Int. J. Electrochem. Sci., 8 **(2013)** 8210 - 8217

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Amplified Voltammetric Strategy for the Detection of Proteases Using Dopamine-Modified Gold Nanoparticles

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Received: 9 April 2013 / *Accepted:* 11 May 2013 / *Published:* 1 June 2013

In this work, we reported a label-free and amplified electrochemical strategy for the detection of proteases using dopamine (DA)-modified gold nanoparticles (DA-AuNPs) as redox probes. To demonstrate the feasibility and sensitivity, thrombin was tested as a model analyte. Specifically, peptide with a sequence of CSGFPRGRY was immobilized onto the gold electrode. Monophenol of tyrosine residue in the peptide was catalytically oxidized by tyrosinase to catechol. The resultant catechol was derivatized with 1,4-phenyldiboronic acid (PDBA) for the attachment of electrochemically active DA-AuNPs through the formation of tight covalent bond between boronic acid and catechol group, which facilities the amplified voltammetric detection. However, the cleavage of peptide by thrombin induced the loss of tyrosine residue, resulting in the decrease in the amount of catechol group and DA-AuNPs on electrode. The magnitude of the decrease in the peak current is related to the concentration and activity of thrombin.

Keywords: Electrochemical biosensor; proteases; dopamine; gold nanoparticles; signal amplification

1. INTRODUCTION

In recent year, an intensive research effort has been performed in the field of analytical electrochemistry seeking the design of electrochemical biosensors duo to its high sensitivity, simplicity, rapid response, and compatibility with miniaturization. To improve the sensitivity, the use of nanomaterials for the construction of biosensing devices becomes one of the most exciting approaches [1,2]. Among kinds of nanomaterimals, gold nanoparticles (AuNPs) have been widely used to facilitate electron transfer between redox tags and electrode surface in view of the high surface-to-volume ratio and surface energy, and the unique combination of chemical and physical properties

[3,4]. Moreover, the rational design of the surface chemistry of AuNPs promotes specific interactions between receptors and analytes, rendering the measurements highly selective and sensitive [5]. Traditionally, electrochemically active thionine (Th) or ferrocene (Fc) was modified onto the antibody-coated AuNPs for signal output and amplification [6,7]. Dopamine (DA) is also an electrochemically active compound. The DA-modified quantum dots show well-defined redox waves [8]. Recently, our group suggested that DA-modified AuNPs (DA-AuNPs) exhibits excellent properties in molecular recognition and signal amplification for the detection of glycoproteins and miRNAs [9,10]. Enzymes, serving a wide variety of functions inside living organisms, are indispensable for signal transduction and cell regulation [11]. Enzymes analytical detection is important for the screening of noxious toxins and pathologies associated with their presence, and for the development of effective and selective therapeutics. The detection of enzyme and its activity using nanoparticles labels has attracted recent research efforts [12]. In this work, we attempted to demonstrate the application of DA-AuNPs in the amplified voltammetric detection of enzyme.

Among enzymes, proteases constituting 2% of the human proteome in mammalian cells are particularly relevant because proteolytic processing is the final step in the expression of the activity of a great variety of proteins [13,14]. Moreover, proteolytic activity of these enzymes is reported to be associated with several widespread diseases, such as HIV-1 protease in HIV-1 virus [15], matrix metal-loproteinases (MMPs) in cancer cells [16], β- and λ-secretase in Alzheimer's disease [17]. Traditional methods for protease detection including high-performance liquidchromatography (HPLC) [18], enzyme-linked immunosorbent assay (ELISA) [19] and gel electrophoresis [20] are usually time-consuming, lack sensitivity and/or require complicated instruments. To overcome these problems, electrochemical approaches using labeled protease substrates have been proposed recently [16,21]. However, the labeling process would make the experiments complex, affect the bioaffinity between the probes and their targets and/or be less sensitive. In this work, we reported a sensitive and label-free electrochemical method for the detection of proteases based on the signal amplification of DA-AuNPs. To demonstrate the feasibility, thrombin was tested as a model analyte.

