Highly sensitive chemiluminescence technology for protein detection using aptamer-based rolling circle amplification platform

Zhi-Juan Cao, Qian-Wen Peng, Xue Qiu, Cai-Yun Liu*, Jian-Zhong Lu

School of Pharmacy, Fudan University, Shanghai 201203, China

Received 26 May 2011; accepted 9 June 2011
Available online 22 July 2011

Abstract A robust, selective and highly sensitive chemiluminescent (CL) platform for protein assay was presented in this paper. This novel CL approach utilized rolling circle amplification (RCA) as a signal enhancement technique and the 96-well plate as the immobilization and separation carrier. Typically, the antibody immobilized on the surface of 96-well plate was sandwiched with the protein target and the aptamer–primer sequence. This aptamer–primer sequence was then employed as the primer of RCA. Based on this design, a number of the biotinylated probes and streptavidin–horseradish peroxidase (SA–HRP) were captured on the plate, and the CL signal was amplified. In summary, our results demonstrated a robust biosensor with a detection limit of 10 fM that is easy to be established and utilized, and devoid of light source. Therefore, this new technique will broaden the perspective for future development of DNA-based biosensors for the detection of other protein biomarkers related to clinical diseases, by taking advantages of high sensitivity and selectivity.

© 2011 Xi'an Jiaotong University. Production and hosting by Elsevier B.V. All rights reserved.

1. Introduction

Detection of protein biomarkers in clinical samples is of great importance for effective diagnosis and prognosis of many diseases [1]. In general, antibody-based immunoassay systems are versatile and powerful tools for various molecular analyses [2]. Enzyme-linked immunosorbent assay (ELISA) is considered as a standard method to detect protein biomarkers [3]. However, the concentrations of many important biological markers in clinical examples for cancer, infectious diseases, or biochemical processes are too low to be detected using conventional immunoassays [4]. Therefore, several innovative approaches have been developed to improve the sensitivity by combining antibody-based molecular recognition with various
amplification detection techniques, such as metal ion deposition [5], enzyme amplification [6], bio-barcode detection [7,8], proximity ligation [9,10], metal nanoparticles [11] and quantum dots [12].

Among them, molecular probes based on nucleic acid platforms are emerging. One of the representative examples is the use of aptamers for protein analysis [13–16]. Aptamers are short synthetic nucleotide sequences (DNA or RNA) that have been selected in in vitro selection experiments via the systematic evolution of ligands by exponential enrichment (SELEX) process [17]. As a novel recognition element, aptamers possess significant advantages including simple synthesis, easy labeling, good stability and design flexibility [18,19]. In addition, aptamer-based assay is easy to combine with other amplification methods, such as gold nanoparticles, polymerase chain reaction (PCR) and rolling circle amplification (RCA) [20–28]. For example, Csordas et al. [22] reported a micromagnetic aptamer PCR (MAP) detection system, which integrated high-gradient magnetic field sample preparation in a microfluidic device with aptamer-based real-time PCR readout, to achieve highly sensitive and quantitative detection of protein targets directly from complex samples.

RCA has also been proven to enhance signals for detecting a variety of analytes without thermal cycling. Additionally this RCA process is of linear kinetic amplification model and the RCA product was a single stranded DNA sequence consisting of tandem repeats of the complement of the circular template.

RCA has also been proven to enhance signals for detecting a variety of analytes without thermal cycling. Additionally this RCA process is of linear kinetic amplification model and the RCA product was a single stranded DNA sequence consisting of tandem repeats of the complement of the circular template.

Therefore, the RCA amplification strategy has been used to receive. The DNA-BIND 96-well plate (Costar, 6573) had a micromagnetic aptamer PCR (MAP) detection system, which integrated high-gradient magnetic field sample preparation in a microfluidic device with aptamer-based real-time PCR readout, to achieve highly sensitive and quantitative detection of protein targets directly from complex samples.

