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A folate receptor electrochemical sensor based on terminal protection and supersandwich DNAzyme amplification

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ABSTRACT

Based on the protecting effect of folate receptor (FR) toward folic acid (FA) modified DNA and the signal amplification of supersandwich DNA structure, we designed an interesting electrochemical biosensor for FR. In the present system, with the increase of FR, protecting more FA bound DNA from hydrolysis by exonuclease I (Exo I), FA bound DNA will hybridize to form more supersandwich DNA structure resulting in an increased electrochemical signal. A relationship between the concentration of the target protein, FR, and the obtained electrochemical signal can be established. The signal was obtained by the catalysis on H_2O_2 in the system containing Fc and hemin/DNAzyme. The detection concentration range of FR was from 1.0 to 20.0 ng/mL with an achieved detection limit of 0.3 ng/mL which approached clinically relevant concentrations of FR.

1. Introduction

Sensitive and specific determination of cancer-related biomarkers is essential in modern medicine and clinic diagnosis (Jans and Huo, 2012; Tang et al., 2012; Joo et al., 2012). Folate receptor (FR), a glycosylphophatidylinositol-linked membrane glycoprotein, is a kind of tumor-associated antigen, which is over-expressed in many human tumors, including ovarian carcinomas, choroid plexus carcinomas, and ependymomas (Buist et al., 1995; Ross et al., 1994; Weitman et al., 1994; Leamon and Reddy, 2004). It is overexpressed in a relatively high percentage in breast cancer patients (Low and Kularatne, 2009; Kalli et al., 2008). Hartmann et al. concluded that extensive folate over-expression is strongly correlated with bad prognosis of breast cancer (Hartmann et al., 2007). However, it is almost absent in most normal tissues. Thus detection of FR is very important to evaluate the evolution of malignancy and can provide information for disease diagnosis. FR as an efficient folate binder can bind folic acid (FA) with high affinity (association constants in the order of 10^9–10^{10}) at 1:1 stoichiometry (McHugh and Cheng, 1979). The high affinity between FR and FA makes them work as a bridge connecting medicine with cancer cell, and an attractive property for tumor therapy (Liu et al., 2007). And meanwhile some reports have demonstrated the use of FA as a bioreceptor for detection of FR such as cyclic voltammetry (Liu et al., 2007), atomic force microscopy (Bhalerao et al., 2007), diffraction (Achary et al., 2007), impedance (Weng et al., 2011), lab on a chip (Lima et al., 2012) and electrogenerated chemiluminescence (Wu et al., 2011) biosensors.

Recently a few novel detecting strategies based on the attractive "terminal protection of single strand DNA", were developed to determine FR (Cao et al., 2012; Wei et al., 2012). These assays were based on the finding that the single-stranded DNA (ssDNA) terminally tethered to a small molecule is protected from the degradation by restriction endonuclease (exonuclease I (Exo I), *Flavobacterium okeanokoites* restriction endonuclease (Fok I) or exonuclease III) when the small molecule moiety is bound to its protein target. However, as FR is added to the test solution, it can bind folate specifically to form the FR-FA-ssDNA and then it will not be degraded by Exo I because the bound FR macromolecules may convey a steric hindrance, which prevents Exo I from approaching and cleaving the phosphodiester bond adjacent to the 3′ end (Cao et al., 2012). These assays combined the unique biochemical properties of DNA with high affinity of small molecule-protein interactions (Zhen et al., 2012; Wei et al., 2012; Cao et al., 2012). Although these methods are novel and interesting, there are still some insufficiencies, such as the necessities of the exorbitant apparatus, specific labeling and without signal amplification (Wei et al., 2012) or amplified with complex process such as cycle cleavage amplification strategy (Wu et al., 2009; Cao et al., 2012). A great deal of effort, therefore, still need to be devoted to developing analytical methods for easy amplified detection of FR.

