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The Specific Binding between Aptamer and Its Target Molecules Investigated by Microcantilever Sensor

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Abstract. Cantilever with modified aptamers on the gold surface was employed to detect platelet-derived growth factor-BB (PDGF-BB) and cocaine. The deflection increased when PDGF-BB and cocaine were added into the flowing cell, respectively. The defection depended on the concentration of PDGF-BB and cocaine. It was also found the microcantiver went back to the same position as without cocaine after adding cocaine aptamer in situ, which proved that the cantilever could be reused. The deflection arose from the specific interaction between aptamer and its target.

Introduction

Since the mid-20th century, as people have deeper understanding of the nature of human life, construction and development of new biological analysis platform is becoming more and more important. Biosensor as an important field of bio-analytical technique has involved multidisciplinary research, full of innovation and vitality. There have been already a large number of various types of biosensors widely used in the medical, food and beverage, environmental and biological drugs. But the traditional biosensors have certain limitations, such as: the preparation of the sensing element is relatively cumbersome; the loss of activity when the sensing element is fixed; limited shelf time; high demands on the working environment and sample conditions; the sensitivity of the transducer is not high; long response time; the information provided is single. Thus, the scientific and business communities are actively exploring new biosensors which have both sensitive components and excellent transducers.

With the in depth research of specific binding between DNA and protein, inspired by the combinatorial chemistry, antibody library and random phage peptide library technology, Gold [1] and his colleagues built the random nucleic acid library and screened with the target molecule to find the specific binding nucleic acid ligands. The technology is Systematic Evolution of Ligands by Exponential Enrichment, referred to as SELEX technology. The aptamer is nucleic acid ligand screened by SELEX process. So far, the specific binding aptamers for metal ions, organic dyes, drugs, amino acids, amino polysaccharides, polypeptides, proteins, whole virus particles and even bacteria have been screened out.

To some extent, aptamer is artificial antibody. Its specificity is comparable to antibody and it also has a lot of advantages over antibody. Antibody analysis techniques are widely used in various fields and have made huge contributions to biological analysis. But antibody preparation techniques still have many defects: antibody for toxins, infection intensive factor and low immunogenicity substance are difficult to prepare; heterophile antibody interference in the preparation of monoclonal antibody; preparation of monoclonal antibodies subject to a lot of screening and purification work; the hybridoma may change, and cause the difference between antibody batches; antibody is very sensitive to temperature and prone to irreversible degeneration; the shelf-life of the antibody is limited.

Aptamer can compensate for the deficiencies of the antibody because of the following advantages: screening is in vitro process, and it does not depend on the cell or animal, so toxins and non-immunogenic molecule can also produce high affinity aptamer; aptamer can be chemically synthesized at high degree of accuracy and repeatability; aptamer is reversible and it can be

activated within minutes after degeneration; it has long shelf life and can be transported at room temperature [2]. Screening of aptamer is entirely in vitro, and it does not involve any animals and cells, the poison molecules or poorly immunogenic molecules can also have high affinity aptamer. Therefore, as a recognition element of the biosensor, aptamer has unique advantages. The biosensor is mainly composed of two parts, the identification element and the signal converter. And aptamer in nature is just a nucleic acid molecule which does not have the function of the signal conversion or reporting, so it has to rely on other means to achieve the function of the sensor. The vast majority of such aptamer sensors reported in the literature are based on fluorescence or electrochemical detection, fluorescence or redox marker. Over the past decade, with the atomic force microscopy technology continues to mature and develop, its sensitivity and accuracy has been recognized by the scientific community worldwide. Its expanding applications to some new areas also attract the attention of scientists. One of the applications is the development of microcantilever sensors based on AFM probe. Microcantilever sensor is different from the traditional sensor in the following aspects: fast response and high sensitivity; can be operated in vacuum, air, and liquid environment; small size, low energy consumption and the process is simple and easy to operate; the microcantilever sensor can be used as a mass sensor, or as a surface stress sensor, with the advantages of sensitive detection of intermolecular forces and molecular conformational changes. This feature makes the cantilever sensitively report and conduct signals, especially with combination of electrochemical and self-assembly technique will further improve the detection sensitivity, selectivity, stability and reproducibility. Microcantilever can be manufactured by the microscopic processing technology and conventional microelectronics technology by mass production with low-energy consumption, high-yield and good reproducibility. Based on the variety of the target molecule of aptamer and the ability of the specific recognition, we envisage that ultrasensitive cantilever combines with aptamer as recognition element to develop novel type of biosensor. Therefore biosensor made up of cantilever and aptamer has broad application prospects and commercial use. It is based on the combination of the two that the paper detected platelet-derived growth factor (PDGF-BB) and cocaine and by aptamer modified cantilever.