RCA has also been proven to enhance signals for detecting a variety of analytes without thermal cycling. Additionally this RCA process is of linear kinetic amplification model and the RCA product was a single stranded DNA sequence consisting of tandem repeats of the complement of the circular template.

Chemiluminescence (CL) is considered one of the most suited optical detection techniques for developing miniaturized and highly sensitive analytical devices [29]. Even though the quantum efficiency of CL reactions is usually low (in the order of 0.01 or less), the use of enzyme labels such as horseradish peroxidase (HRP) or alkaline phosphatase (ALP), ensures signal amplification and high sensitivity [30,31]. Because of the wide dynamic range of the CL measurements (up to 6 orders of magnitude), the target analyte can be detected in a broad concentration range, from femtomolar to millimolar levels, without the need of sample dilution. Finally, the absence of an excitation light source as in fluorescence measurements makes CL detection less sensitive to interferences attributed to sample components (the only background signal derives from the instrumental noise) and allows a wide range of applications in different fields such as environmental chemistries [32], molecular biology [33,34], pathogenic bacteria [35], clinical diagnosis [36] and cell sensors [37], including several research works in our group [38–41].

Herein, we present an example of the aptamer-based RCA assay by coupling of CL detection for the ultrasensitive protein assay. Platelet-derived growth factor B-chain (PDGF-BB), an important protein for cell transformation and tumor growth and progression, was selected as the model protein.

## 2. Materials and methods

### 2.1. Materials and reagents

All chemicals were of analytical grade and were used as received. The DNA-BIND 96-well plate (Costar, 6573) had an N-oxysuccinimide surface and was obtained from Corning Inc. (NY, USA). The PDGF-BB proteins and goat anti-human PDGF-BB antibody were purchased from R&D Systems (Minneapolis, MN, USA). The PDGF proteins were reconstituted in 4 mM HCl with 0.2% BSA prior to use. Phi 29 reagent set was purchased from Epicenter (Madison, WI, USA). *Escherichia coli* DNA ligase was obtained from Takara Biotechnology Co., Ltd. (Dalian, China). Streptavidin–horseradish peroxidase (SA–HRP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Bovine serum albumin (BSA) was obtained from Sino-American Biotechnology Co. and other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). HRP substrate kits were purchased from Millipore Corporation (Billerica, MA, USA). Oligonucleotides were obtained from Invitrogen Biotechnology Co., Ltd. (Shanghai, China), including the following sequences (Table 1).

### 2.2. Buffers

All of the solutions were prepared with water from a Millipore system (Millipore XQ, MA, USA). Binding buffer (BB) was 0.5 M Na2HPO4–NaH2PO4, pH 8.5. PBS consisted of 2.7 mM KCl, 1 mM MgCl2, pH 7.4. PBST was PBS containing 0.05% Tween 20. Blocking buffer was PBS containing 5% BSA. Ligation buffer (LB) consisted of 30 mM Tris–HCl, pH 8.0, 4 mM MgCl2, 10 mM (NH4)2SO4, 1.2 mM EDTA and 0.1 mM b-NAD. RCA reaction buffer was 40 mM Tris–HCl (pH 7.5), 50 mM KCl, 10 mM MgCl2, 5 mM (NH4)2SO4 and 4 mM DTT.

### 2.3. Apparatus

CL measurement was carried out using a PC-controlled Fluoroskan Ascent FL (Thermo Electron Corporation).

### 2.4. Sandwich assay with RCA or without RCA

In a typical experiment, goat anti-human PDGF-BB antibody was diluted to 0.75 μg/mL in BB and added into the wells in a 96-well plate (100 μL each well). The plate was incubated for 1 h with gentle mixing at 37 °C. Excess PDGF-BB antibody was removed by decanting the supernatant. The plate was washed and blocked with blocking buffer (150 μL per sample) for 60 min at 37 °C. After decantation of blocking buffer, series dilutions of PDGF-BB were prepared in 100 μL PBS.

