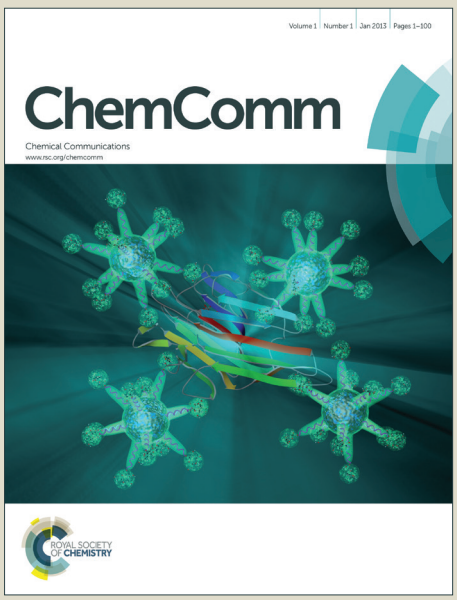
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Chemical Communications

ChemComm

Dynamic Article Links ▶

Cite this: DOI: 10.1039/coxx00000x

ARTICLE TYPE

www.rsc.org/xxxxxx

Target-induced structure switching of DNA for label-free and ultrasensitive electrochemiluminescent detection of protein

Mengli Yang, Ying Chen, Yun Xiang,* Ruo Yuan and Yaqin Chai

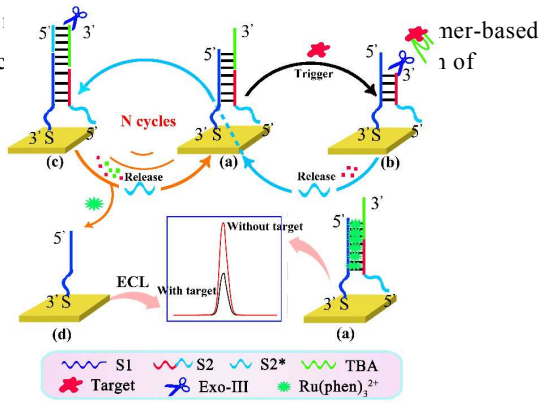
Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

The association of the target thrombin with the corresponding aptamer leads to structure swithcing of the dsDNA probes and the formation of nicking sites for exonuclease III, which causes cyclic cleavage of the dsDNA probes and highly reduced intercalation of the electrochemiluminescent signal indicators for label-free and sensitive detection of thrombin at femtomolar level.

Quantitative detection of specific proteins plays an increasingly important role in the biomedical field, especially in disease diagnosis.[1,2] Various assay protocols aiming at protein determination have been developed, among which the most widely used methods are immunoassays based on antigen-antibody interactions. Despite the fact that many advances have been made in immunoassays for protein detection, these methods usually encounter the problem of complicated procedures for antibody screening and production. Therefore, the development of new alternatives for protein identification and determination is highly demanded. Aptamers, synthetic oligonucleotides (DNA or RNA) selected from random-sequence nucleic acid libraries by an *in vitro* evolution process called systematic evolution of ligands by exponential enrichment (SELEX),[3] have shown overwhelming advantages over antibodies as recognition probes for protein detection, owing to their high affinity and specificity, target versatility, easy synthesis and regeneration. In the past decades, various aptamer-based protein sensing strategies based on different signal transduction means such as fluorescence,[4] colorimetry,[5] surface plasmon resonance (SPR),[6] electrochemistry[7] and electrochemiluminescence (ECL)[8,9] have been developed. Among these aptamer-based protein sensing methods, the ECL-based approach has attracted special attention due to the simplicity, good reproducibility, low background and high sensitivity of the ECL technique.

