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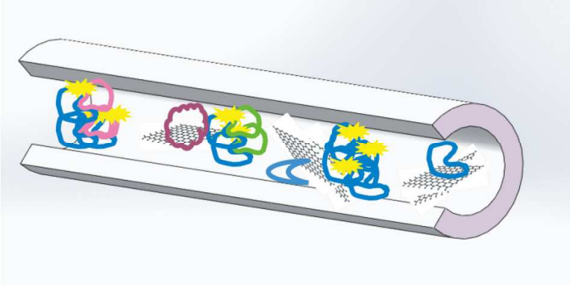

A new sense of parallel label-free target detection based on the combination of chip electrophoresis and nanopartical optical biosensor.

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ARTICLE

A Novel Microfluidic Chip Electrophoresis Strategy for Simultaneous Label-free Multi-Protein Detection Based on Graphene Energy Transfer Biosensor

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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Abstract: A new sense of high throughput and parallel optical sensoring platform with single color probe based on microfluidic chip electrophoresis combined with aptamer-carboxyfluorescein/graphene oxide energy transfer was reported here. Label-free protein multi-targets were detected, even in challenging complex sample without any pre-treatment.

Introduction

When the emission spectrum of donor fluorescent molecule and the absorption spectrum of receptor overlap, and the distance between two molecules within 10nm, Förster resonance energy transfer (FRET) happens as a phenomenon of non-radioactive energy transfer via long-range dipole-dipole interactions. This effect forms the basis of widely application of elaborate-designed FRET with pronounced selectivity and sensitivity [1]. Simultaneous detection of multiple analytes sensoring is appealing for industrial, environmental and biosecurity applications. Sensitive and multiplex protein detection is vitally important in numerous areas because it can provide necessary information for deeper understanding the genomic expression, molecular diagnostics and pharmacoproteomics. Nevertheless, it is noteworthy that simultaneously parallel multiple targets optical sensoring remains challenging. In order to increase the efficiency in this kind of mix-and-detect assay, multicolor DNA probes were synthesized [2, 3]. But the advance towards high-throughput sensoring is hampered by the limited number of color probes available. In the same way as electrochemical sensor array, one kind of sensor array with ensemble aptamers for a wider range of targets was reported [4]. In addition, capillary electrophoresis (CE) was also employed to assist the simultaneous detection of dual single-base mutations in a given DNA oligonucleotide using two probes [5], excepted being used as an effective separation of doner-acceptor complexes from free donor and acceptor to avoids the interference of unhybridized molecular beacons (MB) signal[6, 7]. However, as far as we know, separation technique based label-free multiple detection with individual color probe has never been reported. The striking emphasis of no sample pre-treatment in traditional mix-and-detect method actually increased the difficulty to realize multi-target analysis. Being the most efficient separation technique, chromatography could be combined with RET, instead of repelling each other, to innovate multitarget sensoring. Actually, if the separation ability is high enough, the interaction between probe and target could also be more conveniently demonstrated avoiding the trouble of probe array immobilization. In this sense of mean, chromatography can also regarded as one kind of array technology without the trouble of probe array immobilization.

What is the cause of the no report on this until now? It was reasonable to infer that the dedicated interaction balance among nanomaterial, energy donor and bioconjugate pair made it difficult for the biosensor system to adapt to the interaction with stationary phase and harsh elution conditions in traditional liquid chromatography separation. CE was employed [5-7] due to free solution environment and mild separation conditions. However, in order to further increase the detection number of target in CE, all strategies to improve the separation resolution should be considered [8]. Microfluidics has been greatly advanced since the μ-TAS (miniaturized total analysis systems) was coined by Manz [9]. Common analytical assays, including polymerase chain reactions, protein analysis, DNA separation, and cellular studies, have been performed in microfluidic devices [10]. Among many characteristics of multidimensional chip electrophoresis, including fast, low volume sample consumption and portability etc., the most striking and advantageous characteristics, especially compared with CE, is the higher peak capacity which is expected to allow high throughput and parallel analysis of targets easily. Thanks to these advantages, chip based chromatography might bring us a new result.