The abnormal expression of PDGF-BB and its receptor has close relation to the occurrence and development of a variety of tumors. The identification and detection of PDGF has become the new hot spot of recent research[3]. Particularly after the SELEX technology provides a nucleic acid sequence(aptamer) that can specificically combine with PDGF-BB. Electrochemical and fluorescent methods for identification and detection of the PDGF-BB has been developed[4-7], but these methods need complicate labeling process.

Cocaine is a drug which has great toxicity on the central nervous system and can excite the cerebral cortex to produce pleasure. It causes a lot of damage and complications of cardiovascular and cerebrovascular. Cocaine also has direct effect on the respiratory, digestive, urinary and reproductive system[8]. Due to the sharing of needles and syringes, abusers may not only be infected and spread of hepatitis and HIV, but also may have psychosis disease[9-12]. Cocaine abuse not only brings serious harm to the physical and mental health of the individual, but also seriously endangers social security, resulting in the increase of criminality[13-15]. Therefore, sensitive and rapid detection of cocaine is of great significance. In the literature the vast majority detection of cocaine that have been reported is fluorescent or electrochemical methods which need labeling [16-19]. Microcantilever sensor has the merits of fast response, high sensitivity, small size, low energy consumption and simple process. It can be operated in vacuum, air and liquid environments, and can detect the intermolecular interaction and conformational changes, etc. By surface modification of specific sensitive film, the microcantilever can be used as sensors to detect explosives[20], metal ions[21, 22] and small organic molecules[23], etc.; the application of microcantilever on thiol self-assembled film[24], DNA hybridization[25], protein identification[26] and cell adhesion[27] are the focus of in-depth study. In this article, the interaction processes between platelet-derived growth factor (PDGF-BB) and aptamer, cocaine and aptamer are monitored in real-time. We expect to provide the basis for clinical diagnosis and more convenient detection method.

Experiment

Materials and Reagents. Rectangular silicon microcantilever (length 350μm, width 35μm, thickness 1μm, elastic constant of 0.03 N/m, one side of the microcantilever surface was coated by 10-nm chromium adhesion layer and followed by plating 50 nm gold) was purchased from NT-MDT company; Platelet-derived growth factor-BB (PDGF-BB) from human was purchased from Sigma-Aldrich Company; cocaine hydrochloride reference substance was bought from the National Institute of Control of Pharmaceutical and Biological Products; mercapto-modified PDGF-BB aptamer (5'-HS-$(CH_2)_6$-CAGGCTACGGCACGTAGAGCATCACCATGATCCTG) and thiol-modified cocaine aptamer (HS-$(CH_2)_6$ - 5'-AG ACAA GGA AAATCCTTCAAT-GAAGTGG GTC G-3') were synthesized by Shanghai Biological Engineering Company; Tris-HCl, NaCl and $MgCl_2$ (analytical grade) were purchased Since Sinopharm Chemical Reagent Co., Ltd. in Shanghai. PDGF-BB and PDGF-BB aptamer buffer solution: 200 mM Tris-HCl + 5 mM $MgCl_2$, pH = 7.4. Cocaine and cocaine aptamer buffer solution: 10 mM Tris-HCl +100 mM NaCl + 5mM MgCl2, pH = 7.4. The buffer solutions were sterilized using a portable high-pressure steam sterilizer at 120 degree. Micropipette tips, sample tubes and centrifuge tubes were also sterilized. H_2O_2 (30%) and H_2SO_4 (98%) were of analytical grade and purchased from Beijing Chemical Company. The ultrapure water used in the experiment were prepared by 18.2 MΩ • cm^{-1} Milli-Q system.