| Name                  | Sequence                                      |
|-----------------------|-----------------------------------------------|
| Aptamer-primer        | 5'-TAC TCA GGG CAC TGC AAG CAA TGT TGG TCC CAA TGG GCT GAG TAT TTT TTT TGT CGG TCG TAG AAG GAA ACA GTT AC-3' |
| Padlock probe         | 5'-phosphate-TAG CAC GGA CAT ATA TGA TGG ACC GCA GTA TGA GTA TCT CTC ACT ACT ACT AAG TGG AAG AAG AAA TGT AAC TGT TTC CCT C-3' |
| Biotinylated probe    | 5'-biotin-GTT TTC TTT TAG CAC-3'             |

Table 1 DNA sequences used in this work.
and added into the above wells and incubated for another 40 min with gentle mixing at 37 °C. After washing, 100 μL of 7.5 pmol aptamer-primer complexes in PBS and 5 pmol padlock were added into the wells, which was incubated for 60 min at 37 °C. And then the padlock probe was circularized via ligation by 1 U of E. coli DNA ligase in LB (100 μL each well) for 60 min at 37 °C to form the circular template for RCA. The complex was incubated with 100 μL of 40 U phi29 DNA polymerase and 100 μM dNTPs in RCA reaction buffer for 75 min at 37 °C. After a rinsing step, 100 μL of 7.5 pmol biotinylated probe (at the 5’ end) was applied to and hybridized with the resulted RCA products, which then captured the SA–HRP (1:7500 dilution, 100 μL per well). Finally, the plate was washed three times with 150 μL of PBST. The CL signals on the surface of each conjugates were detected directly with 100 μL of commercial CL HRP substrate.

In the assay without RCA, after the binding of PDGF-BB onto the surface of wells, 7.5 pmol aptamer–primer complexes were added to react with the immobilized PDGF-BB. After the plate was washed three times with PBST (150 μL each time), 7.5 pmol of biotinylated probe in 100 μL PBS and 1:7500 dilution of SA–HRP were added and captured on the surface of the wells sequentially (each for 30 min at 37 °C). Finally, the plate was washed three times with 150 μL of PBST. The CL signals were also detected as described above.

3. Results and discussion

3.1. Design of the aptamer-based assay

A “sandwich-type” detection strategy was employed with HRP as the detection tag in our design (Fig. 1), which involved the sequential immobilization of capture antibody, PDGF-BB and aptamer–primer onto the 96-well plate surface in a classic sandwich assay format. The aptamer–primer had two functional units. The aptamer part was used to recognize and bind PDGF-BB, and the primer part at the 3’-terminus was employed as a universal primer sequence to initiate a linear RCA reaction in the presence of Phi29 polymerase and dNTPs. Both the 3’- and 5’-terminus of a padlock probe could hybridize to the 3’-terminus of the aptamer–primer. Ligation of the padlock probe resulted in a circular template for RCA, which then produced single-stranded tandem repeated copies of the circular template. The tandem repeated copies in the RCA product were complementary to the biotinylated probe. Thus, the RCA product could hybridize a number of biotinylated probes, which then captured SA–HRP. After the CL substrates were added, the CL signal was recorded immediately with the CL measurement. In contrast, for the assay without RCA, the biotinylated probes directly hybridized the aptamer–primer, which had been immobilized on the surface of the 96-well plate as the formation of antibody–antigen–aptamer complex, and then SA–HRP reacted with the captured biotinylated probes. Finally, the CL signal was also obtained by the reaction with CL substrates and recorded by the CL measurement. All experiments were performed at least triplicate.

3.2. Optimization of reaction parameters

Several parameters were optimized systematically for the highly sensitive approach of protein assay, including the amounts of capture antibody, aptamer–primer, padlock, ligase, polymerase, dNTPs, biotinylated probe and SA–HRP.