In general, protein biomarkers are presented in ultralow concentrations in biological samples from patients in the early stage of disease progression. To realize ultrasensitive detection of these trace amounts of proteins, effective signal amplifications are commonly required. In recent years, the polymerase chain reaction (PCR)-like amplification strategies, including rolling circle amplification (RCA)[10] and ligase chain reaction,[11] aptamer-based sensing sc[illegible] of



Scheme 1 Principle of target-induced structure switching of DNA for sensitive and label-free ECL detection of thrombin based on Exo III-assisted recycling amplification.

proteins. Although high sensitivity can be obtained by these PCR-like amplification strategies, these sensing protocols are limited due to the drawbacks of complex handling operation, easy contamination and high cost. While, other signal amplification approaches based on biobarcode,[12] nanomaterial composite[13] and enzyme[14] labels for aptamer-based protein detections require extensive label preparation and conjugation processes. Very recently, a new class of signal amplification strategies based on nuclease (exonuclease or endonuclease)-assisted target recycling has been reported.[15,16] The nuclease with sequence specific activity can degrade the probe strands of the probe/target dsDNA strands to release the target DNA strands to achieve target recycling amplification. This signal amplification strategy has been demonstrated to be useful in detecting trace levels of DNA targets.[17] Unlike endonuclease which requires restricted, specific sequences for enzyme recognition, exonuclease has received increasing interest in target recycling-amplified DNA detection. For example, exonuclease III (Exo III), which catalyzes the stepwise removal of mononucleotides from 3'-hydroxyl termini of dsDNA strands with blunt or recessed 3'-termini, have been successfully employed for amplified detection of DNA,[18] small molecules[19] and metal ions.[20] Despite the wide

application of the nuclease-assisted recycling amplification in sensitive DNA detection, the extension of this technique for protein detection remains a major challenge because nuclease only shows activity on DNA strands.

To explore the integration of nuclease-assisted recycling amplification in sensitive protein detection based on aptamer probes, we report herein a new target-induced DNA structure switching strategy for amplified and label-free electrochemiluminescent detection of thrombin. The association of the thrombin target with the corresponding aptamers of the dsDNA strands leads to the formation of nicking sites for Exo III to trigger cyclic degradation of the dsDNA strands. The digestion of the dsDNA strands further reduces the intercalation of $Ru(phen)_3^{2+}$ into the dsDNA grooves,[21] which subsequently generates significantly minimized ECL emission for amplified thrombin detection down to the femtomolar level in a label-free format.

Our Exo III-assisted, target-induced structure switching of DNA recycling amplification strategy for sensitive and label-free ECL detection of thrombin is illustrated in Scheme 1. The dsDNA strands of S1/S2 are first self-assembled on the gold electrode (AuE) surface through the formation of Au-S bonds. Subsequent partial hybridization of the surface-immobilized S1 with thrombin binding aptamer (TBA) leads to the generation of new dsDNA strands (S1/S2-TBA) and the sensing surface for thrombin. The S1/S2-TBA dsDNA strands have 3'-protruding termini, which is resistant to Exo III digestion in the absence of thrombin due to the fact that Exo III only cleaves dsDNA strands with blunt or 3'-recessing termini. The ECL indicator, $Ru(phen)_3^{2+}$, can effectively intercalate into the grooves of the dsDNA strands and generate strong ECL emission. However, when the target thrombin is introduced into the sensing system, it associates with TBA to form TBA/thrombin complexes and releases TBA from the sensor surface. The release of TBA switches the surface-immobilized dsDNA with 3'-protruding termini to those with 3'-recessing termini, producing nicking sites for Exo III. Exo III then digests part of S2 complementary to S1 (the red part of S2) and releases the secondary target sequence (S2*, the cyan part of S2), which is complementary to part of TBA. S2* hybridizes with the TBA on the sensor surface to further switch the surface-immobilized dsDNA with 3'-protruding termini to those with 3'-blunt termini, which leads to the degradation of TBA and part of S2 by Exo III. This enzymatic digestion results in the release and generation of more S2*. S2* again hybridizes with the TBA on the sensor surface to initiate the cyclic digestion of the dsDNA strands, which significantly reduces the intercalation of $Ru(phen)_3^{2+}$ and generates inhibited ECL emission signal for thrombin detection. Due to Exo III-assisted cyclic cleavage of the dsDNA strands, the ECL emission inhibition effect is substantially amplified and highly sensitive detection of thrombin is thus expected.