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Recently, various strategies have been applied in the amplified detection of sensing (Lu et al., 2012; Wang, 2005; Gill et al., 2006; Scrimin and Prins, 2011; Jeanmaire and Van Duyne, 1977; Lyon et al., 1998; He et al., 2000; Liu and Wang, 2005; Wang et al., 2007; Riskin et al., 2009; Wang and Irudayaraj, 2011) such as using nanoparticles (Wang et al., 2002; Polsky et al., 2006; Zhang et al., 2007; Hansen et al., 2006) and catalyst enzymes (Ivnitski and Rishpon, 1996; Rishpon and Ivnitski, 1997; Caruana and Heller, 1999; Patolsky et al., 2001, 2002; Zhang et al., 2003; Li et al., 2008a,b; Ferapontova et al., 2010; Zhu et al., 2009; Wen et al., 2011; Zhu et al., 2011). The rapid progress in the design of DNAzymes introduced new catalytic tools for the development of amplified sensing assays (Willner et al., 2008; Liu et al., 2009, 2007, Kolpashchikov, 2010; Gerasimova, and Kolpashchikov, 2010; Li et al., 2010a,b, 2007, 2008a,b; Teller et al., 2009; Ali and Li, 2009; Elbaz et al., 2009, 2008; Liu and Lu 2003, 2004). Specially, hemin/G-quadruplex horseradish peroxidase (HRP)-mimicking DNAzyme (Travascio et al., 1998; Kosman, and Juskowiak, 2011; Kong2011) was extensively used as a catalytic label for the amplified colorimetric (Xiao et al., 2004; Teller et al., 2009; Elbaz et al., 2009; Neo et al., 2012; Du et al., 2011; Zhou et al., 2010a,b; Nakayama and Sintim Herman, 2009; Li et al., 2009, 2008a,b,c), chemiluminescence (Niazov et al., 2004; Freeman et al., 2011; Liu et al., 2011), electrocatalytic (Pelossof et al., 2010a,b; Yali et al., 2012; Pelossof et al., 2010a,b; Golub et al., 2011), optical (Pelossof et al., 2011; Simcha et al., 2012; Kong et al., 2010; Zhou et al., 2010a,b) or fluorescence (Li et al., 2011a,b, 2010a,b) detection. Thus, new scheme based on coupling the biocatalytic amplification of horseradish peroxidase (HRP)-mimicking DNAzyme with additional amplification processes are highly desired.

In this paper, based on the specific FA–FR interaction that can prevent DNA enzymolysis by Exo I, we designed a convenient signal amplification electrochemical biosensor for FR. First, sequence1 (S1) was modified onto the gold electrode (GE) with Au–S bond to obtain S1/GE. Then, S2 strands are labeled with FA molecules at the 3′ end. In the absence of FR, the FA-linked DNA (S2-FA) is hydrolyzed into mononucleotides by Exo I in the 3′ to 5′ direction (can not join in the next hybridization). However, as FR is added to the test solution, FR can specifically bind to FA forming the FR-bound S2-FA (S2/FA-FR) which will prevent Exo I from approaching and cleaving (Cao et al., 2012). At last, S1/GE was hybridized with S2/FA-FR and S3. S2 and S3 are designed for the concatamer reaction. S3 is a 48-mer oligonucleotide with ferrocene (Fc) label at the 3′ end. S3 contains 3 parts. The red part, 15 bases at 3′ end of S3 can hybridize with 5′ end of S2. The other 15 bases of yellow part can hybridize with 3′ end of S2. The rest green part with 18 bases can bind with hemin to form the DNAzyme. Thus after the hybridization process it became a "DNAzyme-supersandwich structure" (S1/S2-FA-FR/S3/GE) which contained numerous Fc and hemin/DNAzyme units. Because more FR protects more S2 from hydrolysis, which will lead more hybridization of S1, S2 and S3, and thus form more supersandwich DNA structure with more Fc and hemin/DNAzyme, resulting in an increased electrochemical signal, a relationship between the concentration of the target protein, FR, and the obtained electrochemical signal can be established. With the catalysis on H_2O_2 in the system containing Fc and hemin/DNAzyme, the signal was detected and thus a sensitive method for FR detection is developed.

2. Experimental sections

2.1. Materials and reagents.

HPLC-purified oligonucleotides (sequences are listed in Table S1) were obtained from Sangon Biotechnology Co. Ltd (Shanghai, China). Exonuclease I (Exo I), folate, 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysulf-osuccinimide (Sulfo-NHS), Tris (hydroxymethyl) aminomethane (Tris), and other reagents were also obtained from the Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). FR was purchased from Sigma Aldrich Chemical Co. Doubly distilled water was used throughout the experiments.

2.2. Preparation of folate acid and folate receptor-linked S2 (S2/FA–FR).

Folate acid was conjugated to the 3′-NH_2 of the DNA probe 2 with the method of the succinimide coupling (EDC-NHS). First, 0.5 mL of 20 μM probe 2 was mixed with 0.5 mL of 100 mM Tris–HCl (pH 7.4) including 10 mM folate, 1 mM EDC, and 5 mM Sulfo-NHS and incubated for 3 h at 37 °C in the dark. The solution (FA-S2) was then dialyzed against phosphate buffer solution (PBS, pH 7.4) using a membrane with molecular weight cutoff of 1000 Da to remove excessive folate acid. Afterwards, a series of varying concentrations of folate receptor (FR) were joined into the preceding solutions entirely for sufficiently incubating at 37 °C for 2 h in dark. Finally, through acceding respectively to 400 U/ml Exo I, the aforementioned mixture solutions were hydrolyzed absolutely about 30 min that FA-S2 with different concentrations of FR (S2/FA-FR) was fabricated.