![Figure 1](https://via.placeholder.com/150)

![Figure 1](https://via.placeholder.com/150)

![Figure 1](https://via.placeholder.com/150)

![Figure 1](https://via.placeholder.com/150)

![Figure 1](https://via.placeholder.com/150)

**Figure 1** Schematic representation of aptameric system for the detection of PDGF-BB with or without RCA.

![Figure 2](https://via.placeholder.com/150)

**Figure 2** CL intensity vs. the capture antibody amount. Experimental conditions: 5.0 pmol of aptamer–primer, 2.1 pmol of padlock, 6 U of ligase, 25 U of polymerase, 100 μM of dNTPs, 2.5 pmol of biotinylated probe, 1:5000 dilution of SA–HRP and 10 μM PDGF-BB. The detection procedure was carried out as described in the Experimental Section 2.4.
3.2.1. Effect of the capture antibody amount

The amount of capture antibody on the wells played an important role in the performance of the immunoassay. Several amounts of anti-PDGF-BB antibody were investigated as shown in Fig. 2. Elevated CL intensity was observed with increasing amount of the capture antibody, and peaked at 75 ng of capture antibody. Further increase in the amount of capture antibody to 100 ng resulted in a decrease of the CL intensity. This was attributed to steric and electrostatic hindrances, arising from more tightly packed capture antibody on the surface of wells, limiting access to the surface-bound capture antibody by PDGF-BB antigen. Thus, 75 ng capture antibody was selected for subsequent experiments.

3.2.2. Effects of the amounts of the aptamer–primer and padlock

The effects of aptamer–primer and padlock amounts were subsequently examined and optimized. CL intensity increased in the range of 0.1–7.5 pmol of aptamer–primer and then decreased slightly (Fig. 3A). CL intensity increased with increasing amount of padlock sequences over the range of 0.1–5.0 pmol, and then decreased (Fig. 3B). Thus, 7.5 pmol of aptamer–primer and 5.0 pmol of padlock sequences were selected in further studies.

Figure 3 CL intensity vs. the amounts of aptamer–primer (A) and padlock (B). Experimental conditions: 75 ng of PDGF-BB antibody, 6 U of ligase, 25 U of polymerase, 100 μM of dNTPs, 2.5 pmol of biotinylated probe, 1:5000 dilution of SA–HRP and 10 pM PDGF-BB. A, 2.1 pmol of padlock; B, 7.5 pmol of aptamer–primer. The detection procedure was carried out as described in the Experimental Section 2.4.

Figure 4 CL intensity vs. concentration of RCA reagents. Experimental conditions: 75 ng of PDGF-BB antibody, 7.5 pmol of aptamer–primer, 5.0 pmol of padlock, 2.5 pmol of biotinylated probe, 1:5000 dilution of SA–HRP and 10 pM PDGF-BB. A, 25 U of polymerase, 100 μM of dNTPs; B, 1 U of ligase and 100 μM of dNTPs; C, 1 U of ligase and 40 U of polymerase. The detection procedure was carried out as described in the Experimental Section 2.4.
3.2.3. Dependence of CL intensity on the amount of RCA reagents

The amounts of ligase, polymerase and dNTPs also affected the CL signal to some extent. As shown in Fig. 4, CL intensity started to increase rapidly as the concentration of ligase increased from 0 to 1 U, and reached a maximum with 1 U of ligase. Further increase in the amount of ligase to 10 U caused a decrease in the CL intensity. Thus, 1 U of ligase was selected for subsequent experiments. Similarly, 40 U of polymerase and 100 μM of dNTPs were selected respectively in further studies.

3.2.4. Dependence of CL intensity on the amount of report reagents

The amounts of biotinylated probe and SA–HRP were two vital factors for the amplification of CL signal. Therefore, these parameters were also investigated. As shown in Fig. 5, the range of 0.5–10.0 pmol of biotinylated probe and 1:1000 to 1:15 000 dilution of SA–HRP were studied for the optimal condition. Finally, the maximum CL intensity was obtained using 7.5 pmol of biotinylated probe and 1:7500 dilution of SA–HRP, which were thus employed for the subsequent experiments.

