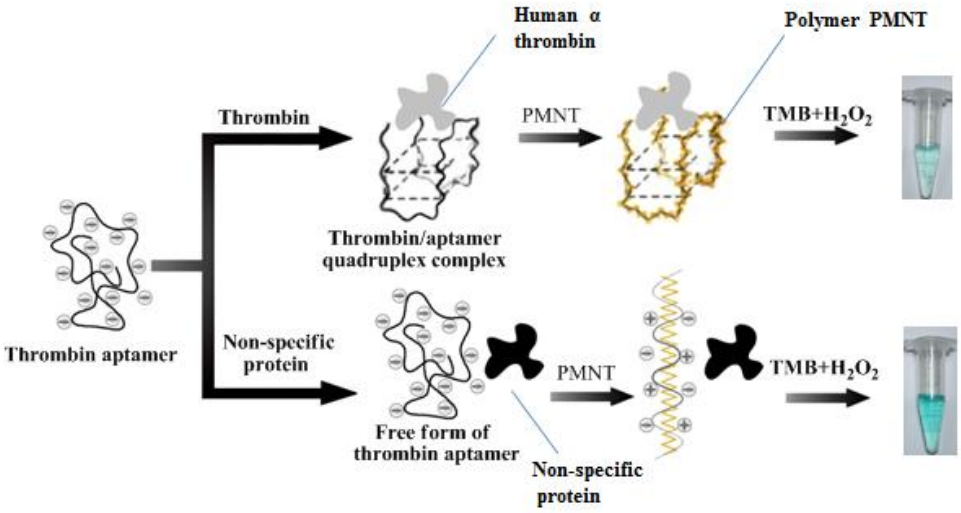
Figure 1 The absorption spectra of oxidation color reaction of TMB by H_2O_2 under the different assays. (a) without PMNT, (b) in the presence of PMNT, (c) in the presence of TBA/PMNT. Experimental conditions: 5 mM TMB, 20 mM H_2O_2, 250 mM HAc-NaAc buffer (pH 3.5), 0.1 μM PMNT, 0.1 μM TBA.

Figure 2 The absorption spectra of TMB–H_2O_2 mixed solution in the presence of (a) PMNT alone, (b) complex of TBA/PMNT, (c) complex of thrombin/TBA/PMNT. Experimental conditions: 5 mM TMB, 20 mM H_2O_2, 250 mM HAc-NaAc buffer (pH 3.5), 0.1 μM TBA, 0.1 nM thrombin.

Figure 3 (A)The UV–vis absorption spectra of TMB–H_2O_2 in the presence of 5 mM TMB, 20 mM H_2O_2, 0.1 μM PMNT and 0.1 μM TBA in 250 mM HAc–NaAc buffer (pH 3.5) containing different concentrations of Thrombin (0.01 nM–1 nM). (B) The linear calibration plot between the absorbance at 652 nm on the thrombin concentration. The experiment was done three times.

Figure 4 Specificity of aptasensor for thrombin. The absorbance changes

of A_{652} for other competing proteins (BSA, IgG and lysozyme) were measured at 0.1 nM human thrombin; 1.0 nM BSA; 1.0 nM lg G; 1.0 nM lysozyme.



