Talanta ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Aptamer-based microcantilever biosensor for ultrasensitive detection of tumor marker nucleolin

Huiyan Li [a,b], Xiaojing Bai [a,c], Nan Wang [a,c], Xuejuan Chen [a,c], Jing Li [b], Zhe Zhang [a,c], Jilin Tang [a,*]

[a] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China
[b] School of Public Health, Jilin University, Changchun 130021, PR China
[c] University of Chinese Academy of Sciences, Beijing 100049, PR China

ARTICLE INFO

Article history:
Received 8 April 2015
Received in revised form
9 June 2015
Accepted 13 June 2015

Keywords:
Microcantilever biosensor
Nucleolin
Aptamer
AS1411
Tumor markers

ABSTRACT

We present an aptamer-based microcantilever biosensor for label-free detection of nucleolin. The sensor cantilevers in the microcantilever array were functionalized with nucleolin aptamer (AS1411) while the reference cantilevers were modified by 6-mercapto-1-hexanol (MCH) to eliminate environmental disturbances. The interaction between nucleolin and AS1411 induced surface stress changes, resulting in a differential deflection between sensor and reference cantilevers. The amplitude of differential cantilever deflection had a good linear relationship with the nucleolin concentration ranging from 10 nM to 250 nM with a correlation coefficient of 0.999. The detection limit was about 1.0 nM, at a signal-to-noise ratio of 3. The aptamer-based microcantilever sensor demonstrated good selectivity and was facile, rapid, and reagentless. Our results show the potential for the application of microcantilever biosensor system as a powerful tool to detect tumor markers with high sensitivity and specificity.

1. Introduction

Early and ultrasensitive detection of tumor markers play important roles in clinical diagnosis and evaluation of recovery of patients with certain cancer-related diseases [1]. Nucleolin is a multifunctional protein ubiquitously expressed in exponentially growing eukaryotic cells, which involved in the regulation of ribosome biogenesis and maturation. Nucleolin is overexpressed on cancer cells surface compared to normal ones, including renal cell carcinoma [2], breast cancer cells [3], lymphocytic leukemia [4], etc. [5,6]. Nucleolin can be selectively bound and internalized into cancer cell by anti-nucleolin aptamer. Because of these properties, we hypothesized an aptamer-based microcantilever biosensor for label-free detection of nucleolin.

Aptamers are artificial DNA or RNA oligonucleotides, which can be screened through SELEX (systematic evolution of ligands by exponential enrichment) processes from a random sequence bank [7,8]. They can bind to molecular targets such as drugs, viruses, proteins, cells and other small molecules with high affinity and specificity [9]. As an alternative to antibodies, aptamers have potential advantages over antibodies in analytical and diagnostic assays, such as easily screened, longer shelf-life, easy modification and high stability. These advantages make them good candidates for utilizing as recognition elements in biosensor applications, such as surface plasmon resonance (SPR) [10], fluorescent sensor [11], quartz crystal microbalance (QCM) [12], electrochemical sensors [13], and microcantilever biosensors [14,15]. Among these, microcantilever biosensors are attractive tools for detection of chemical and biological molecules. They have attracted considerable attention owing to the advantages including rapid response, higher sensitivity, simple procedures and lower sample volume [16,17]. Microcantilever biosensors are normally modified with receptor that exhibits high affinity to the target ligand on one surface of the cantilevers. Binding of the target on the sensitized surface changes the surface stress of the cantilevers and deflects the cantilevers [18].