Instruments and Experimental methods. The flowing cell in the experiment was self-made and the effective volume is about 1mL[28] and the detailed structure shown in Fig. 1. Peristaltic pump (Japan, Rikakikai) was used as injection device and the flowing rate was controlled at about 0.7mL/min during the testing process; atomic force microscopy (AFM, SPA-400 & SPI3800N Japan Seiko) optical system was used for detection of microcantilever deflection; the deflection signal was converted to digital signals by self-made data acquisition box[28]; the samples were injected into the flowing cell after the baseline was stable. The whole experimental setup was on the optical shockproof platform. The article defined microcantilever deflection towards the gold side as positive direction, compared with negative direction towards the silicon surface.

Microcantilever Modification. Microcantilever was soaked in piranha solution (volume ratio of H_2O_2 (30%) and H_2SO_4 (98%) is 3:7) for 5 to 10 minutes, rinsed by the Milli-Q water, ethanol and blown dry with argon. Then it was incubated in 1 μM mercapto-modified PDGF-BB aptamer or mercapto-modified cocaine aptamer in Tris-HCl buffer solution at 25°C in a thermostatic bath for two hours. Then the cantilever was rinsed with buffer solution and stored in Tris-HCl buffer before use.

Results and Discussion

The interaction between PDGF-BB aptamer modified cantilever and PDGF-BB. The result of detection of PDGF-BB by aptamer modified microcantilever was as shown in Fig. 2. The microcantilever was put into the flowing cell and the Tris-HCl buffer was injected by a peristaltic pump. The flowing rate was maintained constant throughout the testing process at 0.7mL/min. When the cantilever was balanced in buffer solution and baseline was stable, at about 360 seconds, as shown in Fig. 2, PDGF-BB solution was added and caused negative deflection. As the reaction proceeded, the number of bound molecules gradually increased, and also increased the surface stress of the cantilever. The deflection continued for 500 seconds before it reached equilibrium; concentrations of l nM, 100 pM, and 10 pM PDGF-BB were detected in the sample solution, corresponding approximately 40nm, 30nm, 20nm deflection response of the microcantilever, the deflection rate is about 0.08nm/s, 0.06 nm/s, and 0.04 nm/s. The greater concentration of PDGF-BB caused the greater value of displacement of the cantilever deflection, and the faster of deflection rate.

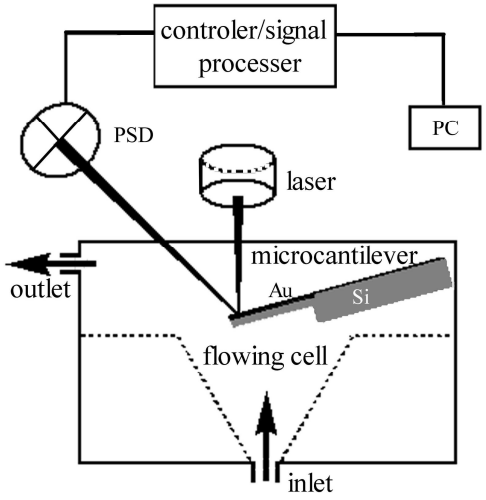

Fig. 1 Schematic diagram of flowing cell[28]