2.3. Substrate DNA S1 self-assembled on gold electrode

Prior to modification, the gold working electrode (2 mm diameter) was first polished sequentially with 0.3 and 0.05 μm alumina powder followed by ultrasonic cleaning with ethanol, and ultrapure water for 3 min each. Then the electrode was scanned in 0.1 M H_2SO_4 between −0.2 and 1.55 V at 100 mV/s 20 circles. After that, a steady-state redox wave was observed. The immobilization of the capture probe (S1) was performed by dropping 30 μL of 10 mM Tris–HCl buffer (pH 7.4) containing 1.0 μM S1 probe and 0.4 M NaCl on the cleaned gold electrode in a humidified chamber. The self-assembly through the thiol of S1 and Au atom was proceeded for 10 h. Then the gold electrode was rinsed with water and incubated in 2 mM 6-mercaptohexanol in Tris–HCl buffer, for 2 h to eliminate the nonspecific-bonded DNA. After being thoroughly rinsed with the washing buffer, the S1 probe modified gold electrode (S1/GE) was stored at 4 °C in HEPES buffer for further use.

2.4. Preparation of supersandwich DNA structure linked G-quadruplex/hemin peroxidase

The S1 probe modified electrodes were then incubated in the hybridization buffer 0.05 M Tris–HCl buffer (pH 7.4) and 0.2 mM hemin solution containing 3 μM S2/FA–FR, S3 probe at 37 °C to form the supersandwich structure modified electrode (S1/S2-FA-FR/S3/GE). When finishing assembly, the modified electrode was thoroughly rinsed, dried with N_2, and then incubated in 1 M $NaClO_4$ before electrochemical measurements. Under the same conditions, S4 or S5 substituting S3 was prepared for no Fc labeled DNA structure (S1/S2/S4/GE) and no G-quadruplex contained in DNA structure (S1/S2/S4/GE) modified gold electrode, respectively. Likely, we choose S6 to replace S3, and then S2 and S6 were prepared for traditional sandwich DNA structure (S1/S2/S6/GE) modified electrode.

2.5. Measurement procedure

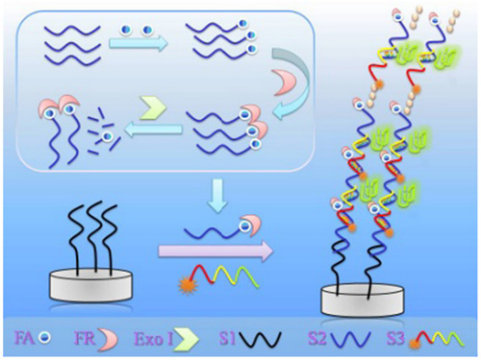
All the electrochemical experiments were carried out at room temperature. Cyclic voltammetry (CV) was performed in 0.1 M

PBS (pH 7.4) within the potential range from −0.4 to 0.4 V using scan rate 100 mV/s. Electrochemical impedance spectra (EIS) was performed in 1/15 M PBS (pH 7.4) containing 5 mM $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ and 0.1 M KCl in the frequency range from 0.1 Hz to 100 kHz with 5 mV as the amplitude at a polarization potential of −0.18 V.

3. Results and discussion

3.1. Strategy of the assay

Scheme 1 depicts the fabrication of the supersandwich DNA assay based on the high specific affinity between FA and FR. First, S2 is modified with FA. In the absence of FR, the FA-linked DNA (S2-FA) is hydrolyzed into mononucleotides by Exo I. As FR is added to the test solution, the FR-bound S2-FA (S2/FA-FR) will be formed due to the specifically binding of FR with FA, which will prevent Exo I from approaching and cleaving. At the same time, S1 was modified onto the gold electrode (GE) with Au–S bond. Thus, in the presence of hemin, S1/GE was hybridized with S2/FA-FR and S3, and then because of the special DNA sequences, "DNAzyme-supersandwich structure" (S1/S2-FA-FR/S3/GE) will be formed after the hybridization process. With the increase of FR, protecting more S2 from hydrolysis, it will lead more hybridization of S1, S2 and S3, and thus form more supersandwich DNA structure with more Fc and hemin/DNAzyme, resulting in an increased electrochemical signal, a relationship between the concentration of the target protein, FR, and the obtained electrochemical signal can be established.