There are many kinds of functional nanoparticles, such as quantum dots, metal nanoparticles, and carbon nanomaterial used into optical biosensoring. Graphene is receiving great interest in various areas because of its extraordinary electronic, thermal, and mechanical properties [11]. As a result of p-stacking interactions, graphene oxide (GO) sheets can strongly adsorb single stranded nucleic acids, and then quench the fluorescence of organic dye, which is labelled to ssDNA [12-14].Very recently, biological applications of GO, have been very active research field in the development of biosensors because of the ultrahigh quenching efficiency of GO [2,4,15-19]. And then, in order to investigate the possibility of multi-

detection based on chip-CE, in the same way as literature, in this paper GO was anticipated to serve as an energy acceptor of fluorescein FRET system to construct a fluorescent sensor for sensitive detection of the biorecognition event between aptamer and protein. Three kinds of proteins were chosen as target proteins considering their aptamers had been utilized by others demonstrating their stronger binding affinity [20-22]. Furthermore, since most sensing work using oligonucleotide probes has been performed on model systems, and few clinically relevant targets have actually been tested [23], the ability of this proposed multiplex detection in complex matrix was also demonstrated here.

Experimental

Reagents

Three kinds of DNA aptamers targeting thrombin (5'FAM-AGTCCGTGGTAGGGCAGGTTGGGGTGACT-3') [21], cytochrome c (5'FAM-AGTGTGAAATATCTAAACTAAATGTGGAGGGTGGGACGGGAAGAAGTTTATTTTTCACACT-3') [22] and lysozyme (5'FAM-ATCTACGAATTCATCAGGGCTAAAGAGTGCAGAGTTACTTAG-3') [23] respectively were synthesized by Sangon Biotech (Shanghai, China), with a 6-FAM label was attached to the 5' end of each apatmer. Thrombin, cytochrome c and lysozyme and carrier ampholytes (pH 3–10) were purchased from were all purchased from Sigma-Aldrich (St. Louis, MO). Other reagents were purchased from Beijing Chemical Works (Beijing, China). Graphene oxide (GO) was synthesized from graphite powder using Hummer's method [24]. The electrophoresis chip utilized a spin-coated chromium plate (SG2506, UM grade, chromium layer thickness 145 nm; Az-1805 photoresist, 570 nm thickness; Hunan Changsha Shaoguang Microelectronics Co.) as substrate, and the same type of sheet glass as a cover slip. All aqueous solutions were prepared with purified water using a Millipore Purification System (Billerica, MA).

Microchip fabrication

Glass chips were fabricated according to standard procedures involving photolithography, wet chemical etching, hole drilling, and high-temperature bonding [25]. A photomask with multi-dimensional microchannels structure designed using Illustrator was placed onto a glass substrate with chromium and photo-resistant coating via UV exposure using a model JKG-2A lithographic device (Photomechanical, Shanghai, China). Then, the glass plate was immersed into 0.15% NaOH for 2 min, followed by water flushing, drying at 110 °C for 15min and chromium removal. Then, the microchannels were etched in HF/HNO3. After etching, the etched substrate and cover plate were washed sequentially with acetone and water and chromium was removed again. The plate was dried by heating, and then drilled. The plate was activated in concentrated sulphuric acid for 8 h, followed by washing with water, bonding and heating at 60 °C. Finally, the chip was placed in an oven at 110 °C for high-temperature bonding.

The experimental device and the microfluidic chip with double T structure were shown in Fig.1. One kind of two-dimensional chip with a double "T" structure was shown, including a 263 μm wide and 1.4 cm long straight horizontal IEF channel intersected by a secondary dimensional CGE separation channel 130 μm wide and 3.5 cm long. Another kind of two-dimensional chip in which the IEF channel (263 μm wide and 1.4 cm long) were joined by 4 narrower channels (130 μm wide and 3.5 cm long) for secondary CGE separation was also given.