AS1411 (also known as AGRO100) is a 26-mer guanine-rich (G-rich) oligonucleotides DNA, commonly known as anti-nucleolin aptamer, which could form a stable dimeric G-quadruplex structure to specifically target to nucleolin that highly expressed on cancer cells surface [19,20]. It has been developed for treating relapsed or refractory acute myeloid leukemia and renal cell carcinoma in phase II clinical trials [21]. In this work, we used AS1411 for label-free detecting nucleolin. The detection principle is illustrated in Fig. 1. The sensor cantilevers (shown in yellow) were functionalized with thiolated-AS1411, while the reference cantilevers (shown in turquoise green) were modified with 6-mercapto-1-hexanol (MCH) to eliminate the influence of other

* Corresponding author. Fax: +86 431 85262734.
E-mail address: jltang@ciac.ac.cn (J. Tang).

http://dx.doi.org/10.1016/j.talanta.2015.06.034
0039-9140/

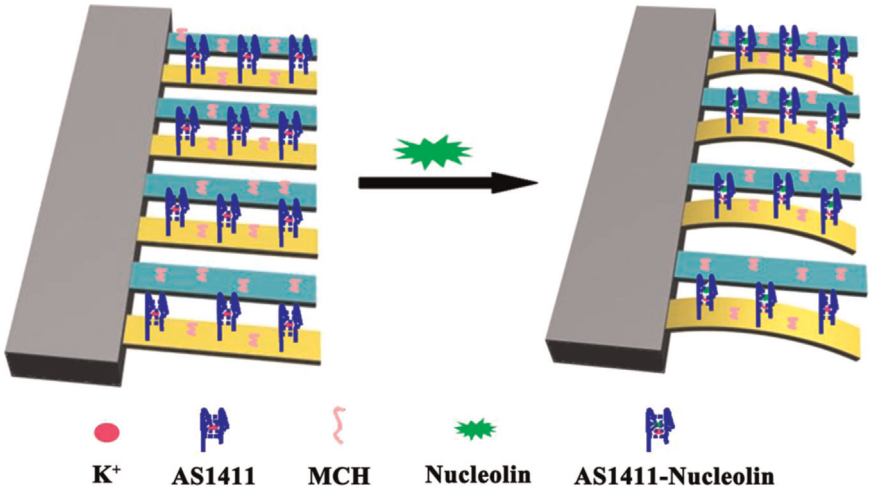

Fig. 1. Schematic representation of the microcantilever biosensor for nucleolin detection. Sensor cantilevers (yellow) were functionalized with AS1411 and reference cantilevers (turquoise green) were modified with MCH. Upon injection of nucleolin solution, the interaction between nucleolin and AS1411 causes a change of surface stress, resulting in a differential deflection of the sensor cantilevers compared with the reference cantilevers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

disturbances, such as nonspecific adsorption, ionic strength and temperature. The interaction of AS1411 and nucleolin changed the surface stress of the cantilevers and resulted in a differential deflection between sensor and reference cantilevers. On the basis of the deflection behavior of cantilevers, an ultrasensitive method for detecting nucleolin using the aptamer-based microcantilever biosensor was proposed. The approach provides a means to quantitative analysis of target proteins and produces an ultrasensitive method for convenient detection of tumor markers.

2. Materials and methods

2.1. Materials

Nucleolin aptamer (thiolated-AS1411) (5′-GGTGGTGGTGGTTG-TGGTGGTG GTGGTTTTTT-$(CH_2)_6$-SH-3′) was synthesized by Sangon (Shanghai, China). Nucleolin was purchased from Shanghai Wuhao Trade Co., Ltd. (Shanghai, China). Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) was obtained from Alfa Aesar (Tianjin, China). 6-Mercapto-1-hexanol (MCH) was purchased from Sigma-Aldrich. All other reagents were of analytical grade and used without further purification. The running buffer was prepared with 50 mM phosphate buffered saline (PBS) containing 100 mM KCl, pH 7.4. The functionalization buffer contained 20 mM Tris–HCl, 100 mM KCl, and 1 mM TCEP, pH 7.4. TCEP was used to cleave the disulfide bond on the aptamers, generating a free-sulfhydryl group to form self-assembled monolayers (SAMs) [22]. All solutions in the experiments were prepared with ultrapure water from a Millipore Milli-Q water system (18.2 MΩ cm) and stored at 4 °C prior to use.