Scheme 1. Fabrication process of the electrochemical amplified determination of FR based on DNAzyme-supersandwich structure.

3.2. Electrochemical impedance spectroscopy of the modification procedure

To prove our design, we have first conducted electrochemical impedance measurements (EIS) to study the interface properties of different modified electrode. As shown in Fig. 1A (curve a), the impedance spectrum of the bare GE includes almost no semicircle domain. After the immobilization of S1, a semicircle can be observed due to the formation of a negatively charged interface, which may electrostatically repel the also negatively charged redox species $[Fe(CN)_6]^{3-/4-}$ (curve b). When the electrode is further hybridized with S2/FA-FR and S3, the diameter of the semicircle continues to increase (curve c), indicating the further loading of negatively charged DNA strands. This may also disclose the fact that FR may protect S2 from the digestion by Exo I; otherwise, the S2 strands are all digested, and thus no more DNA strands can be loaded on the electrode surface via hybridization. In a control experiment, as shown in curve d of Fig. 1A, if S1/GE only hybridized with S2, the diameter of the semicircle increased compared with curve b, but decreased relative to curve c. All these impedance spectra are coherent as predicted.

3.3. Feasibility investigation of this assay for detection of FR activity

In order to verify our proposal, we studied the cyclic voltammograms (CV) response of different electrodes. As shown in Fig. 2B curve a, a large response is obtained on S1/S2-FA-FR/S3/GE, indicating that FR is able to protect S1-FA from digesting of Exo I. In the control experiments, if in the absence of FR, the CV signal shows a negligible change (curve b), implying that S2-FA is easy to be digested by Exo I without the protection of FR which is in accordance with literature (Cao et al., 2012; Zhen et al., 2012; Wei et al., 2012). However, in the absence of FR and Exo I, an apparent signal appeared as curve c shows, due to the fact that there is no Exo I to digest S2-FA and thus S2-FA are loaded on the electrode surface and the supersandwich DNA structure will also be fabricated by hybridization of S1, S2 and S3. When S1/GE hybridizes with S2-FA-FR without S3 (curve d), a small CV response is observed, implying that without Fc and DNAzyme, there is no signal. Consequently, the proposed sensor is effective to detect FR.

3.4. The electrochemical catalysis on H_2O_2

This structure was also used to study the electrochemical catalysis on hydrogen peroxide. As curve a of Fig. 2A shows, in the absence of H_2O_2, the CV response is weak on S1/S2-FA-FR/S3/GE. With the addition of 5 μM H_2O_2 to the buffer, an increase in CV

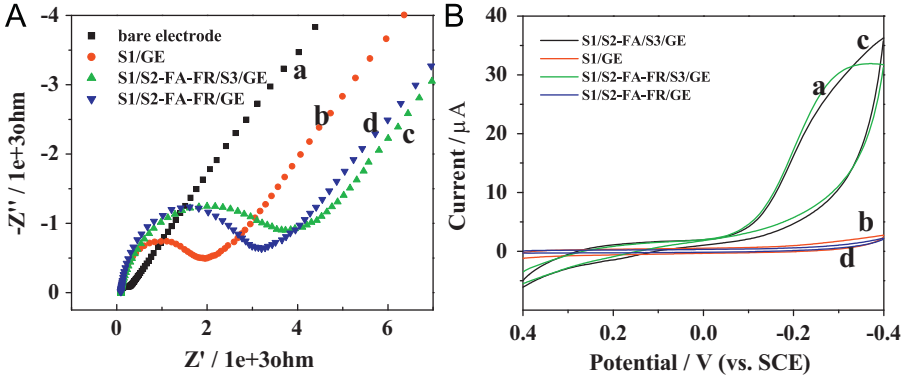


Fig. 1. (A) Nyquist plot for EIS measurements for different modified electrodes: bare GE (a), S1/GE (b), S1/S2-FA-FR/S3/GE (c), and S1/S2-FA-FR/GE (d); (B) CV of different conditions in PBS (pH 7.4) containing 100 μM H_2O_2: S1/S2-FA-FR/S3/GE in the presence of Exo I (a); S1/GE in the present of Exo I (b) and S1/S2-FA/S3/GE in the absence of Exo I (c) (20 ng/mL FR used as an example).