2. EXPERIMENTAL

2.1 Chemicals and reagents

Thrombin, tyrosinase, dithiobis(succini-midylpropionate) (DSP), tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and 6-mercapto-1-hexanol (MCH) were obtained from Sigma–Aldrich. Peptide with a sequence of CSGFPRGRY was synthesized and purified by ChinaPeptides Co., Ltd (Shanghai, China). Dopamine hydrochloride was purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). 1,4-Phenyldiboronic acid (PDBA) was obtained from Heowns Biochem Technologies LLC (Tianjing, China). The 5 mM PDBA stock solution was prepared with 50% methanol and diluted to the desired concentration before use. All aqueous solutions were prepared with a Millipore system (Simplicity Plus, Millipore Corp.).

2.2 Preparation of DA-AuNPs

The preparation and characterization of DA-AuNPs has been reported by us previously [9,10]. Brief, The DA-DSP complex was prepared by mixing two-equivalent DA (20 mM) and one-equivalent DSP (10 mM) in anhydrous DMF containing 20 mM triethylamine for 12 h. The DA-DSP solution was then diluted with deionized water to 500 μM before use. To avoid the oxidation of catechol tags, 1 mM Na_2SO_3 was added into the diluted DA-DSP solution. DA-AuNPs was prepared by mixing 1.4 μM DA-DSP and 3.2 nM citrated-stabilized AuNPs at room temperature for 2 h. The synthesized DA-AuNPs suspension was stored in a clean environment at 4 °C.

2.3 Preparation of peptide-modified electrode

The gold disk electrode with a diameter of 2 mm was polished with diamond pastes down to 3 μm and alumina pastes down to 0.3 μm, and then sonicated in ethanol and water. The peptide-modified electrode was prepared by immersing the cleaned gold electrodes in a solution of 10 μM peptide containing 50 μM TCEP in the darkness for 12 hours. This step was followed by washing the electrode thoroughly with water and soaking the electrode in a 1 mM MCH for 1 hour to block the unreacted gold surface. Again, the electrode was rinsed with ethanol/water to rid any non-specifically adsorbed substance.

2.4 Detection of thrombin

For the detection of thrombin, the peptide-modified electrode was first incubated with 1 U tyrosinase for 30 min to generate the o-diphenol group on electrode. The experimental conditions for tyrosinase-mediated reaction were chosen according to the well-established protocol [22,23]. Then, 10 μL of PBS (pH 7.8) comprising a given concentration of thrombin and 0.1 mM PDBA containing 1 mM Na_2SO_3 was cast onto the electrode surface. The electrode was rinsed with water to rid any non-specifically adsorbed substance. This step was followed by exposing to 10 μL of DA-AuNPs suspension for 20 min. After the electrode had been rinsed with water, voltammetric determination in phosphate-buffered saline solution (PBS buffer, 10 mM, pH 7.4) containing 50 mM Na_2SO_4 was performed on a DY2013 electrochemical workstation (Digi-Ivy, Inc., Austin, TX) using a homemade plastic electrochemical cell. A platinum wire and a Ag/AgCl electrode were used as the auxiliary and the reference electrodes, respectively.

3. RESULTS AND DISCUSSION

3.1 Principle of the amplified voltammetric detection of thrombin

The schematic representation of the amplified electrochemical detection of thrombin via oxidation of DA tags on the AuNPs is illustrated in Fig. 1. Peptide CSGFPRGRY was immobilized onto the gold electrode through the Au-S bonds. The unreacted gold surface was blocked by MCH. The monophenol group in the tyrosine residue of peptide was catalytically oxidized by tyrosinase to o-diphenol and o-quinol in the presence of O_2 [22]. To ensure that all of surface of the monolayer is in

the o-diphenol state, Na_2SO_3 was added into the PDBA solution in the PDBA capture step. One boronic acid group of PDBA was reacted with o-diphenol via the formation of diol-boronate ester, while the other one allowed DA-AuNPs to be attached in the same interaction. Since each gold nanoparticle can be modified with a large number of DA molecules, the electrochemical signals will be greatly amplified. However, the cleavage of peptide by thrombin at Arg residue will induce the loss of tyrosine residue on the electrode [22], making the PDBA impossible to be anchored on the surface for the attachment of DA-AuNPs. The magnitude of the decrease in the peak current is related to the amount of removed tyrosine residue on electrode, which depends upon the concentration and activity of thrombin.