Scheme 1

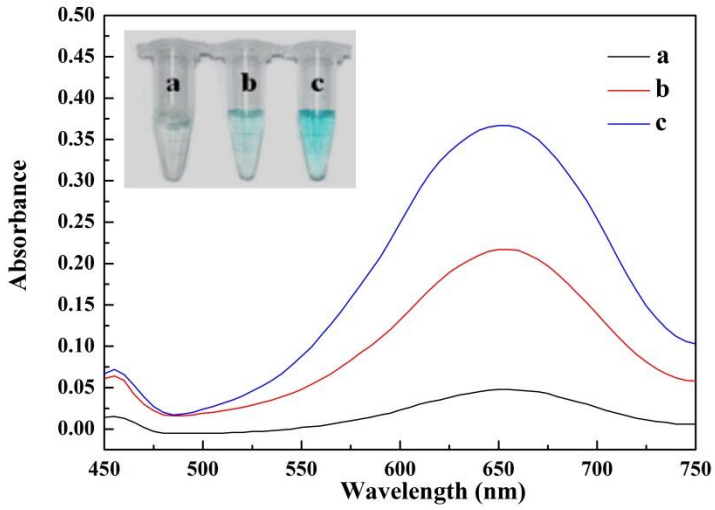


Figure 1

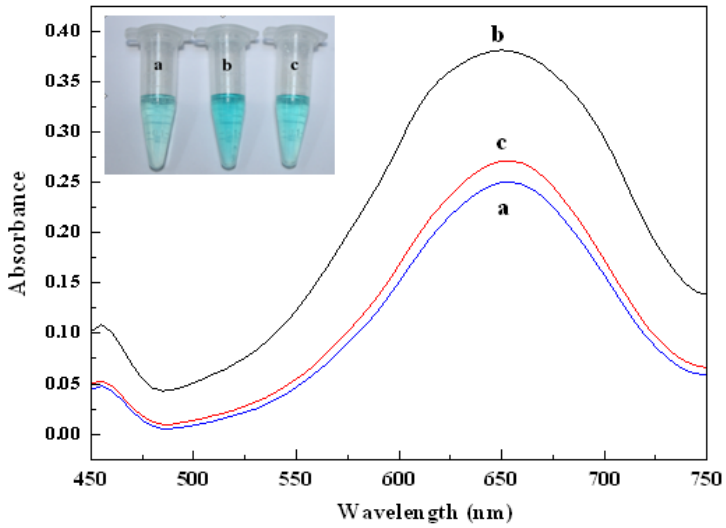


Figure 2

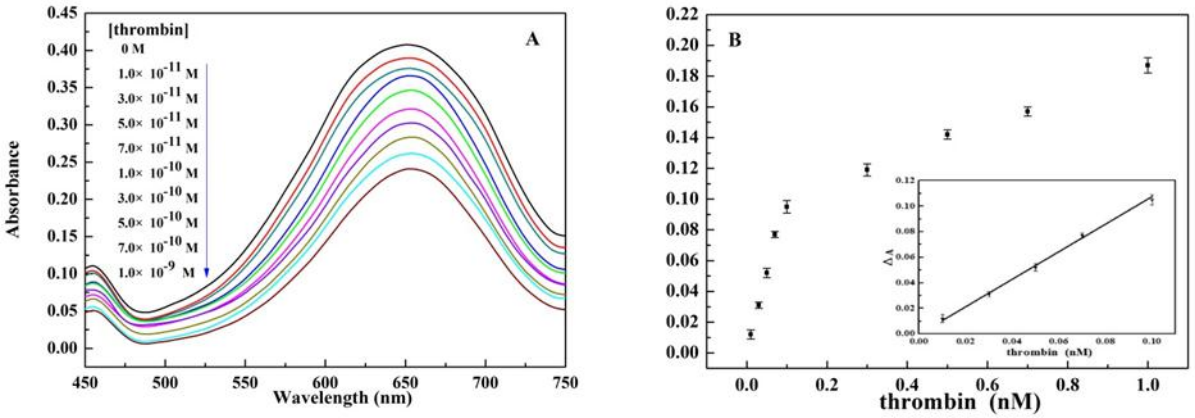


Figure 3

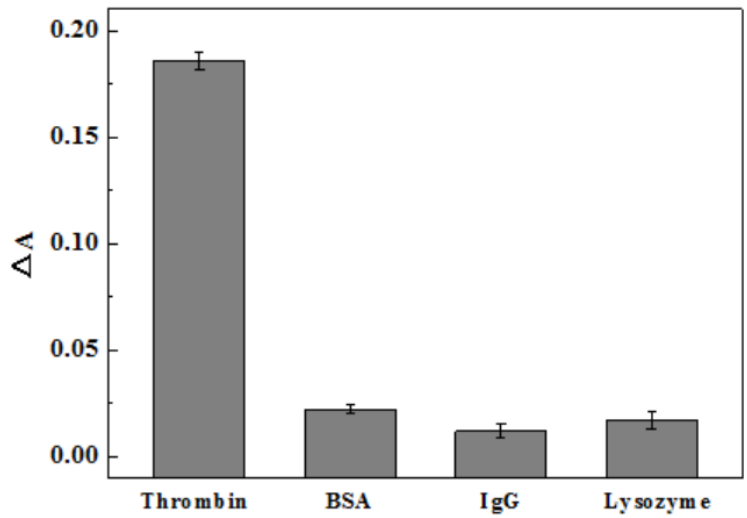


Figure 4

Highlights

- A colorimetric assay for the ultrasensitive determination of thrombin was proposed.
- The catalytic ability of PMNT derived from decomposing H_2O_2 into ·OH radicals.
- The catalytic activity of PMNT was closely relevant to the conformation of PMNT.