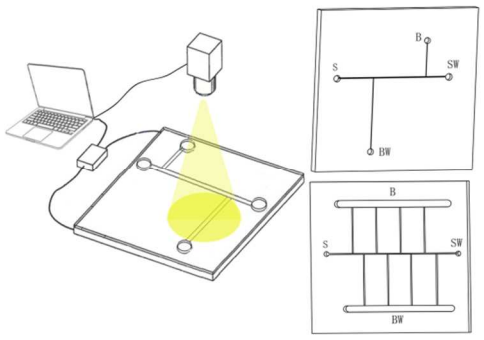

Figure1. (a) The scheme of the experimental device; (b) The chip for electrophoresis with simple double T structure (upper) and another kind of chip structure with multichannel (below).

Microchip electrophoresis for protein analysis

Sample preparation

Firstly, 2μM aptamer of thrombin, 10μM aptamer of cytochrome c and 10μM aptamer of lysozyme were added into 0.3mg/mL GO solution, followed by incubating in oven at 90℃ for 5min. The mixture was cooled at room temperature. After that, thrombin, cytochrome c and lysozyme (0.25mg/mL) were put into the solution with the volume ratio of GO, the three fluorescently-labelled aptamers and three proteins of 1:2:2:2:2:2:2. Finally, ampholyte was mixed into the solution with the volume ratio of 10% v/v.

In the preparation of complex protein sample, the complex protein samples were prepared by extraction from normal bacteria cells. The cultured E.coli (DH5α) cells were centrifuged at 4℃, 10,000 g for 15 min to precipitate the thallus. The precipitate was collected and re-suspended in PBS buffer (pH 7.5) followed by addition of cell lysis buffer. The whole proteins of E. coli (0.25mg/mL) were introduced into the incubation solution with GO, thrombin, cytochrome c and lysozyme, and their specific FAM-labelledaptamer in the volume ratio of 2:1:2:2:2:2:2:2 respectively. Finally, ampholyte (10% v/v) was mixed into the solution. After that, the sample could be injected into the chip microchannel for further analysis.

Two-dimensional microfluidic chip electrophoresis

As indicated in Fig. 1, isoelectric focusing (IEF) was performed between reservoir S and SW, and the secondary capillary zone electrophoresis (CZE) was performed between reservoir B and BW. The microfluidic chip electrophoresis was carried out as follows: (a) Before each electrophoresis, the microchannels were washed sequentially with 1 mol/L NaOH for 20 min, water for 10 min and the 0.5% methyl cellulose dissolved in Tris-HCl 10 min; (b) The 0.5% MC solution in the first-dimension channel was pumped out and changed with sample prepared above; (c) An electric potential was then applied to the microchannels by means of platinum electrodes placed in the reservoirs, using a computer-controlled high-voltage power supply (XCDY microfluidic chip electrical source purchased from Shandong Normal University), between the acid reservoir

(reservoir S, anode) and the alkaline reservoir (reservoir SW, ground), for 30 s to initiate protein IEF; (d)After IEF, the electrical voltage was switched to reservoir B(ground) and reservoir BW (anode) for second-dimensional CZE separation. The typical separation medium in secondary channels was 0.5% MC solution. The progress of applied voltage and time for two-dimensional electrophoresis listed in Table 1 (in ESI).

Data collection and Image analysis

The whole process was monitored and recorded using an IX70 inverted fluorescence stereomicroscope equipped with a CCD camera (Olympus, Japan). The field of view of the inverted microscope was aimed at the downstream of the secondary dimensional channel as demonstrated at Fig.2. The spacing from the first channel to the fourth channel was in the diameter size of the microscope field. And then the separation behaviours in all channels could be captured and recorded by microscope. ImageJ software (http://rsb.info.nih.gov/ij) was used to convert the photographs captured from the videos of electrophoresis into 3D electropherograms based on the fluorescence intensities in the channels.