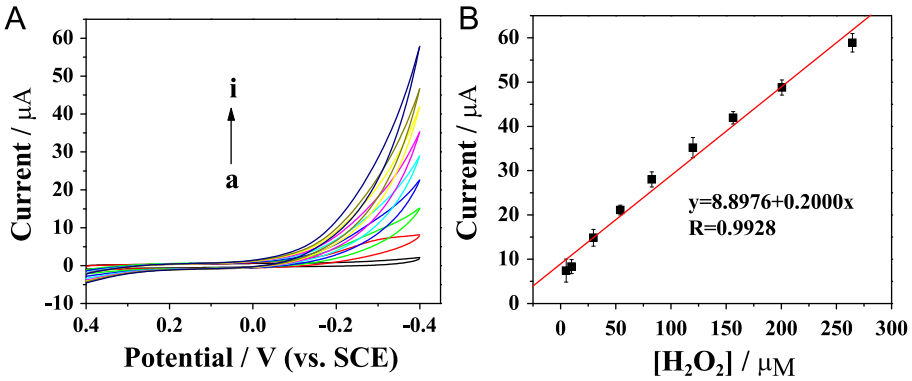


Fig. 2. (A) CV of bioelectrocatalytic reduction of various concentrations of H_2O_2 by the DNAzyme-supersandwich on S1/S2-FA-FR/S3/GE in PBS (a–i) 0, 10, 30, 55, 80, 120, 160, 200, 260 μM; (B) the corresponding calibration curve of current vs. the concentration of H_2O_2.

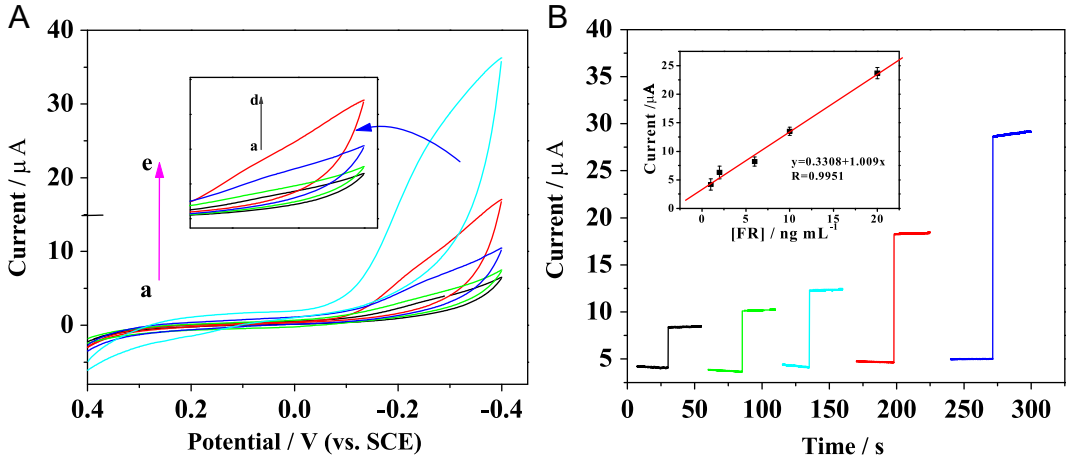


Fig. 3. (A) CV and (B) steady state amperometric current at −0.4V on S1/S2-FA-FR/S3/GE in the presence of various concentrations of FR: (a–f) 1, 2, 6, 10, 20 ng/mL in PBS (pH 7.4) with 100 μM H_2O_2. The corresponding calibration curve of current vs. the concentration of FR (inset).

response appeared in the negative potential range that most likely reflects the onset of peroxide reduction (curve b Fig. 2A). With the concentration of H_2O_2 increasing, the bioelectrocatalytic currents enlarged. The calibration curve corresponding to the CV responses of the modified electrode at different H_2O_2 concentrations is shown in Fig. 2B. The response current was linear with the H_2O_2 concentration in the range of 10–260 μM. The detection limit was 3 μM (n=3).

3.5. Detection of FR activity

Under the optical conditions (discussed in SI), we could anticipate a quantitative nature for FR based on the terminal protection assay and supersandwich DNAzyme structure. Fig. 3 gives CV responses of FR with varying concentrations. We observed dynamically increased CV peaks in response to FR of increasing concentrations within the range from 1.0 to 20.0 ng/mL with an achieved detection limit of 0.3 ng/mL which approached clinically relevant concentrations of FR (Wei et al., 2012).