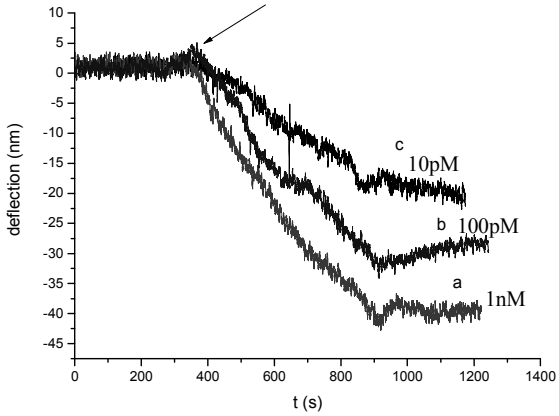

Fig. 2 Deflection curve of PDGF-BB aptamer modified microcantilever with injection of different concentrations of PDGF-BB in buffer solution.

The specific reaction between PDGF-BB and aptamer modified on the gold surface of the microcantilever caused the bending deformation of the cantilever. The number of molecules bound to the microcantilever increased with PDGF-BB solution flowing through the cell driven by peristaltic pump, resulting in increase in the bending deformation of the microcantilever; when the concentration of PDGF-BB binding to the microcantilever was in a dynamic equilibrium with the concentration of PDGF-BB in the flowing cell, microcantilever bending deformation would tend to balance. When reaching equilibrium, the greater concentration of the sample solution led to more molecules binding to the cantilever, thus caused greater deflection displacement of the cantilever.

Fig. 3 shows the schematic diagram of the interaction between PDGF-BB aptamer modified cantilever and PDGF-BB. The specific binding force between aptamer and its target is mainly derived from the high specificity of false base pair stacking interactions, hydrogen bonding, electrostatic interaction, etc. The specific recognition between aptamer and its target molecule is dependent on the formation of three-dimensional structure of its single-chain nucleotide molecule. Aptamer may form three dimensional structures such as hairpin, pseudoknot, bulge and G-quartet, and combine with the target molecule via hydrogen bonding, hydrophobic stacking interactions, and van der Waals forces, etc. The combination of aptamer with the target molecule is the mutual induction of adaptive identification that the single-stranded DNA can form adaptive folding at the presence of target molecule to identify each other. Stacking interaction plays a key role in most nucleic acid / ligand complex, especially in aptamer / protein complexes, followed by hydrogen bonding and other interactions. The false base pair stacking refers to a ligand parallel to the plane of the aromatic ring and there is an overlap between aromatic ring and the bases of the nucleic acid molecule, similar to the base stacking interactions in the nucleic acid molecules. This specific recognition of the aptamer and PDGF-BB is the result of a variety of forces. The microcantilever is essentially a force sensor and sensitive enough to conduct the force. The combination of the two can improve the specificity and sensitivity of the protein detection.

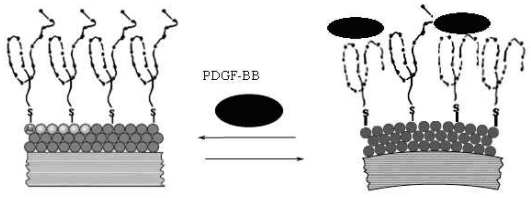

Fig. 3 The deflection diagram of cantilever caused by the interaction between PDGF-BB and PDGF-BB aptamer

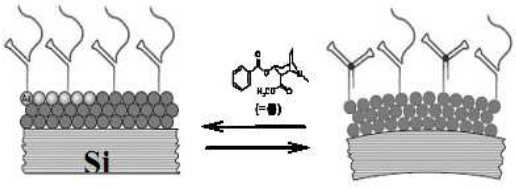

Fig. 4 Deflection schematic diagram of microcantilever caused by the interaction between cocaine and cocaine aptamer modified cantilever aptamer