3.2.5. Analytical performance with or without RCA

Under the above-optimized experimental conditions, the quantitative behavior of the method was assessed by monitoring the dependence of the CL intensity on the concentration of the target PDGF-BB. As shown in Fig. 6, a linear relationship was obtained between the CL intensity and the amount of PDGF-BB in the range of 10 fM–1 nM ($\log I = 0.836 \log C + 0.928$).

![Figure 5](image)

**Figure 5** CL intensity vs. the concentration of biotinylated probe and SA–HRP. Experimental conditions: 7.5 ng of PDGF-BB antibody, 7.5 pmol of aptamer-primer, 5.0 pmol of padlock, 1 U of ligase, 40 U of polymerase, 100 μM of dNTPs and 10 pM PDGF-BB. A, 1:5000 dilution of SA–HRP; B, 2.5 pmol of biotinylated probe. The detection procedure was carried out as described in the Experimental Section 2.4.

![Figure 6](image)

**Figure 6** Log–log calibration data for PDGF-BB by using the protocol with RCA (up) and without RCA (down). The detection procedure was carried out as described in the Experimental Section 2.4.

| Analytical method | Label | Detection limit |
|-------------------|-------|-----------------|
| Fluorescence      | Cy3   | 0.4 nM [25]     |
| Fluorescence      | FAM and DABCYL | 6.8 pM [26] |
| Fluorescence      | Fluorescein | 110 pM [42] |
| Fluorescence      | TOTO  | 0.1 nM [44]     |
| Fluorescence      | SYBR-green | 0.1 ng/mL [45] |
| Fluorescence      | Dyes  | 10 ng/μg [46]   |
| Electrochemical    | AP    | 10 fM [27]      |
| Electrochemical    | Methylene blue | 63 pM [28] |
| Electrochemical    | Fc    | 1.0 μg/mL [43]  |
| Densitometry       | Gold nanoparticles (AuNPs) | 83 aM [20] |
| Diffractometric    | Microbeads | 10 pM [23] |
| Photoluminescence  | Au-nanodots NPs | 10 pM [47] |
| Colorimetric       | AuNPs | 35 nM [48]      |
| Capillary          | FAM   | 50 pM [49]      |
| Electrophoresis    |       |                 |
| Luminescence       | [Ru(phen)$_2$(dppz)]$^{2+}$ | 1.0 nM [50] |
| Chemiluminescence  | HRP   | 10 fM (this work) |

**Table 2** Comparison of sensitivity for different aptamer-based assays for PDGF-BB.
reported previously (Table 2). The detection specificity was also determined and evaluated by the aptamer recognition function. As shown in Fig. 7, compared with 100 pM PDGF-BB, the CL intensity from 1 nM of the non-target proteins (IgG, IgA, IgM, interferon and thrombin) was low. It was demonstrated that the CL signal was specifically triggered by the aptamer/target binding.

3.2.6. Detection specificity
Sensitivity and specificity were the two critical factors for a successful assay system. In the proposed strategy, the sensitivity of our assay compared favorably with previous efforts. The detection specificity was also determined and evaluated by the aptamer recognition function. As shown in Fig. 7, compared with 100 pM PDGF-BB, the CL intensity from 1 nM of the non-target proteins (IgG, IgA, IgM, interferon and thrombin) was low. It was demonstrated that the CL signal was specifically triggered by the aptamer/target binding.

4. Conclusion
A new CL platform for the determination of protein has been demonstrated by the combination of the aptamer as the identification unit and RCA as the amplification unit. This aptamer-RCA based CL technique has significant advantages, including improved sensitivity and high selectivity. The detection limit of this novel protocol was demonstrated down to 10 fM, which is a 1000-fold improvement compared to non-RCA assay, and also comparable to or even better than what was provided by most previous reports. Furthermore, our protocol might discriminate the target protein from 10-fold concentration of other proteins. In summary, this aptamer-RCA based CL technique possesses great potential to serve as the PDGF-BB diagnostic tool in clinical test by virtue of its sensitivity and selectivity. In addition, this new approach can also be easily extended to the detection of other biomarkers relating to other diseases, such as IgE or thrombin, etc.