3.6. Selectivity, stability and application of the sensor

Specificity of the method for FR is exhibited in Fig. 4, compared with 20 ng/mL FR by employing other common proteins: 0.1 mg/mL of BSA, 0.1 mg/mL of IgG and 10 mg/mL of thrombin. Obviously, we can see the presence of the interference proteins exhibits no significant effect on the electrochemical intensity, revealing the high selectivity of the proposed approach. Therefore, the proposed aptasensor presents remarkably high sensitivity and selectivity.

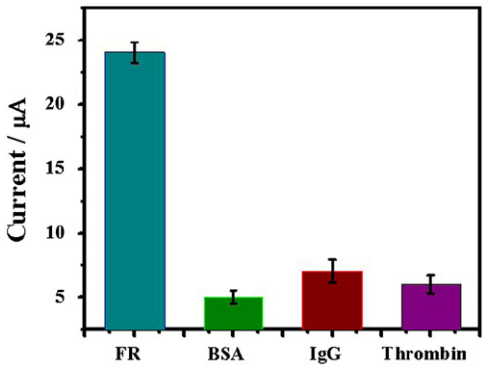


Fig. 4. Selectivity of the supersandwich assay for FR analysis in PBS (pH 7.4) with 100 μM H_2O_2.

Considering the high sensitivity and selectivity of this amplified electrochemical FR sensor, it was expected to hold great promise in disease diagnosis. In the experiments, FR is added in normal human serum at various concentrations and assayed according to the above method. As shown in Table 1, comparing the assay results and the given concentrations in serum, the average relative error is less than 4%, showing that detection of FR in sample testing is also feasible. After the biosensors were stored in refrigerator for a period of 2 weeks, 91.7% of the initial DPV response for FR (15 ng/mL as an example) could be remained indicating that the proposed method possess an acceptable stability.

Table 1
Comparison of our results using proposed method with the standard FR concentrations in serum samples.

Samples	FR concentration detected by proposed method (ng/mL)	Standard FR concentration (ng/mL)	Relative error (%)
1	14.33	15	4.6
2	4.81	5	3.8
3	0.96	1	4

4. Conclusions

In conclusion, this work describes a novel electrochemical biosensor for detection of FR by using DNAzyme supersandwich structure amplification and terminal protection strategy. The highlight of this work is to adequately utilize the signal amplification based on one step supersandwich structure amplification for the determination of FR.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.10.066.