The interaction between cocaine aptamer modified cantilever and cocaine. The results of detection of cocaine by aptamer modified microcantilever were as shown in Fig. 5, 6 and 7. The microcantilever was put into the flowing cell and the Tris-HCl buffer was injected by a peristaltic pump. The flowing rate was maintained constant throughout the testing process at 0.7mL/min. When the cantilever was balanced in buffer solution and baseline was stable, at about 3.4 minutes, as shown in Fig. 5, 100 μM cocaine was added and caused negative deflection. As the reaction proceeded, the number of binding molecules gradually increased, and also increased the surface stress of the cantilever. The deflection continued for 19 minutes before it reached equilibrium; the deflecting displacement was 30 nm and the deflection rate was about 1.6nm/min. In Fig. 6, after about 4.3 minutes, 10 μM cocaine was added and caused negative deflection. The deflection continued for 16 minutes then gradually tends to balance and the deflection displacement is about 20nm, during which the deflection rate is approximately 1.25nm/min.

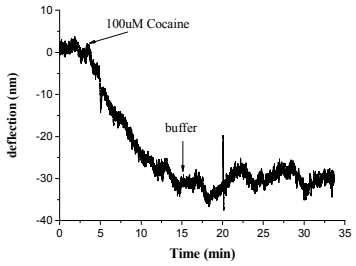

Fig. 5 Deflection curve of cocaine aptamer modified microcantilever with injection of 100 μM cocaine in buffer solution.

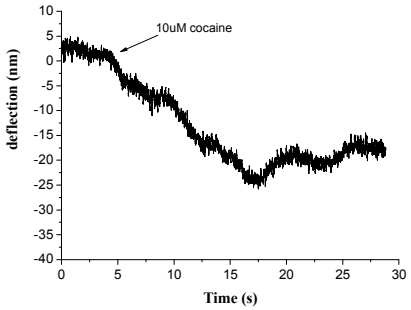

Fig. 6 Deflection curve of cocaine aptamer modified microcantilever with injection of 10 μM cocaine in buffer solution.

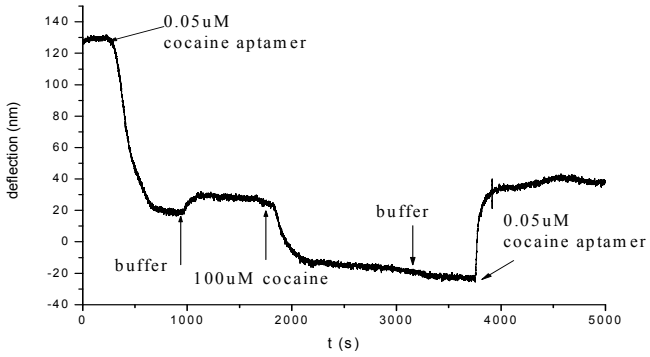

Fig. 7 Deflection curves of the process of self assembly cocaine aptamers on microcantilever in situ and the interaction between cocaine and aptamer.

Before the cocaine solution was added, the cantilever was in a relatively steady state; the specific reaction between cocaine and aptamer modified on the gold surface of the microcantilever caused the bending deformation of the cantilever. The number of molecules bound to the microcantilever increased with cocaine solution flowing through the cell driven by peristaltic pump, resulting in an increase in the bending deformation of the microcantilever; when the concentration of cocaine binding to the microcantilever was in a dynamic equilibrium with the concentration of cocaine in the flowing cell, microcantilever bending deformation would tend to balance. The greater concentration of the sample solution led to more molecules binding to the cantilever when reaching equilibrium, thus caused greater deflection displacement of the cantilever. As can be seen from Fig. 5 and 6, 100 μM cocaine caused 30nm deflection displacement response, and 10 μM cocaine caused the 20nm deflection displacement response, and with the increase of the concentration of the sample, the deflection rate also increased.