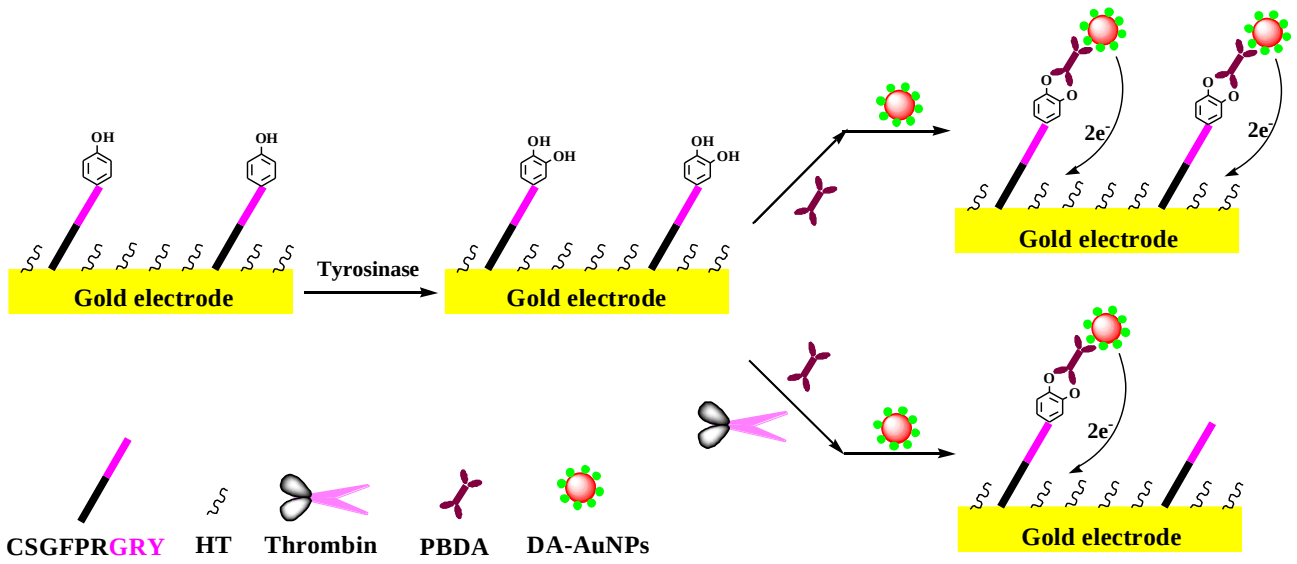

Figure 1. Schematic illustration of the amplified electrochemical strategy for thrombin detection using DA-AuNPs.

3.2 Feasibility of the amplified voltammetric detection

Fig. 2 shows the representative CVs acquired at peptide-modified electrodes after the treatment with tyrosinase (black curve), tyrosinase/PDBA/DA-AuNPs (red curve), tyrosinase/thrombin /PDBA/DA-AuNPs (blue curve). No redox peak was observed after the electrode surface was treated with tyrosinase, indicating that the catechol tags produced by tyrosinase-mediated catalysis cannot be detected directly, which is in agreement with that reported previously [22]. After the electrode was incubated with PDBA and DA-AuNPs, a couple of well-defined redox waves were obtained. The redox peaks with E_{pa} = 0.176 V and E_{pc} = 0.129 V were attributed to the oxidation/reduction of the catechol moieties on DA-AuNPs [9,10]. The results indicated that the electrochemical signal was amplified by DA-AuNPs. However, if the electrode was incubated with PDBA and thrombin together, a significant decrease in the current was observed, indicated that the tyrosine residue was removed due to thrombin-assisted cleavage of peptide. Additionally, we investigated the effect of cleavage time of thrombin and found that the oxidation current decreased with the increase of the reaction time and reached the minimum after 50 min (Fig. 3), indicating that the thrombin-catalyzed cleavage is