Acknowledgments
We acknowledge financial support from the National Drug Innovative Program (2009ZX09301-011) and the Research Fund for the Doctoral Program of Higher Education (200802461096, 2009007110056).

References
[1] L. Hartwell, D. Mankoff, A. Paulovich, et al., Cancer biomarkers: a systems approach, Nat. Biotechnol. 24 (8) (2006) 905–908.
[2] J.P. Gosling, A decade of development in immunoassay methodology, Clin. Chem. 36 (8) (1990) 1408–1427.
[3] K.N. Baker, M.H. Rendall, A. Patel, et al., Rapid monitoring of recombinant protein products: a comparison of current technologies, Trends Biotechnol. 20 (4) (2002) 149–156.
[4] G.P. Manukyan, K.A. Ghazaryan, Z.A. Ktsoyall, et al., Cytokine profile of Armenian patients with familial Mediterranean fever, Clin. Biochem. 41 (10–11) (2008) 920–922.
[5] X. Chu, X. Fu, K. Chen, et al., An electrochemical stripping metalloimmunoassay based on silver-enhanced gold nanoparticle label, Biosens. Bioelectron. 20 (9) (2005) 1805–1812.
[6] J. Wang, G. Liu, M.R. Jan, Ultrasensitive electrical biosensing of proteins and DNA: carbon-nanotube derived amplification of the recognition and transduction events, J. Am. Chem. Soc. 126 (10) (2004) 3010–3011.
[7] J.M. Nam, C.S. Thaxton, C.A. Mirkin, Nanoparticle-based barcode codes for the ultrasensitive detection of proteins, Science 301 (5641) (2003) 1884–1886.
[8] H.D. Hill, C.A. Mirkin, The bio-barcode assay for the detection of protein and nucleic acid targets using DTT-induced ligand exchange, Nat. Protoc. 1 (1) (2006) 324–336.
[9] M. Gullberg, S.M. Gustafsdottir, E. Schallmeiner, et al., Cyto kine detection by antibody-based proximity ligation, Proc. Natl. Acad. Sci. USA 101 (22) (2004) 8420–8424.
[10] O. Soderberg, M. Gullberg, M. Jarvisiu, et al., Direct observation of individual endogenous protein complexes in situ by proximity ligation, Nat. Methods 3 (12) (2006) 995–1000.
[11] J.E. Smith, C.D. Medley, Z. Tang, et al., Aptamer-conjugated nanoparticles for the collection and detection of multiple cancer cells, Anal. Chem. 79 (8) (2007) 3075–3082.
[12] J.A. Hansen, J. Wang, A.N. Kawde, et al., Quantum-dot/aptamer-based ultrasensitive multi-analyte electrochemical biosensor, J. Am. Chem. Soc. 128 (7) (2006) 2228–2229.
[13] D. Proske, M. Blank, R. Buhmann, et al., Aptamers-basic research, drug development, and clinical applications, Appl. Microbiol. Biotechnol. 69 (4) (2005) 367–374.
[14] S. Tombelli, M. Mimunni, M. Mascini, Analytical applications of aptamers, Biosens. Bioelectron. 20 (12) (2005) 2424–2434.
[15] M. Famulok, G. Mayer, M. Blind, Nucleic acid aptamers-From selection in vitro to applications in vivo, Acc. Chem. Res. 33 (9) (2000) 591–599.
[16] D.H.I. Bunka, P.G. Stockley, Aptamers come of age at last, Nat. Rev. Microbiol. 4 (8) (2006) 588–596.
[17] C. Tuerk, L. Gold, Systematic evolution of ligands by exponential enrichment—RNA ligands to bacteriophage-T4 DNA-polymerase, Science 249 (4968) (1990) 505–510.
[18] S.D. Jayasena, Aptamers: an emerging class of molecules that rival antibodies in diagnostics, Clin. Chem. 45 (9) (1999) 1628–1650.