References

Achary, G., Chang, C.L., Doorneweerd, D.D., Vlashi, E., Henne, W.A., Hartmann, L.C., Low, P.S., Savran, C.A., 2007. Journal of the American Chemical Society 129, 15824–15829.
Ali, M.M., Li, Y., 2009. Angewandte Chemie-International Edition 48, 3512–3515.
Bhalerao, K.D., Lee, S.C., Soboyejo, W.O., Soboyejo, A.B.O., 2007. Journal of Materials Science: Materials in Medicine 18, 3–8.
Buist, M.R., Molthoff, C.F.M., Kenemans, P.C.J., Meijer, L.M., 1995. Journal of Clinical Pathology 48, 631–636.
Cao, Y., Zhu, S., Yu, J.C., Zhu, X.J., Yin, Y.M., Li, G.X., 2012. Analytical Chemistry 84, 4314–4320.
Caruana, D.J., Heller, A., 1999. Journal of the American Chemical Society 121, 769–774.
Du, Y., Li, B.L., Guo, S.J., 2011. Analyst 136, 493–497.
Elbaz, J., Moshe, M., Shlyahovsky, B., Willner, I., 2009. Chemistry—A European Journal 15, 3411–3418.
Elbaz, J., Shlyahovsky, B., Willner, I., 2008. Chemical Communications, 1569–1571.
Ferapontova, E.E., Hansen, M.N., Saunders, A.M., Shipovskov, S., Sutherlandc, D.S., Gothelf, K.V., 2010. Chemical Communications 46, 1836–1838.
Freeman, R., Liu, X., Willner, I., 2011. Journal of the American Chemical Society 133, 11597–11604.
Gerasimova, Y.V., Kolpashchikov, D.M., 2010. Chemistry & Biology 17, 104–106.
Gill, R., Polsky, R., Willner, I., 2006. Small 2, 1037–1041.
Golub, E., Freeman, R., Willner, I., 2011. Angewandte Chemie, International Edition 50, 11710–11714.
Hansen, J.A., Wang, J., Kawde, A.N., Xiang 3w, Y., Gothelf, K.V., Collins, G., 2006. Journal of the American Chemical Society 128 2228-2228.
Hartmann, L.C., Keeney, G.L., Lingle, W.L., Christianson, T.J., Varghese, B., Hillman, D., Oberg, A.L., Low, P.S., 2007. International Journal of Cancer 121, 938–942.
He, L., Musick, M.D., Nicewarner, S.R., Sallinas, F.G., Benkovic, S.J., Natan, M.J., Keating, C.D., 2000. Journal of the American Chemical Society 122, 9071–9077.
Ivnitski, D., Rishpon, J., 1996. Biosensors and Bioelectronics 11, 409–417.
Jans, H., Huo, Q., 2012. Chemical Society Reviews 41, 2849–2866.
Jeanmaire, D.L., Van Duyne, R.P., 1977. Journal of Electroanalytical Chemistry 84, 1–20.
Joo, J., Kwon, D., Yim, C., Jeon, S., 2012. ACS Nano 6, 4375–4381.
Kalli, K.R., Oberg, A.L., Keeney, G.L., Christianson, T.J., Low, P.S., Knutson, K.L., Hartmann, L.C., 2008. Gynecologic Oncology 108, 619–626.
Kolpashchikov, D.M., 2010. Chemical Reviews 110, 4709–4723.
Kong, D., 2011. Progress in Chemistry 23, 2119–2131.
Kong, D.M., Xu, J., Shen, H.X., 2010. Analytical Chemistry 82, 6148–6153.
Kosman, J., Juskowiak, B., 2011. Analytica Chimica Acta 707, 7–17.
Leamon, C.P., Reddy, J.A., 2004. Advanced Drug Delivery Reviews 56, 1127–1141.
Li, C.L., Liu, K.T., Lin, Y.W., 2011a. Analytical Chemistry 83, 225–230.
Li, Y., Li, X., Ji, X., Li, X., 2011b. Biosensors & Bioelectronics 26, 4095–4098.
Li, D., Shlyahovsky, B., Elbaz, J., Willner, I., 2007. Journal of the American Chemical Society 129, 5804–5805.
Li, D., Wieckowska, A., Willner, I., 2008a. Angewandte Chemie, International Edition 47, 3927–3931.
Li, J.W., Chu, Y.Z., Lee, B.Y.H., Xie, X.L.S., 2008b. Nucleic Acids Research 36 36-36.
Li, T., Wang, E.K., Dong, S.J., 2008c. Chemical Communications 44, 3654–3656.
Li, T., Dong, S.J., Wang, E.K., 2009. Analytical Chemistry 81, 2144–2149.
Li, T., Wang, E.K., Dong, S.J., 2010a. Analytical Chemistry 82, 1515–1520.
Li, W., Liu, Z.L., Lin, H., Nie, Z., Chen, J.H., Xu, X.H., Yao, S.Z., 2010b. Analytical Chemistry 82, 1935–1941.
Lima, R.S., Piazzetta, M.H.O., Gobbi, A.L., Rodrigues-Filho, U.P., Nascente, P.A.P., Coltrobe, W.K.T., Carrilho, E., 2012. Lab on a Chip 12, 1963–1966.
Liu, J., Cao, Z., Lu, Y., 2009. Chemical Reviews 109, 1948–1998.
Liu, J., Lu, Y., 2003. Journal of the American Chemical Society 125, 6642–6643.
Liu, J., Lu, Y., 2004. Journal of the American Chemical Society 126, 12298–12305.
Liu, L., Zhu, X.L., Zhang, D.M., Huang, J.Y., Li, G.X., 2007. Electrochemistry Communications 9, 2547–2550.