2.2. Preparation of microcantilever array

The microcantilever array was obtained from Concentris GmbH (Basel, Switzerland). The microcantilever array consist of eight rectangular silicon cantilevers, which are 500 μm in length, 100 μm in width, and 1 μm thick. Each array was coated with 3 nm of titanium followed by a 20 nm gold layer, resulting in a bimetallic structure [23]. Before functionalization, the microcantilever array was cleaned using an UV-ozone cleaner for 30 min. Then the microcantilever array was rinsed thoroughly with pure water and ethanol, respectively. The freshly cleaned microcantilever array was individually functionalized using four glass capillaries filled with 1 μM thiolated-AS1411 for 3 h at room temperature. The capillaries allowed the thiolated-AS1411 solution to flow onto the cantilevers surface through the microchannels. After functionalization, the microcantilever array was rinsed thoroughly with water. Subsequently, the microcantilever array was incubated in 2 mM MCH for 1 h to block the remaining bare region. Finally, the microcantilever array was rinsed again with ethanol and water for three times.

2.3. Measurements and apparatus

The deflection measurements were performed by using a commercial Cantisens sensor platform (Concentris GmbH, Basel, Switzerland). The functionalized microcantilever array was mounted in a microcantilever array holder and then inserted into a liquid chamber in which running buffer was pumped past the microcantilever array via a syringe pump. The temperature in the liquid chamber was kept constant at 37 ± 0.01 °C. The running buffer was flowed past the functionalized microcantilever array and pumped to the waste reservoir via a syringe pump at a rate of 0.42 μL/s during the experiment. The microcantilever array was equilibrated in running buffer until a stable baseline was obtained. Then the microcantilever array was exposed to the nucleolin solution with concentrations ranging from 0.1 nM to 250 nM. Real-time deflection of each cantilever was monitored via the optical beam deflection system of the platform. The cantilever deflection is result of a surface stress change induced by molecular interaction which occurs on only one of the cantilever surface. Stoney's equation describes the relationship between the deflection Δz (m) and surface stress change $\Delta\sigma$ (N/m) of the cantilevers [24]:

$$\Delta\sigma = \frac{Et^2}{3(1-\nu)L^2}\Delta z \quad (1)$$

where L and t are the cantilevers length and thickness, respectively. E is Young's modulus, ν is Poisson's ratio. Here, we define the bending of cantilever toward gold side as positive, while toward the silicon side as negative.

3. Results and discussion

3.1. Deflection of cantilevers induced by AS1411–nucleolin

interaction

In this work, microcantilever biosensor working in static mode was used to investigate the binding between AS1411 and nucleolin. The sensor cantilevers were individually functionalized with thiolated-AS1411 via Au–S bond. To prevent environmental disturbances (nonspecific adsorption, ionic strength and temperature), the reference cantilevers were modified with MCH. The functionalized microcantilever array was inserted into the liquid chamber and the deflection of each cantilever was monitored in real-time via a scanning laser diode. The average deflection signal in Fig. 2A represents the mean value of four identically functionalized cantilevers within an array. The differential deflection in Fig. 2B is derived by subtracting the averaged signal of the four reference cantilevers from that of the four sensor cantilevers. It represents the specific interactions between nucleolin and AS1411.