[19] S. Song, L. Wang, J. Li, et al., Aptamer-based biosensors, Trac-Trends Anal. Chem. 27 (2) (2008) 108–117.

[20] Y.Y. Li, C. Zhang, B.S. Li, et al., Ultrasonic densitometry detection of cytokines with nanoparticle-modified aptamers, Clin. Chem. 53 (6) (2007) 1061–1066.

[21] C.C. Huang, S.H. Chiu, Y.F. Huang, et al., Aptamer-functionalized gold nanoparticles for turn-on light switch detection of platelet-derived growth factor, Anal. Chem. 79 (13) (2007) 4798–4804.

[22] A. Csordas, A.E. Gerdon, J.D. Adams, et al., Detection of proteins in serum by micromagnetic aptamer PCR (MAP) Technology, Angew. Chem. Int. Ed. 49 (2) (2010) 355–358.

[23] J. Lee, K. Icoz, A. Roberts, et al., Diffractionometric detection of proteins using microbead-based rolling circle amplification, Anal. Chem. 82 (1) (2010) 197–202.

[24] L.J. Ou, S.J. Liu, X. Chu, et al., DNA encapsulating liposome based rolling circle amplification immunoassay as a versatile platform for ultrasensitive detection of protein, Anal. Chem. 81 (23) (2009) 9664–9673.

[25] L.T. Yang, C.W. Fung, E.J. Cho, et al., Real-time rolling circle amplification for protein detection, Anal. Chem. 79 (9) (2007) 3230–3239.

[26] Z.S. Wu, H. Zhou, S. Zhang, et al., Universal aptameric system for highly sensitive detection of protein based on structure-switching-triggered rolling circle amplification, Anal. Chem. 82 (6) (2010) 2221–2227.

[27] S. Song, L. Wang, J. Li, et al., Aptamer-based rolling circle amplification: a platform for electrochemical detection of protein, Anal. Chem. 79 (19) (2007) 7492–7500.

[28] Z.S. Wu, H. Zhou, S. Zhang, et al., Electrochemical aptameric recognition system for a sensitive protein assay based on specific target binding-induced rolling circle amplification, Anal. Chem. 82 (6) (2010) 2282–2289.

[29] L. Zhou, L.J. Ou, X. Chu, et al., Aptamer-based rolling circle amplification: a platform for electrochemical detection of protein, Anal. Chem. 79 (19) (2007) 7492–7500.

[30] Z.S. Wu, H. Zhou, S. Zhang, et al., Electrochemical aptameric recognition system for a sensitive protein assay based on specific target binding-induced rolling circle amplification, Anal. Chem. 82 (6) (2010) 2282–2289.

[31] A.P. Fan, Z.J. Cao, H. Li, et al., Chemiluminescence platforms in immunoassay and DNA analysis, Anal. Sci. 25 (5) (2009) 587–597.

[32] L.X. Zhao, L. Sun, X.G. Chu, Chemiluminescence immunoassay, Trac-Trend Anal. Chem. 28 (4) (2009) 404–415.

[33] E. Marzocchi, S. Grilli, L. Della Ciana, et al., Chemiluminescent detection systems of horseradish peroxidase employing nucleophilic acylation catalysts, Anal. Biochem. 377 (2) (2008) 189–194.

[34] Y.P. Li, Electrogenerated chemiluminescence detection of mercury (II) ions based on DNA probe labeled with ruthenium complex, Anal. Sci. 27 (2) (2011) 193–196.

[35] M. Yamashita, T. Shibata, T. Kabashima, et al., Chemiluminescence detection of telomere DNA in human cells on a membrane by using fluorescein-5-isothiocyanate-labeled primers, Anal. Biochem. 413 (1) (2011) 50–54.

[36] H.Q. Wang, W.Y. Liu, Z. Wu, et al., Homogeneous label-free genotyping of single nucleotide polymorphism using ligation-mediated strand displacement amplification with DNAzyme-based chemiluminescence detection, Anal. Chem. 83 (6) (2011) 1883–1889.