completed within 50 min. The current did not finally drop to the background level, implying that not all the peptides assembled onto the electrode surface were cut by thrombin. Note that the formation of diol-boronate ester between catechol and boronic acid is completed immediately in solution (data not shown).

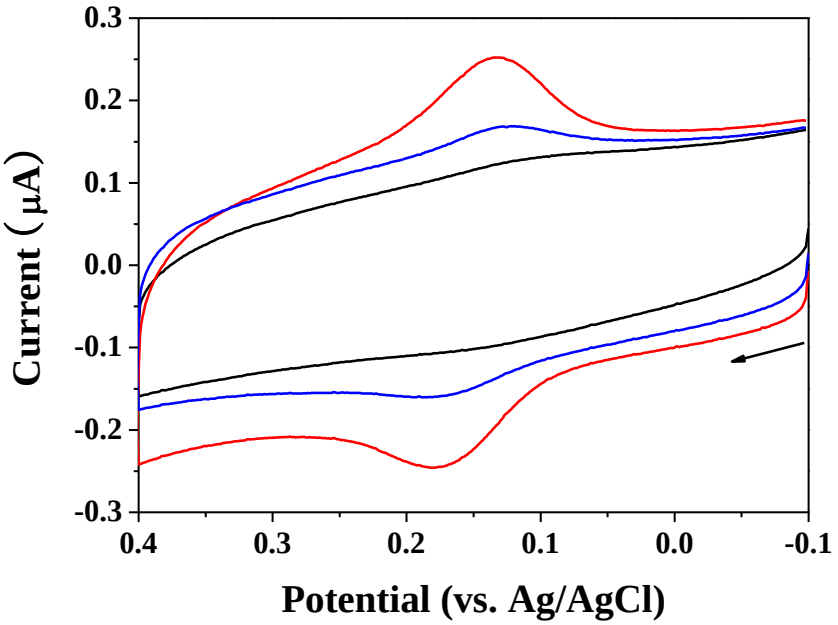

Figure 2. CVs acquired at peptide-modified electrodes after the treatment with tyrosinase (black curve), tyrosinase/PDBA/DA-AuNPs (red curve), tyrosinase/thrombin /PDBA/DA-AuNPs (blue curve). The concentration of thrombin was 100 ng/mL. The arrow indicates the scan direction and the scan rate was 100 mV/s.