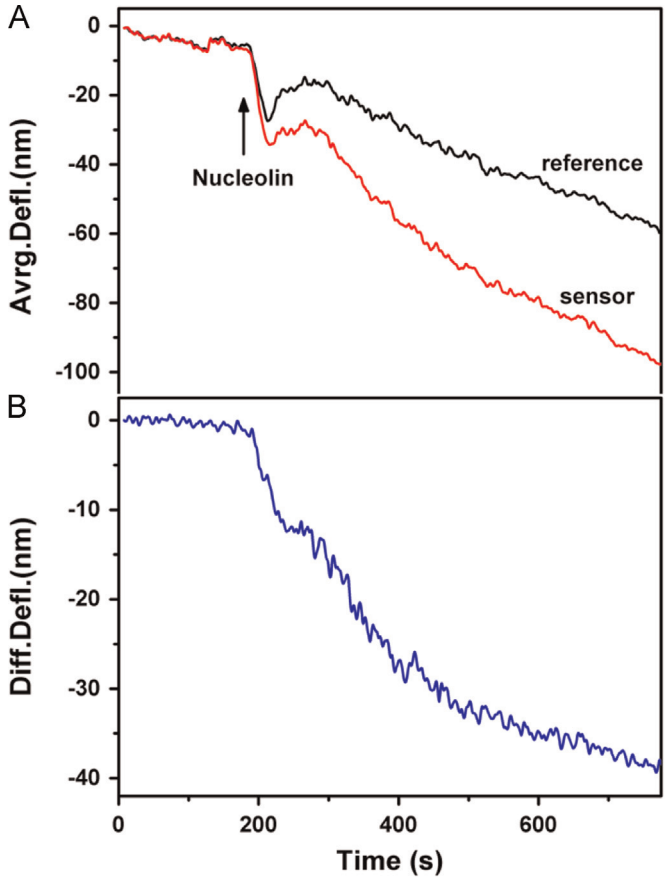

Fig. 2. Detection of nucleolin with AS1411 functionalized microcantilever array. (A) Average deflections (Avrg.Defl) response of aptamer coated cantilevers (shown in red) and MCH-coated cantilevers (shown in black) against time. Upon injection of 100 nM nucleolin solution, the deflection of the cantilevers was measured in situ. (B) Differential deflection (Diff. Defl) reveals the specific interaction between AS1411. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Before nucleolin was introduced, the microcantilever array was only exposed to the running buffer until a stable baseline was obtained. Then 100 nM nucleolin was injected into the liquid chamber. Time-dependent cantilever deflection is shown in Fig. 2. As can be seen from Fig. 2A, upon introduction of 100 nM nucleolin solution (at about 180 s), the sensor cantilevers bent toward the silicon side exhibiting a significant negative deflection (red, Fig. 2A) while a relatively weaker negative deflection can be seen for the reference cantilevers (black, Fig. 2A). The weaker deflection of reference cantilevers can be ascribed to the influence of environmental changes. As illustrated in Fig. 2B, a differential deflection of about 38 nm was obtained at about 775 s after 250 μL nucleolin solution was injected. According to the Stoney's formula (Eq. (1)), the bending signal corresponded to a surface stress change of ~10.07 mN/m. The surface stress changes as a result of the interaction between AS1411 and nucleolin. The formation of nucleolin–AS1141 complexes may cause physical steric crowding of the high density aptamers that immobilized on the gold side of the cantilevers. As a result, the nucleolin–AS1141 complexes were trying to expand which finally led to an increased repulsion between the neighboring chains. The interchain repulsion induces a change in the surface stress, causing the sensor cantilevers to bend toward the silicon side.

3.2. Cantilevers response to different concentrations of nucleolin

The sensitivity of microcantilever biosensor detection was investigated by determining the response of the microcantilever array to different concentrations of nucleolin. The real-time detection signals for different concentrations of nucleolin are shown as in Fig. 3A. It was demonstrated that the differential cantilever deflections increased from about 9 nm to 71 nm with the increase of nucleolin concentrations from 0.1 nM to 250 nM at about 775 s.