Liu, X., Freeman, R., Golub, E., Willner, I., 2011. ACS Nano 5, 7648–7655.
Liu, Y.C., Wang, C.C., 2005. Journal of Physical Chemistry B 109, 5779–5782.
Low, P.S., Kularatne, S.A., 2009. Chemistry & Biology 13, 256–262.
Lu, C.H., Wang, F., Willner, I., 2012. Journal of the American Chemical Society 134, 10651–10658.
Lyon, L.A., Musick, M.D., Natan, M.J., 1998. Analytical Chemistry 70, 5177–5183.
McHugh, M., Cheng, Y.C., 1979. Journal of Biological Chemistry 254, 11312–11318.
Nakayama, S., Sintim Herman, O., 2009. Journal of the American Chemical Society 131, 10320–10333.
Neo, J.L., Kamaladasan, K., Uttamchandani, M., 2012. Current Pharmaceutical Design 18, 2048–2057.
Niazov, T., Pavlov, V., Xiao, Y., Gill, R., Willner, I., 2004. Nano Letters 4, 1683–1687.
Patolsky, F., Lichtenstein, A., Willner, I., 2001. Nature Biotechnology 19, 253–257.
Patolsky, F., Weizmann, Y., Willner, I., 2002. Journal of the American Chemical Society 124, 770–772.
Pelossof, G., Tel-Vered, R., Elbaz, J., Willner, I., 2010a. Analytical Chemistry 82, 4396–4402.
Pelossof, G., Tel-Vered, R., Elbaz, J., 2010b. Analytical Chemistry 82, 4396–4402.
Pelossof, G., Tel-Vered, R., Liu, X., Willner, I., 2011. Chemistry—A European Journal 17, 8904–8912.
Polsky, R., Gill, R., Kaganovsky, L., Willner, I., 2006. Analytical Chemistry 78, 2268–2271.
Riskin, M., Tel-Vered, R., Lioubashevski, O., Willner, I., 2009. Journal of the American Chemical Society 131, 7368–7378.
Rishpon, J., Ivnitski, D., 1997. Biosensors & Bioelectronics 12, 195–204.
Ross, J.F., Chaudhuri, P.K., Ratnam, M., 1994. Cancer 73, 2432–2443.
Scrimin, P., Prins, L.J., 2011. Chemical Society Reviews 40, 4488–4505.
Simcha, S., Wang, F., Orbach, R., 2012. Analytical Chemistry 84, 1042–1048.
Tang, J., Hou, L., Tang, D.P., Zhang, B., Zhou, J., Chen, G.N., 2012. Chemical Communications 48, 8180–8182.
Teller, C., Shimron, S., Willner, I., 2009. Analytical Chemistry 81, 9114–9119.
Travascio, P., Li, Y.F., Sen, D., 1998. Chemistry & Biology 5, 505–517.
Wang, J., 2005. Small 1, 1036–1043.
Wang, J., Xu, D., Polsky, R., 2002. Journal of the American Chemical Society 124, 4208–4209.
Wang, Y.L., Irudayaraj, J., 2011. Chemical Communications 47, 4394–4396.
Wang, Y.L., Wei, H., Li, B.L., Ren, W., Guo, S.J., Dong, S.J., Wang, E.K., 2007. Chemical Communications 43, 5220–5222.
Weng, J., Zhang, Z., Sun, L., Wang, J.A., 2011. Biosensors & Bioelectronics 26, 1847–1852.
Wen, Y.L., Pei, H., Wan, Y., Su, Y., Huang, Q., Song, S.P., Fan, C.H., 2011. Analytical Chemistry 83, 7418–7423.
Wei, X.F., Lin, W.L., Ma, N., Luo, F., Lin, Z.Y., Guo, L.H., Qiu, B., Chen, G.N., 2012. Chemical Communications 48, 6184–6186.
Weitman, S.D., Frazier, K.M., Kamen, B.A., 1994. Journal of Neuro-Oncology 21, 107–112.
Willner, I., Shlyahovsky, B., Zayats, M., 2008. Chemical Society Reviews 37, 1153–1165.
Wu, M.S., Xu, B.Y., Shi, H.W., Xu, J.J., Chen, H.Y., 2011. Lab on a Chip 11, 2720–2724.
Wu, Z., Zhen, Z., Jiang, J.H., Shen, G.L., Yu, R.Q., 2009. Journal of the American Chemical Society 131, 12325–12332.
Xiao, Y., Pavlov, V., Niazov, T., Dishon, A., Kotler, M., Willner, I., 2004. Journal of the American Chemical Society 126, 7430–7431.
Yali, Y., Yuan, R., Chai, Y.Q., 2012. Chemical Communications 48, 4621–4623.
Zhang, J., Song, S., Wang, L., Pan, D., Fan, C., 2007. Nature Protocols 2 2888-2888.
Zhang, Y., Kim, H.H., Heller, A., 2003. Analytical Chemistry 75, 3267–3269.
Zhen, Z., Li, J.T., Lin, J., Jiang, J.H., Yu, R.Q., Xiong, X.L., Tan, W.H., 2012. Analytical Chemistry 84, 5708–5715.
Zhou, X.H., Kong, D.M., Shen, H.X., 2010a. Analytica Chimica Acta 678, 124–127.
Zhou, X.H., Kong, D.M., Shen, H.X., 2010b. Analytical Chemistry 82, 789–793.
Zhu, C.F., Wen, Y.Q., Li, D., Wang, L.H., Song, S.P., Fan, C.H., Willner, I., 2009. Chemistry—A European Journal 15, 11898–11903.
Zhu, C.L., Lu, C.H., Song, X.Y., Yang, H.H., Wang, X.R., 2011. Journal of the American Chemical Society 133, 1278–1281.