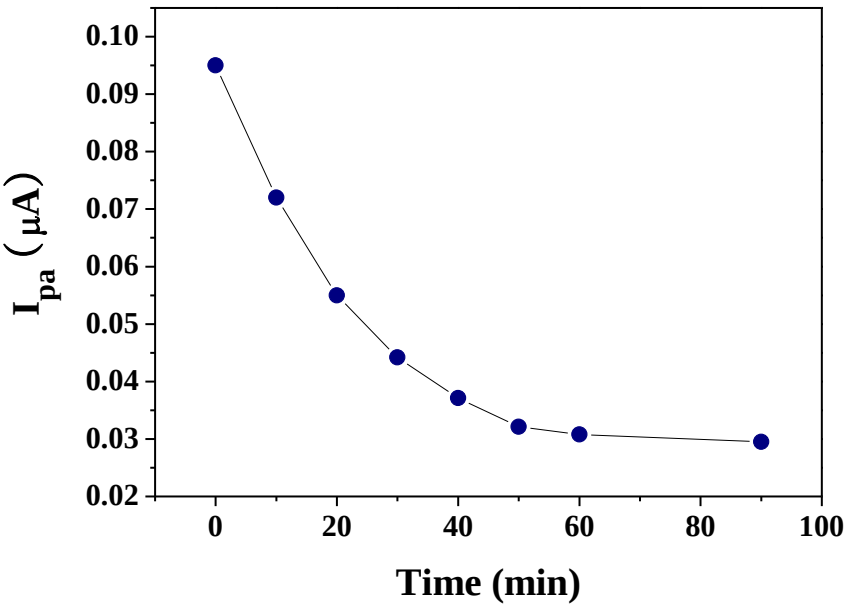

Figure 3. Time-dependence of thrombin-mediated cleavage reaction. The other experimental conditions are the same as those in Fig. 2.

3.3 Effect of pH

The activity of thrombin and stability of boronate ester are pH-dependent [24,25]. The effect of pH on the formation of boronate ester and the activity of thrombin was therefore carried out. As shown in Fig. 4, the oxidation peak current in the absence of thrombin increases with the increase of pH and reaches the maximum at pH 7.2 ~ 7.8, indicating that basic pH is preferred to the binding of PDBA. This is understandable because the affinity of diol-boronic acid complex is small at acidic pH [25]. ΔIpa (the change of oxidation current at 0.176 V (Ipa^0-Ipa'), where Ipa^0 and Ipa' represent the current in the absence and presence of thrombin, respectively) was used here to evaluate the performance of the sensor. As shown in Fig. 4B, ΔIpa reaches the maximum at pH 7.8. Thus, we chose a pH 7.8 PBS buffer solution as the reaction media.

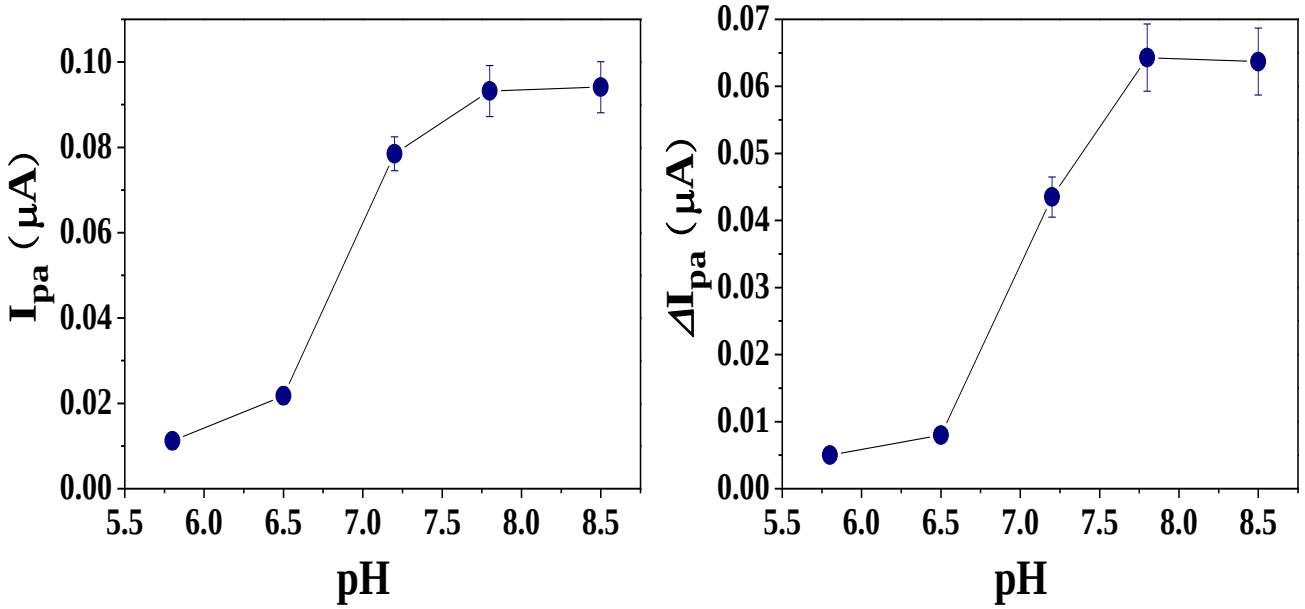

Figure 4. Effect of pH on the formation of boronate ester (A) and the thrombin activity (B). The relative standard deviations (RSDs) from at least three replicates were shown as the error bars. The other experimental conditions are the same as those in Fig. 2.