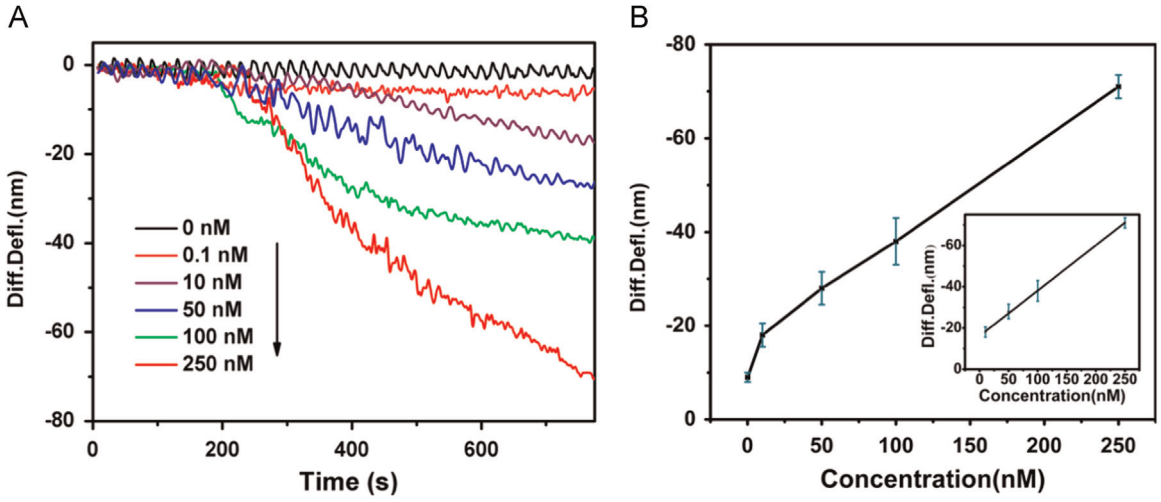

Fig. 3. (A) Differential deflection as a function of time for different concentrations of nucleolin: 0, 0.1, 10, 50, 100, 250 nM. (B) Calibration curve of differential deflection for different concentrations of nucleolin: 0.1, 10, 50, 100, 250 nM. Inset: linear relationship between the differential deflection of cantilevers and different concentrations of nucleolin: 10, 50, 100, 250 nM.

Please cite this article as: H. Li, et al., Talanta (2015), http://dx.doi.org/10.1016/j.talanta.2015.06.034

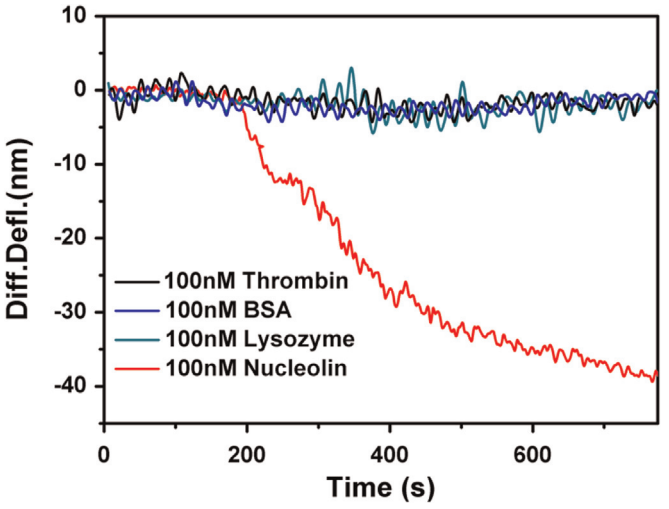

Fig. 4. The selectivity of the microcantilever biosensor for nucleolin. The concentration of nucleolin and nonspecific proteins (BSA, lysozyme, thrombin) are all 100 nM.

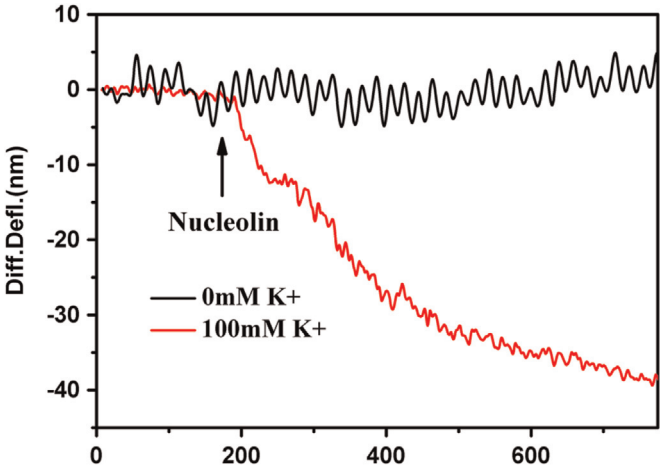

Fig. 5. Differential deflection as a function of time for 100 nM nucleolin in two different running buffers (50 mM PBS and 50 mM PBS + 100 mM KCl).