[37] S.C. Donhauser, R. Niessner, M. Seidel, Sensitive quantification of Escherichia coli O157:H7, Salmonella enterica, and Campylobacter jejuni by combining stopped polymerase chain reaction with chemiluminescence flow-through DNA microarray analysis, Anal. Chem. 83 (8) (2011) 3153–3160.

[38] A. Roda, M. Mirasoli, L.S. Dolci, et al., Portable device based on chemiluminescence lensless imaging for personalized diagnostics through multiplex bioanalysis, Anal. Chem. 83 (8) (2011) 3178–3185.

[39] H. Kong, D. Liu, S.C. Zhang, et al., Protein sensing and cell discrimination using a sensor array based on nanomaterial-assisted chemiluminescence, Anal. Chem. 83 (6) (2011) 1867–1870.

[40] Y. Zhou, Y.H. Zhang, C.W. Lau, et al., Sequential determination of two proteins by temperature-triggered homogeneous chemiluminescent immunoassay, Anal. Chem. 78 (16) (2006) 5920–5924.

[41] Z.J. Cao, Z.X. Li, Y.J. Zhao, et al., Magnetic bead-based chemiluminescence detection of sequence-specific DNA by using catalytic nucleic acid labels, Anal. Chim. Acta. 557 (1–2) (2006) 152–158.

[42] J.R. Miao, Z.J. Cao, Y. Zhou, et al., Instantaneous derivatization technology for simultaneous and homogeneous determination of multiple DNA targets, Anal. Chem. 80 (5) (2008) 1606–1613.

[43] X.L. Yan, Z.J. Cao, M. Kai, et al., Label-free aptamer-based chemiluminescence detection of adenosine, Talanta 79 (2) (2009) 383–387.

[44] X. Fang, A. Sen, M. Vicens, et al., Synthetic DNA aptamers to detect protein molecular variants in a high-throughput fluorescence quenching assay, Chembiochem 4 (9) (2003) 829–834.

[45] Y.L. Zhang, Y. Huang, J.H. Jiang, et al., Electrochemical aptasensor based on proximity-dependent surface hybridization assay for single-step, reusable, sensitive protein detection, J. Am. Chem. Soc. 129 (50) (2007) 15448–15449.

[46] C.S. Zhou, Y.X. Jiang, S. Hou, et al., Detection of oncoprotein platelet-derived growth factor using a fluorescent signaling complex of an aptamer and TOTO, Anal. Bioanal. Chem. 384 (5) (2006) 1175–1180.

[47] J. Zhu, Y. Huang, X.P. Jiang, et al., A new fluorescence immunosensing method based on aptamer-plasmid complex amplification, Chin. J. Anal. Chem. 37 (11) (2009) 1596–1600.

[48] M.C. Vicens, A. Sen, A. Vanderlaan, et al., Investigation of molecular beacon aptamer-based bioassay for platelet-derived growth factor detection, Chembiochem 6 (5) (2005) 900–907.

[49] C.C. Huang, C.K. Chiang, Z.H. Lin, et al., Bioconjugated gold nanodots and nanoparticles for protein assays based on photoluminescence quenching, Anal. Chem. 80 (5) (2008) 1497–1504.

[50] C.C. Huang, Y.F. Huang, Z.H. Cao, et al., Aptamer-modified gold nanoparticles for colorimetric determination of platelet-derived growth factors and their receptors, Anal. Chem. 77 (17) (2005) 5735–5741.

[51] H. Zhang, X.F. Li, X.C. Le, Differentiation and detection of PDGF isomers and their receptors by tunable aptamer capillary electrophoresis, Anal. Chem. 81 (18) (2009) 7795–7800.

[52] Y.X. Jiang, X.H. Fang, C.L. Bai, Signaling aptamer/protein binding by a molecular light switch complex, Anal. Chem. 76 (17) (2004) 5230–5235.