Results and discussion

As we assumed that, the well-established FRET process involved two basic steps in this proposed system. First, fluorescein-based dye (FAM) labelled ssDNA of aptamers assembled noncovalently onto GO. Due to the strong interaction between aptamers and GO, the fluorescence of labelled FAM was quenched by FRET between GO and FAM. Second, upon the introduction of proteins, the specific binding between aptamer and its corresponding protein target resulted the change of the interaction between aptamer and GO, and then the release of ssDNA probe from the surface of GO. As a result, fluorescence of FAM-labelled aptamer was restored. Otherwise, there was no recovery of fluorescence without the target protein. The schematic diagram of chip-CE-assisted biosensor for simultaneous detection of protein was given in Fig.2.

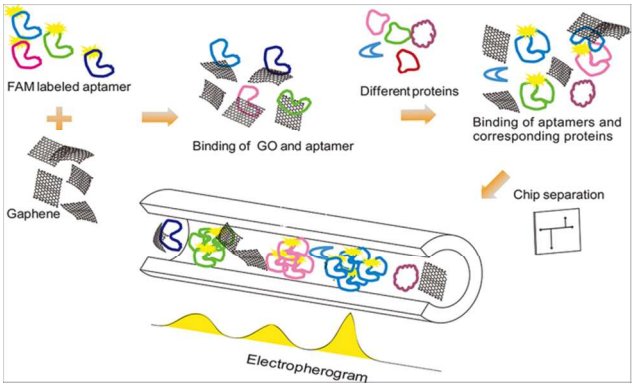

Figure 2.The schematic diagram of chip-CE-assisted biosensor for simultaneous detection of protein.

Initially, experiments were carried out to optimize the ratio of GO and FAM labelled aptamer. On one hand, the interference of fluorescent background brought by free FAM labelled aptamers was thus avoided. On the other hand, the proper ratio of between GO and FAM labelled aptamer made it efficient for the aptamer-protein complex to release from the surface of GO, so that an obvious FRET signal change could be observed in the presence of target analyte. 0.3mg/mL was finally chosen the as optimized GO concentration because fluorescence intensity was only 0.4% of the original one. In addition, the ratio of aptamer to GO conjugates added into the mixture was not the same among different aptamer because it was found that the extent of fluorescence recovery after the binding of aptamer and protein was different. According to literature [20-22], it may be ascribed to the diverse affinity between these three pairs of protein-aptamer whose sequence structure was designed. With only one kind of fluorescent probe being used, the existence of multi-targets could not be determined by current fluorescence spectra based ensemble measurement. Chip-CE played a crucial role in achieving multiplex detection. One of the first issues confronting the experiment was the enough separation ability. In this paper, one kind of isoelectric focusing (IEF) and capillary zone electrophoresis (IEF-CZE) coupled two-dimensional electrophoresis chip designed with double T structure (Fig.3 upper right) was used. It was found through the fluorescent electrophoresis images collected after first dimensional IEF, that the mixture was failed to be separated by only the IEF separation mode between S and SW (data in ESI), whereas it was not the case with multidimensional separation. Fortunately, taking advantage of high performance IEF/CGE coupled 2D chip electrophoresis with the double T structure chip, as shown in Fig. 3, three protein-aptamer complexes were baseline separated after being further separated by the secondary dimension of CZE. The existence of target protein could be determined according to the occurrence of electrophoresis peak which was correspondent to the complex of aptamer and protein. In this way, three proteins can be detected simultaneously and parallel. As for other chromatography based strategy, such as western-blotting and CE (5-7) in which all the components are detected without selectivity, and then the successful separation of all components is prerequisite for target analysis. In our system, only these targets specifically binding with aptamer could create the fluorescent component which could be observed through next electrophoresis separation. The limited amount of fluorescent components need to be detected certainly greatly reduced the burden on following separation.

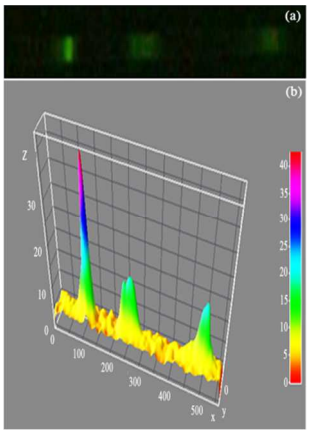

Figure 3. (a) Fluorescent separation images using IEF and CGE coupled two-dimensional microchip electrophoresis (thrombin, cytochrome c and lysozyme from left to right); (b) The 3D plot spectrum of (a).