To demonstrate the feasibility of the proposed sensing strategy, we first investigated the effect of Exo III on the dsDNA assemblies on the sensor surface in the absence of the target thrombin. For this purpose, the sensor were incubated with/without the presence of Exo III followed by further incubation with 20 mM $Ru(phen)_3^{2+}$. From Fig. 1A, we can see that the addition of Exo III has negligible effect on the ECL response (curve b vs a), owing to the fact that the dsDNA strands with 3'-protruding termini are resistant to Exo III digestion. The mechanism of the ECL emission of $Ru(phen)_3^{2+}$ with TPrA as the coreactant is proposed as follows:[22,23]

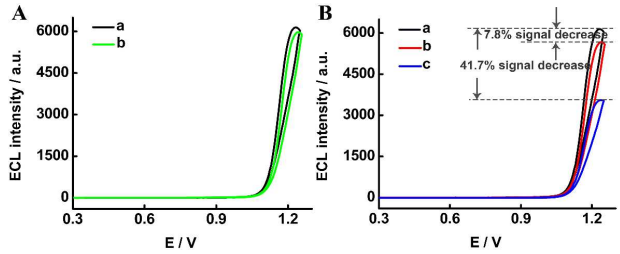


Fig. 1 (A) ECL responses of the sensor without (a) and with (b) the addition of Exo III (10 U) in the absence of thrombin, followed by incubation with 20 mM $Ru(phen)_3^{2+}$. (B) ECL responses of the sensors incubated with (a) Exo III (10 U), (b) thrombin (20 pM) and (c) thrombin (20 pM) and Exo III (10 U) at 37 °C for 60 min. ECL measurements were recorded in 0.1 M PB (pH 7.5) containing 20 mM TPrA. Scan rate: 50 $mV\ s^{-1}$.

$TPrAH^+$ (DNA) → $TPrA^{\bullet+}$ + H^+ (DNA) + e^-

$TPrA^{\bullet+}$ → $(C_3H_7)_2N(CHC_2H_5^{\bullet})$ + H^+

$(Ru(phen)_3^{2+}$-DNA) - e^- → $(Ru(phen)_3^{3+}$-DNA)

$(Ru(phen)_3^{3+}$-DNA) + $(C_3H_7)_2N(CHC_2H_5^{\bullet})$ → $(Ru(phen)_3^{2+*}$-DNA) + $[(C_3H_7)_2N{=}CHC_2H_5]^+$

$(Ru(phen)_3^{2+}$-DNA) + $(C_3H_7)_2N(CHC_2H_5^{\bullet})$ → $(Ru(phen)_3^{+}$-DNA) + products

$(Ru(phen)_3^{+}$-DNA) + $TPrA^{\bullet+}$ → TPrA + $(Ru(phen)_3^{2+*}$-DNA)

$(Ru(phen)_3^{2+*}$-DNA) → $(Ru(phen)_3^{2+}$-DNA) + $h\upsilon$

At basic condition (pH 7.5), TPrA exists in the form of $TPrAH^+$, which can be easily absorbed on the anionic phosphate backbones of the dsDNA stands via electrostatic interactions. Subsequent proton transfer from $TPrAH^+$ to the phosphate ions of the DNA backbones oxidizes TPrA at 0.89 V and generates active radicals of $TPrA^{\bullet}$. The $TPrA^{\bullet}$ radicals further react with the oxidized $Ru(phen)_3^{3+}$ and generate the excited state of $Ru(phen)_3^{2+*}$. At the low potential, $TPrA^{\bullet}$ can reduces $Ru(phen)_3^{2+}$ to $Ru(phen)_3^{+}$ which reacts with $TPrA^{\bullet+}$ to produce excited-state $Ru(phen)_3^{2+*}$. Strong ECL emission occurs when the excited state of $Ru(phen)_3^{2+*}$ returns to the ground state.