To further understand and monitor the process of the cocaine aptamer self assembling, and also examine the regeneration of the cantilever, cocaine aptamer assembling process was monitored by microcantilever, and then 100μM cocaine was injected as shown in Fig. 7. The clean microcantilever was put into the flowing cell, and 0.05μM thiol modified cocaine aptamer was injected after the microcantilever was balanced in the buffer solution and the baseline was stable. The strong interaction between thiol modified cocaine aptamer and the gold surface caused immediate negative deflection of the microcantilever. Thus the self-assembly of cocaine aptamer on the gold surface of the cantilever by chemisorption was monitored. After the baseline was stable, the buffer solution was again injected into the flowing cell; the deflection of cantilever restored a little towards the gold surface because some aptamers of physical adsorption were flushed away by the buffer solution. When the cantilever equilibrated again, 100μM cocaine solution was added and the specific reaction between cocaine and aptamer caused the negative deflection of the cantilever. When the bending deformation of the cantilever reached a steady state, the buffer solution was again rinsed the flowing cell and microcantilever. For the second time 0.05μM thiol modified cocaine aptamer was injected, because of the specific affinity between aptamer and cocaine, cocaine was taken away from the microcantilever, the cantilever deflection recovered to the previous position before 100μM cocaine solution was added, which proved the modified cantilever can be reused. The specific recognition between aptamer and its target molecule is dependent on the formation of three-dimensional structure of its single-chain nucleotide molecule. Fig. 4 shows the combination of aptamer with the target molecule is the mutual induction of adaptive identification that the single-stranded DNA can form adaptive folding at the presence of target molecule to identify each other. During the interaction between aptamer and cocaine, the small molecule was wrapped in the nucleic acid chain by folding.

Studies have shown that the specific binding between biological molecules not only needs both the matching of each other's space structure, but also requires a variety of “forces” such as hydrogen bonding, ionic bond or van der Waals forces to stabilize the combination that has been formed. When these two conditions have been met, all stable integration is possible. The infinite diversity of the nucleic acid library provides selectivety of spatial structure, the nucleic acid molecules with the negatively charged phosphate groups can bind with a variety of positively charged groups by the electrostatic interaction to form stable combination. Nitrogen and hydroxyl group are important elements for forming hydrogen bond, and these two groups are rich in the nucleic acid molecules. The nucleic acid library can satisfy the two requirements of the specific interaction of biological molecules - space and force, which is the theory foundation for screening the aptamer of various molecules in recent years. The aptamer with this specific recognition of cocaine is precisely based on the results of the various "force" and spatial structure matching to achieve the specificity and sensitivity of detection.

Summary

In this article, a novel sensor platform was constructed through aptamer (thiolated nucleic acid sequence) modification of gold surface of microcantilever by self-assembly method to achieve the identification and low concentration detection of PDGF-BB and cocaine, while the processes of interaction between them were monitored in situ. The above experiments were based on one of the directions of biosensor technology development - the use of artificial antibody (aptamer) to substitute the natural antibody, to simplify the preparation process of the sensitive element and improve its stability, which is particularly meaningful for those detections involving using antibodies previously and other related research. As can be seen from the experiments, the microcantilever sensor technique can be used as an experimental platform for biochemical research. The most important feature is that the entire process of biochemical reactions between molecules can be monitored in real-time, so by combination with traditional biochemical experimental methods, such as quartz crystal microbalance (QCM) and surface plasmon resonance (SPR), it has far-reaching significance for the awareness of the microscopic mechanism of biological, chemical phenomena, and establishment of theoretical model.

References

[1] C. Tuerk and L. Gold: Science, vol. 249(1990), p. 505.

[2] Shao Ningsheng, Huang Yanping.: Biochemistry and Biophysics, vol. 33(2006), p. 329.

[3] Sun Shujun: Chinese Medical Abstracts · Oncology,vol. 20(2006), p. 62.

[4] Y. Huang, X. M. Nie, S. L. Gan, J. H. Jiang, G. L. Shen, and R. Q. Yu: Analytical Biochemistry, vol. 382(2008), p. 16.

[5] Y. Jiang, X. Fang, and C. Bai: Anal. Chem., vol. 76(2004), p. 5230.

[6] J. Wang, W. Meng, X. Zheng, S. Liu, and G. Li: Biosensors and Bioelectronics, vol. 24(2009), p. 1598.