The sizes of GO particles existing in the buffer background during electrophoresis should be small enough to avoid influence on protein separation and subsequent fluorescent detection with light scattering. At the same time, it was assumed by us that the main difference in our experiment among the various sizes of GO smaller than the one-size limit was the adsorption ability. Therefore, different ratios of GO and fluorescent-labeled aptamer should be optimized respectively. In the study described in this paper, GO was synthesized from graphite powder using Hummers' method [24]. The clear GO aqueous water solution was also prepared in the same way as literatures for FRET-based optical sensors. Through atomic force microscopic (AFM) analysis, the size of graphene used for this experiment was about 2 μm (data in the ESI). From our experiment, satisfactory electrophoresis separation and fluorescent detection was obtained when this kind of GO was used, thus demonstrating that this size of GO was appropriate; accordingly, this size of GO was chosen for investigation. Based on that, we could further optimize the ratio of GO and fluorescent-labeled aptamer to realize multi-target detection. Another thing worth mentioning is that after IEF, how to transfer focus fluorescent protein-aptamer complexes from IEF channel into the secondary dimension. Some components might focused outside the two junctions of the first and secondary channels, for example the pI value of proteins was outside the pI range of ampholine used for IEF(thrombin (pI=6.6, MW=37,000), cytochrome c (pI=10.7, MW=12,000) and lysozyme (pI=11, MW=14,300)). In this experiment, the positions of the fluorescent protein-aptamer complex focused were within the two junctions of the first and secondary channels. There might be a degree of variation to pI value of proteins after binding with aptamer. In case of transferring focused band outside the junctions, the adjustment of the position of focused band with TEMED [26] could be applied.

The next goal was to confirm the selectivity and the robustness of the assay for multiplex protein sensoring in real complex mixture. This method was then used to detect three target proteins in the whole proteins of E. coli cell. There is the possibility of the existence of non-specific interaction interference by E. coli proteins. However, a minimal fluorescence increase was observed in the presence of interference proteins, while a significant fluorescence enhancement was observed for target proteins. It was illustrated that the non-specific interaction between aptamers and E. coli was not strong enough; that is, it could not compete with the specific interaction between aptamers and their corresponding protein targets to release ssDNA from GO. The interference could also be corrected from the background through control experiments. In addition, for those blank GO substrates without adsorbed aptamer, the non-specific interaction between GO and other proteins did not influence the binding between aptamers and their corresponding protein targets, and thus, analysis of the target protein. Therefore, our system was not influenced by non-specific interactions. The selectivity and specificity of the aptamer-GO-based FRET biosensor in complex samples has also been described in other reports.

All that remains now is the chip electrophoresis separation in which the achievement of three target pair separation within complex protein matrix was the key challenge then. In the case of extremely complicated sample, the chip electrophoresis with simple double T structure (Fig.1 b upper) could not realize well separation of the target complexes suggesting the limited separation capacity (Fig.4(a)). Still, the consistent position of electrophoresis peak with the analysis in simple sample matrix in 3.2 displayed the separation reproducibility of our proposed assay. To further increase the resolving power of this sensor system, another kind of chip structure of multichannel in secondary CZE dimension (Fig.1 below right) with greatly increased separation capacity was employed. Significantly, three fluorescence bands corresponding to three aptamer target protein could be observed in the microchannels (Fig. 4(b)).The peak identifications were confirmed by separating a cell homogenate sample spiked with the three respective proteins. Since the peaks in the electropherogram were ascribed to protein and FAM-labeled aptamer complexes, therefore the migration behaviour cytochrome c and lysozyme with similar pI value and molecular weight were not the same. Their migration behaviours (especially the intervals between peaks) were not only influenced by the pI value and molecular weight of the protein alone, but also by non-selectively labeled FAM, especially the longer-bound ssDNA chains with 61 and 42 bases