This indicates that more aptamer–nucleolin complexes are formed on the sensor cantilevers surface with the increase of the nucleolin concentration. The calibration curve for quantitative measurement of nucleolin is shown in Fig. 3B. The plot illustrates a good linear correlation between differential deflection and concentrations of nucleolin in the range of 10–250 nM with a correlation coefficient of 0.999 (inset of Fig. 3B). The linear regression equation is $Y = -16.1 - 0.21X$, where X is the concentration of nucleolin in nM, and Y is the differential deflection (nm). Additionally, the detection limit (LOD) is calculated to be about 1.0 nM using a signal-to-noise ratio of 3. The error bars illustrate the relative standard deviation (RSD) for three replicates.

3.3. Selective detection of nucleolin using microcantilever biosensor

In order to investigate the selectivity of aptamer-based microcantilever biosensor for nucleolin detection, we introduced three other proteins (thrombin, lysozyme and bovine serum albumin (BSA)) to the functionalized microcantilever array. As displayed in Fig. 4, no significant deflection was observed after adding the other three proteins (100 nM) into the liquid chamber. However, the addition of nucleolin resulted in dramatic deflection of the cantilevers. It indicates that the designed aptamer-based microcantilever biosensor has high selectivity to nucleolin.

3.4. Effect of K^+ on the interaction of AS1411 with nucleolin

It was reported that potassium ion concentration had an effect on the interaction between aptamer and target ligand [25,26]. The effect of potassium ion cantilever deflection was studied. As illustrated in Fig. 5, in the presence of 100 mM potassium ion in running buffer, 100 nM nucleolin solution induced a significant deflection of the cantilevers, while in the absence of potassium ion, the response of the cantilevers to 100 nM nucleolin solution was negligible. This phenomenon demonstrates that potassium ions are essential for the interaction between AS1411 and nucleolin. It might be due to that AS1411 can dimerize to form a highly symmetric bimolecular G-quadruplex consisting of four pairs of stacked G-quartets [27–29] in the presence of K^+ and it can specifically bind to nucleolin to form aptamer–nucleolin complexes. We also found, a dense self-assembled thiolated-AS1411 monolayer was formed on gold substrate. The high density of aptamers on cantilevers may enhance the possibility of a dimer formation (Supporting information, Fig. S1).

4. Conclusions

We have developed an aptamer-based microcantilever biosensor for label-free detecting nucleolin. The interaction between nucleolin and AS1411 changes the surface stress, resulting in a differential deflection of the microcantilever array. It had shown a good linear relationship between different deflection of the cantilevers and nucleolin concentration over the range from 10 nM to 250 nM, and the detection limit was about 1.0 nM. The microcantilever biosensor system showed high sensitivity and selectivity. It demonstrates that our proposed approach is facile, rapid, and reagentless. Moreover, this sensor also exhibits the potential applications to detect various biomolecules, such as proteins, cells, virus, drugs, and other small molecules, as long as aptamers can be available for the molecules of interest.

Acknowledgments

This work was supported by National Basic Research Program of China (973 Program; No. 2011CB935800), the National Natural Science Foundation of China (Nos. 21275140 and 21405152), and the Science and Technology Development Plan of Jilin Province (20130101126JC).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2015.06.034.

References

[1] D. Faraggi, A. Kramar, Urol. Oncol. 5 (2000) 211–213.
[2] J.E. Rosenberg, R.M. Bambury, E.M. Van Allen, H.A. Drabkin, P.N. Lara Jr., A. L. Harzstark, N. Wagle, R.A. Figlin, G.W. Smith, L.A. Garraway, T. Choueiri, F. Erlandsson, D.A. Laber, Invest. New Drugs. 32 (2014) 178–187.
[3] S. Soundararajan, W. Chen, E.K. Spicer, N. Courtenay-Luck, D.J. Fernandes, Cancer Res. 68 (2008) 2358–2365.
[4] Y. Otake, S. Soundararajan, T.K. Sengupta, E.A. Kio, J.C. Smith, M. Pineda-Roman, R.K. Stuart, E.K. Spicer, D.J. Fernandes, Blood 109 (2007) 3069–3075.
[5] A. Pich, L. Chiusa, E. Margaria, Micron 31 (2000) 133–141.
[6] D. Trere, M. Derenzini, V. Sirri, L. Montanaro, F. Grigioni, G. Faa, G.M.