To verify the signal amplification capability of our Exo III-assisted recycling amplification strategy, the ECL intensities of the sensors incubated with/without Exo III in the presence of thrombin (20 pM) were further compared. As depicted in Fig. 1B, the presence of thrombin (without Exo III) leads to a slight decrease (7.8%) in ECL intensity compared with the blank test (with the absence of thrombin and the presence of Exo III). Since the presence of Exo III has negligible effect on the ECL intensity of the sensor with the absence of thrombin (curve b vs a in Fig. 1A), such decrease is basically due to the release of TBA from the sensing surface upon association with thrombin, which causes reduction of the intercalation of $Ru(phen)_3^{2+}$ into the grooves of the dsDNA strands. Moreover, when the sensor is incubated with the mixture of thrombin (20 pM) and Exo III (10 U), significant decrease (41.7%) in ECL intensity is observed (curve c vs a in Fig. 1B). This comparison clearly demonstrates that the addition of Exo III to the sensing system can amplify the inhibition of the

ECL emission in the presence of thrombin due to the cyclic cleavage of the dsDNA strands on the sensor surface.

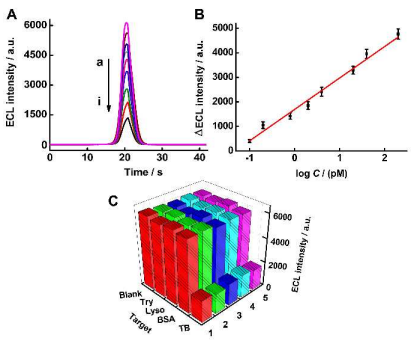


Fig. 2 (A) Typical ECL curves of the sensor for the detection of different concentrations of thrombin: (a) 0, (b) 100 fM, (c) 200 fM, (d) 800 fM, (e) 2 pM, (f) 4 pM, (g) 20 pM, (h) 40 pM, (i) 200 pM. (B) The corresponding calibration plot of the logarithm of thrombin concentration vs ΔECL intensity. Error bars, SD, n=3. (C) Selectivity investigation of the proposed sensing method for thrombin (100 pM) against other interference molecules of BSA (1 nM), Lyso (1 nM), and Try (1 nM). The cleavage time of Exo III was 90 minutes and the amount of Exo III was 8 U. ECL conditions, as in Fig. 1.

Under the optimized conditions, the dependence of the ECL response upon the concentration of thrombin was examined. As shown in Fig. 2A, when the concentration of thrombin increases from 0.1 pM to 200 pM, increasing inhibition of ECL emission is observed and the intensity of the ECL peak decreases accordingly (Fig. 2A, curve b to i). It is found that the ECL intensity difference (ΔECL) between the presence and absence of thrombin is proportional to the logarithmic concentration of thrombin in the range from 0.1 pM to 200 pM (Fig. 2B), and an estimated detection limit of 45 fM is obtained (based on the 3σ rule). Such detection limit for thrombin is comparable or more sensitive than those methods based on the employment of complicated labels for signal amplifications.[24,25] In addition, the reproducibility of the assay protocol was investigated and the ECL responses of six different sensors for 100 pM thrombin gave a relative standard deviation of 9.3%, indicating the good reproducibility of the sensors.

Selectivity investigation of the sensor was performed by carrying out control experiments between thrombin and other interference molecules, including bovine serum albumin (BSA), lysozyme (Lyso), and trypsin (Try). Fig. 2C shows that the presence of 10-fold (1 nM) excess of the interference molecules leads to almost negligible ECL changes compared to the blank test, while the presence of low levels of thrombin (100 pM) exhibits an significant ECL signal decrease. These results demonstrate that our sensing method is highly selective toward thrombin against other interference molecules, due to the highly specific binding capability of TBA to thrombin.

In summary, we have developed a label-free and highly sensitive ECL method for thrombin detection based on target-induced switching of DNA for recycling signal amplification. The presence of the thrombin target switches the structure of the dsDNA probes on the sensor surface and generates nicking sites for Exo III to proceed with cyclic cleavage of the dsDNA strands, which highly reduces the intercalation of the ECL indicators and significantly suppresses the ECL emission. This Exo III-assisted, amplified inhibition of ECL emission leads to highly sensitive detection of thrombin down to 45 fM. Besides, the proposed method is also highly selective toward thrombin against other interference molecules. Moreover, by coupling different aptamer probe sequences, the developed method can be easily adopted to detect various types of target molecules (e.g., small molecules, inorganic ions and cells).

This work was supported by NSFC (Nos. 21275004, 20905062, 21075100 and 21275119), the New Century Excellent Talent Program of MOE (NCET-12-0932).