The necessity of the high performance separation ability was then indicated again. The simultaneous detection of multi-targets depends not only on the selective recognition of aptamers for the targets but also on the separation ability of chip electrophoresis. The proposed method was not affected by the other thousands of proteins. Also strikingly, the raw complex sample could be analysed directly without any pre-treatment and pre-labelling. For this experiment, due to the existence of high fluorescence background in blood sample, the whole protein of E.coli., instead of extra-blood sample, was employed here to prepare simulated real complex sample. Its great advance toward real clinical assay could be prospected without doubt. Considering there are certain separation capacity correspondent to certain channel structure chip, simply increase of GO and aptamer concentration [4] which might influence separation was not a proper solution. There are a lot of reasonable choices, for example fluorescent acceptor [27] or chemiluminescence resonance energy transfer [28] can be employed to overcome the background interfere brought by matrix.

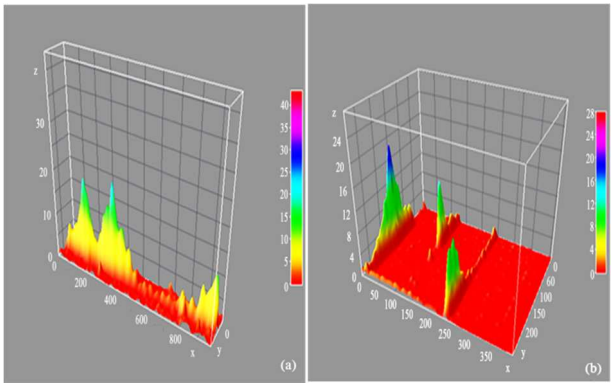

Figure 4.The 3D plot of the fluorescent separation images of IEF-CGE coupled two-dimensional microchip with (a) double T structure and (b) with multichannel in secondary dimension.

Conclusions

Without the trouble of probe array immobilization, a new idea of chromatography based probe array technology was proposed here. The novel combination of resonance energy transfer (RET) based sensor method with high performance chip

electrophoresis offers several advantages. First, the investigation here open a new way to realize a new sense of high throughput and parallel sensoring by the combination of chromatography with RET based optical sensor assay. There are two striking advantages. One is that there was no sample pre-treament and pre-label involved. Another is that no multicolor DNA probes are required. At the same time, there was no immobilization involved unlike the strategy of sensors array biochip. This methodology should have a wide applicability because in theory with sufficient specific sensing elements, the number of targets would be considerable, as long as the separation resolution is allowed. Secondly, high detection sensitivity with low background noise was ensured by superquenching ability of GO. Furthermore, ratiometric imaging is also expected to increase the signal-to-background ratio, and thus afforded greater sensitivity in fluorescent digital imaging. Meanwhile, the irreplaceable advantage of integration make the microfluidic chip a good candidate to realize high resolution separation with further improved sensitive detection. The lowest detection limit of our method and how to increase the number of targets detected are now under way with further experiment optimization. Third, one emphasis in today bioassay is the move to methods that are quick, simple, small sample size and portability equipment. Micro- and nanofluidic devices are becoming the focus of investigations. The unique characteristics of fast, high separation performance, low volume sample consumption and simple operation owned by chip, combined with the design flexibility of the recognition biosensors promises great potential capacity for any high throughput recognitions in real complex sample assays.