3.4 Sensitivity to thrombin

Differential pulse voltammetry can decrease the background charging currents and in turn increase the detection sensitivity. Therefore, we evaluated the sensitivity and dynamic ranges of the proposed method using differential pulse voltammetry. As shown in Fig. 5A, the current decreases with the increase of thrombin concentration. A linear relationship was found over the range of 10 ~ 80 ng/mL, which can be expressed as Current (μA) = 0.272 – 0.0026 $C_{Thrombin}$ (ng/mL) (R^2 = 0.99). We also found that the current began to level off when the thrombin concentration was higher than 80 ng/mL. The detection limit (3σ) of the method was determined to be 1 ng/mL (n = 11).

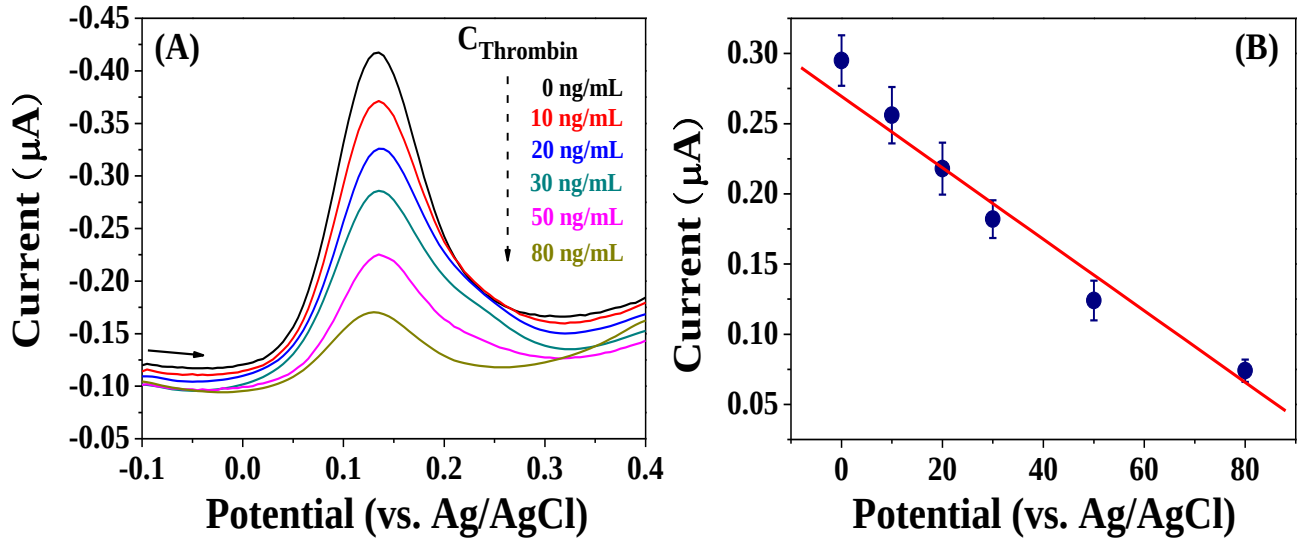

Figure 5. (A) DPVs in the presence of different concentrations of thrombin. Pulse height: 50 mV; pulse width: 50 ms. (B) Plots of peak current versus thrombin concentration. The relative standard deviations (RSDs) from at least three replicates were shown as the error bars.

ACKNOWLEDEMENTS

Partial support of this work by the National Natural Science Foundation of China (21205003) and the Science & Technology Foundation of Henan Province (122102310517) is gratefully acknowledged.