[7] J. Zhu, Y. Huang, X.-P. Jiang, Z.-Y. Tan, J.-H. Jiang, G.-L. Shen, and R.-Q. Yu: Chinese Journal of Analytical Chemistry, vol. 37(2009), p. 1596.

[8] B. A. Johnson, M. A. Javors, Y. W. F. Lam, L. T. Wells, M. Tiouririne, J. D. Roache, N. Ait-Daoud, and K. Lawson: Progress in Neuro-Psychopharmacology & Biological Psychiatry, vol. 29(2005), p. 15.

[9] P. Fagergren, D. H. Overstreet, M. Goiny, and Y. L. Hurd: Neuroscience, vol. 132(2005), p. 1159.

[10] S. I. Dworkin, N. E. Goeders, J. Grabowski, and J. E. Smith: NIDA Res Monogr, vol. 76(1987), p. 221.

[11] M. Daras, B. S. Koppel, and E. Atosradzion: Neurology, vol. 44(1994), p. 751.

[12] F. C. Crawford, M. L. Wood, S. E. Wilson, V. S. Mathura, T. R. Hollen, F. Geall, D. N. Kolippakkam, and M. J. Mullan: Journal of Neurochemistry, vol. 97(2006), p. 662.

[13] C. Rubio, S. Strano-Rossi, M. J. Tabernero, L. Anzillotti, M. Chiarotti, and A. M. Bermejo: Forensic Science International, vol. 227(2013), p. 60.

[14] T. R. Kosten, G. Wu, W. Huang, M. J. Harding, S. C. Hamon, J. Lappalainen, and D. A. Nielsen: Biological Psychiatry, vol. 73(2013), p. 219.

[15] L. V. Fuscaldo, L. W. Bisol, and D. R. Lara: Addictive Behaviors, vol. 38(2013), p. 1859.

[16] Y. Li, H. L. Qi, Y. Peng, J. Yang, and C. X. Zhang: Electrochemistry Communications, vol. 9(2007), p. 2571.

[17] X. X. Li, H. L. Qi, L. H. Shen, Q. Gao, and C. X. Zhang: Electroanalysis, vol. 20(2008), p. 1475.

[18] J. Elbaz, B. Shlyahovsky, D. Li, and I. Willner: Chembiochem, vol. 9(2008), p. 232.

[19] J. Zhou, A. V. Ellis, H. Kobus, and N. H. Voelcker: Analytica Chimica Acta, vol. 719(2012), p. 76.

[20] P. G. Datskos, N. V. Lavrik, and M. J. Sepaniak: Sensor Letters, vol. 1(2003), p. 25.

[21] S. Cherian, A. Mehta, and T. Thundat: Langmuir, vol. 18(2002), p. 6935.

[22] X. H. Xu, T. G. Thundat, G. M. Brown, and H. F. Ji: Analytical Chemistry, vol. 74(2002), p. 3611.

[23] S. Shin, J. K. Paik, N. E. Lee, J. S. Park, H. D. Park, and J. Lee: Ferroelectrics, vol. 328(2005), p. 59.

[24] M. Watari, J. Galbraith, H. P. Lang, M. Sousa, M. Hegner, C. Gerber, M. A. Horton, and R. A. McKendry: Journal of the American Chemical Society, vol. 129(2007), p. 601.

[25] S. M. Yang, C. Chang, T. I. Yin, and P. L. Kuo: Sensors and Actuators B-Chemical, vol. 130(2008), p. 674.

[26] W. M. Shu, S. Laurenson, T. P. J. Knowles, P. K. Ferrigno, and A. A. Seshia: Biosensors & Bioelectronics, vol. 24(2008), p. 233.

[27] J. Zhang and H. F. Ji: Analytical Sciences, vol. 20(2004), p. 585.

[28] Y. M. Xu, B. L. Zhang, S. H. Wu, and Y. Xia: Analytica chimica acta, vol. 649(2009), p. 117.