Notes and references

Key Laboratory on Luminescence and Real-Time Analysis, Ministry of Education, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715 , P. R. China; Fax: +86-23-68252277; Tel: +86-23-68253172; E-mail: yunatswu@swu.edu.cn (Y.X.)

† Electronic Supplementary Information (ESI) available: Experimental details. See DOI: 10.1039/b000000x/

1 J. M. Liu, Y. Li, Y. Jiang and X. P. Yan, *Proteome Res.*, 2010, **9**, 3545.
2 Y. W. Zhang and X. P. Sun, *Chem. Commun.*, 2011, **47**, 3927.
3 C. Tuerk and L. Gold, *Science*, 1990, **249**, 505.
4 R. Nutiu and Y. F. Li, *Angew. Chem., Int. Ed.*, 2005, **44**, 1061.
5 C. C. Huang, Y. F. Huang, Z. H. Cao, W. H. Tan and H. T. Chang, *Anal. Chem.*, 2005, **77**, 5735.
6 V. Ostatna, H. Vaisocherova, J. Homola and T. Hianik, *Anal. Bioanal. Chem.*, 2008, **391**, 1861.
7 I. Willner and M. Zayats, *Angew. Chem., Int. Ed.*, 2007, **46**, 6408.
8 H. Y. Wang, W. Gong, Z. A. Tan, X. X. Yin, L. Wang, *Electrochim. Acta*, 2012, **76**,416.
9 L. Z. Hu, Z. Bian, H. J. Li, S. Han, Y. L. Yuan, L. X. Gao and G. B. Xu, *Anal. Chem.*, 2009, **81**, 9807.
10 Z. S. Wu, S. B. Zhang, H. Zhou, G. L. Shen and R. Q. Yu, *Anal. Chem.*, 2010, **82**, 2221.
11 P. Tong, W. W. Zhao, L. Zhang, J. J. Xu and H. Y. Chen, *Biosens. Bioelectron.*, 2012, **33**, 146.
12 X. R. Zhang, B. P. Qi, Y. Li, S. S. Zhang, *Biosens. Bioelectron.*, 2009, **25**, 259.
13 B. R. Baker, R. Y. Lai, M. S. Wood, E. H. Doctor, A. J. Heeger and K. W. Plaxco, *J. Am. Chem. Soc.*, 2006, **128**, 3138.
14 J. Zhao, Y. Y. Zhang, H. T. Li, Y. Q. W, X. Y. F, F. B. L, L. Tan and S. Z. Yao, *Biosens. Bioelectron.*, 2011, **26**, 2297.
15 C. Q. Zhao, L. Wu, J. S. Ren and X. G. Qu, *Chem. Commun.*, 2011, **47**, 5461.
16 Q. Fan, J. Zhao, H. Li, L. Zhu and G. X. Li, *Biosens. Bioelectron.*, 2012, **33**, 211.
17 D. Wu, B. C. Yin and B. C. Ye, *Biosens. Bioelectron.*, 2011, **28**, 232.
18 Y. Chen, B. Y. Jiang, Y. Xiang, Y. Q. Chai and R. Yuan, *Chem. Commun.*, 2011, **47**, 12798.
19 Y. Y. Xu, J. Xu, Y. Xiang, R. Yuan and Y. Q. Chai, *Biosens. Bioelectron.*, 2014, **51**, 293.
20 F. Xuan, X. T. Luo and I. M. Hsing, *Anal. Chem.*, 2013, **85**, 4586.
21 Y. Chen, J. Xu, J. Su, Y. Xiang, R. Yuan and Y. Q. Chai, *Anal. Chem.*, 2012, **84**, 7750.
22 W. J. Miao, J. P. Choi and A. J. Bard, *J. Am. Chem. Soc.*, 2002, **124**, 14478.
23 X. B. Yin, Y. Y. Xin and Y. Zhao, *Anal. Chem.*, 2009, **81**, 9299.
24 H. P. Huang and J. J. Zhu, *Biosens. Bioelectron.*, 2009, **25**, 927.
25 H. Yang, J. Ji, Y. Liu, J. L. Kong and B. H. Liu, *Electrochem. Commun.*, 2009, **11**, 38.