References

1. J. Wang, *Anal. Chim. Acta*, 500 (2003) 247.
2. R. M. Iost, J. M. Madurro, A. G. Brito-Madurro, I. L. Nantes, L. Caseli and F. N. Crespilho, *Int. J. Electrochem. Sci.*, 6 (2011) 2965.
3. J. M. Pingarrón, P. Yáñez-Sedeño and A. González-Cortés, *Electrochim. Acta*, 53 (2008) 5848.
4. M. T. Castaneda, A. Merkoci, M. Pumera and S. Alegret, *Biosens. Bioelectron.* , 22 (2007) 1961.
5. L. Liu, S. Li, L. Liu, D. Deng and N. Xia, *Analyst*, 137 (2012) 3794.
6. L. Liu, J. Du, S. Li, B. Yuan, H. Han, M. Jing and N. Xia, *Biosens. Bioelectron.*, 41 (2013) 730.
7. L. Liu, G. Wang, Q. Feng, Y. Xing, H. Han and M. Jing, *Int. J. Electrochem. Sci.*, 8 (2013) 3814.
8. X. Ji, G. Palui, T. Avellini, H. B. Na, C. Yi, J. Knappenberger, K. L. and H. Mattoussi, *J. Am. Chem. Soc.*, 134 (2012) 6006.
9. N. Xia, D. Deng, L. Zhang, B. Yuan, M. Jing, J. Du and L. Liu, *Biosens. Bioelectron.*, 43 (2013) 155.
10. N. Xia, L. Zhang, G. Wang, Q. Feng and L. Liu, *Biosens. Bioelectron.*, 10.1016/j.bios.2013.03.074 (2013).
11. M. Drag and G. S. Salvesen, *Nat. Rev. Drug Discov.*, 9 (2010) 690.
12. E. Katz and I. Willner, *Angewandte Chemie International Edition*, 43 (2004) 6042.
13. H. Neurath, *J. Cell. Biochem.* , 32 (1986) 35.
14. H. Neurath, *Proc. Natl. Acad. Sci. USA*(1999) 10962.
15. K. Kerman and H.-B. Kraatz, *Analyst*, 134 (2009) 2400.
16. G. Liu, J. Wang, D. S. Wunschel and Y. Lin, *J. Am. Chem. Soc.*, 128 (2006) 12382.

17. C. R. Burton, J. E. Meredith, D. M. Barten, M. E. Goldstein, C. M. Krause, C. J. Kieras, L. Sisk, L. G. Iben, C. Polson, M. W. Thompson, X.-A. Lin, J. Corsa, T. Fiedler, M. Pierdomenico, Y. Cao, A. H. Roach, J. L. Cantone, M. J. Ford, D. M. Drexler, R. E. Olson, M. G. Yang , C. P. Bergstrom, K. E. McElhone, J. J. Bronson, J. E. Macor, Y. Blat, R. H. Grafstrom, A. M. Stern, D. A. Seiffert, R. Zaczek, C. F. Albright and J. H. Toyn, *J. Biol. Chem.*, 283 (2008) 22993.
18. M. M. Vestling, C. M. Murphy and C. Fenselau, *Anal. Chem.*, 62 (1990) 2391.
19. S. Patel, G. Sumitra, B. C. Koner and A. Saxena, *Clin. Biochem.*, 44 (2011) 869.
20. K. Shimura, H. Matsumoto and K. Kasai, *Electrophoresis*, 19 (1998) 2296.
21. H. Xiao, L. Liu, F. Meng, J. Huang and G. Li, *Anal. Chem.*, 80 (2008) 5272.
22. D. Li, R. Gill, R. Freeman and I. Willner, *Chem. Commun.*(2006) 5027.
23. N. Xia, L. Zhang, Q. Feng, D. Deng, X. Sun and L. Liu, *Int. J. Electrochem. Sci.*, 8 (2013) 5487.
24. R. Machovich, *The Thrombin*, 1 (1984) 111.
25. G. Springsteen and B. Wang, *Tetrahedron* (2002) 5291.