Acknowledgements

This project was supported by the National Nature Science Foundation of China (No. 20705005 and 21174017) and the Special-funded programme on national key scientific instruments and equipment development (2012YQ04014006) and the Cultivation Project for Technology Innovation Program of BIT (2011CX01032)

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

References

1 Song, S., Qin, Y., He, Y., Huang, Q., Fan, C. and Chen, H., Chem. Soc. Rev. 2010, 39, 4234-4243
2 W. Yang, K. R. Ratinac, S. P. Ringer, P. Thordarson, J. J. Gooding and F. Braet, Angew. Chem. Int. Ed. 2010, 49, 2114–2138
3 Lu, C. H., Yang, H. H., Zhu, C. L., Chen, X., Chen, G. N., Angew. Chem. Int. Ed. 2009, 48, 4785–4787.
4 Varghese, N.,Mogera, U.,Govindaraj, A., Das, A.,Maiti, P. K.,Sood, A. K.,Rao, C. N. R., Phys. Chem. Chem. Phys. 2009, 10, 206–210.
5 Mohanty, N.; Berry, V., Nano.Lett. 2008, 8, 4469–4476.
6 He, S., Song, B., Li, D., Zhu, C., Qi, W., Wen, Y., Wang, L., Song, S., Fang, H. and Fan, C., Adv. Funct. Mater. 2010, 20, 453-459
7 Pei, H., Li, J., Lv, M., Wang, J., Gao, J., Lu, J., Li, Y., Huang, Q., Hu, J. and Fan, C., J. Am. Chem. Soc. 2012, 134, 13843-13849
8 Wen, F., Xing, S., He, S., Song, L., Wang, Y., Long, D. Li and C. Fan, Chem. Commun.2010, 46, 2596–2598.
9 Y. Wang, Z. H. Li, D. H. Hu, C.-T. Lin, J. H. Li, Y. H. Lin, J. Am. Chem. Soc. 2010, 132, 9274–9276
10 S. Guo and S. Dong, Chem. Soc. Rev. 2011, 40, 2644–2672
11 Myung S, Solanki A, Kim C, Park J, Kim K S, Lee K B, Adv. Mater. 2011, 23, 2221-2225
12 Sai Bi, Tingting Zhao and BaoyuLuo, Chem. Commun. 2012, 48, 106-108
13 Kim, J.H., Chaudhary, S., and Ozkan, M., Nanotechnology.2007, 18, 195105–1951
14 Y. Q. Li, J. H. Wang, H. L. Zhang, J. Yang, L. Y. Guan,. H. Chen, Q. M. Luo and Y. D. Zhao, Biosens.Bioelectron. 2010, 25, 1283–1289.
15 Ramachandran, A., Zhang, M.Q., Goad, D., Olah, G., Malayer, J.R., El Rassi, Z., Electrophoresis.2003, 24, 70–77.
16 Wang, J.H.,Wang,W., Liu, Y.H., Duo, L.B., Huang, L.J., Jiang, X.F., Mol. Biol. Rep. 2009, 36, 1903–1908.
17 Y. Q. Li, L. Y. Guan, J. H. Wang, H. L. Zhang, J. Chen, S. Lin, W. Chen and Y. D. Zhao, Biosens. Bioelectron.2011, 26, 2317–2322
18 Manz, A., Graber, N., Widmer, H. M., Sens. Actuators B. 1990, 1, 244–248.
19 H. Chen, Z. H. Fan, Electrophoresis. 2009, 30, 758–765
20 B. Juskowiak, Anal. Bioanal. Chem. 2011, 399, 3157–3176
21 Tasset, D. M., Kublik, M. F., and Steiner, W., J. Mol. Biol. 1997, 272, 688–698
22 Chinnapen, D. J. F.,Sen, D., Biochemistry. 2002, 41, 5202–5212
23 J.ColinCox,Andrew D Ellington,Bioorg. Med. Chem. 2001,9(10), 2525–2531.
24 Hummers, W. S.,Offeman, R. E. J., Am. Chem. Soc. 1958, 80,1339−1339.
25 Charles A. Emrich, Igor L. Medintz, Wai K. Chu, Richard A.Mathies., Anal. Chem.2007, 79, 7360-7366.
26 Manabe T, Miyamoto H, Inoue K, Nakatsu M, Arai M, Electrophoresis, 1999, 20, 2677.
27 Juskowiak, B., Anal.Bioanal. Chem. 2011, 399, 3157–3176.
28 S. Bi, T. T. Zhao and B. Y. Luo, Chem. Commun.2012, 48, 106–108.