L. Columbano, A. Columbano, Hepatology 24 (1996) 1269–1273.
[7] C. Tuerk, L. Gold, Science 249 (1990) 505–510.
[8] A.D. Ellington, J.W. Szostak, Nature 346 (1990) 818–822.
[9] G. Yan, Y. Wang, X. He, K. Wang, J. Liu, Y. Du, Biosens. Bioelectron. 44 (2013) 57–63.
[10] M. Piliarik, M. Vala, I. Tichy, J. Homola, Biosens. Bioelectron. 24 (2009) 3430–3435.
[11] R. Nutiu, Y.F. Li, J. Am. Chem. Soc. 125 (2003) 4771–4778.
[12] I.S. Park, N. Kim, Biosens. Bioelectron. 13 (1998) 1091–1097.
[13] B.R. Baker, R.Y. Lai, M.S. Wood, E.H. Doctor, A.J. Heeger, K.W. Plaxco, J. Am. Chem. Soc. 128 (2006) 3138–3139.
[14] X. Bai, H. Hou, B. Zhang, J. Tang, Biosens. Bioelectron. 56 (2014) 112–116.
[15] H. Hou, X. Bai, C. Xing, N. Gu, B. Zhang, J. Tang, Anal. Chem. 85 (2013) 2010–2014.
[16] K.R. Buchapudi, X. Huang, X. Yang, H.F. Ji, T. Thundat, Analyst 136 (2011) 1539–1556.
[17] G.H. Wu, R.H. Datar, K.M. Hansen, T. Thundat, R.J. Cote, A. Majumdar, Nat. Biotechnol. 19 (2001) 856–860.
[18] K. Kang, A. Sachan, M. Nilsen-Hamilton, P. Shrotriya, Langmuir 27 (2011) 14696–14702.
[19] J.J. Liu, X.R. Song, Y.W. Wang, A.X. Zheng, G.N. Chen, H.H. Yang, Anal. Chim. Acta 749 (2012) 70–74.
[20] J. Ai, Y. Xu, B. Lou, D. Li, E. Wang, Talanta 118 (2014) 54–60.
[21] L. Feng, Y. Chen, J. Ren, X. Qu, Biomaterials 32 (2011) 2930–2937.
[22] Y. Chen, T.S. Pui, P. Kongsuphol, K.C. Tang, S.K. Arya, Biosens. Bioelectron. 53 (2014) 257–262.
[23] K.W. Liu, S.L. Biswal, Anal. Chem. 83 (2011) 4794–4801.
[24] G.G. Stoney, Proc. R. Soc. Lond. Ser. A 82 (1909) 172–175.
[25] K.N. Luu, A.T. Phan, V. Kuryavyi, L. Lacroix, D.J. Patel, J. Am. Chem. Soc. 128 (2006) 9963–9970.
[26] A.T. Phan, K.N. Luu, D.J. Patel, Nucleic Acids Res. 34 (2006) 5715–5719.
[27] V. Dapic, V. Abdomerovic, R. Marrington, J. Peberdy, A. Rodger, J.O. Trent, P. J. Bates, Nucleic Acids Res. 31 (2003) 2097–2107.
[28] F. Boan, M.G. Blanco, P. Barros, A.I. Gonzalez, J. Gomez-Marquez, FEBS Lett. 571 (2004) 112–118.
[29] T. Li, L. Shi, E. Wang, S. Dong, Chem. Eur. J. 15 (2009) 1036–1042.

Please cite this article as: H. Li, et al., Talanta (2015), http://dx.doi.org/10.1016/j.talanta.2015.06.034