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Label-free and highly sensitive electrochemiluminescence biosensing using quantum dots/carbon nanotubes in ionic liquid†

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Combining with the synergic effect of carbon nanotubes and ionic liquids for enhancing electrochemiluminescence (ECL) response of CdSe QDs, a universal strategy for highly sensitive biosensing was designed. Using alpha-fetoprotein as a model and monitoring the variation of ECL intensity before and after immunoreaction, a label-free ECL biosensor was developed.

Electrochemiluminescence (ECL) measurement has attracted significant attention because it combines the advantages of luminescence analysis with the merits of electrochemical assay, such as absence of background optical signal, ease of reaction control by applying electrode potential, high sensitivity, selectivity and wide response range.^{1–6} Quantum dot (QD)-based ECL sensor has become a popular analytical tool in the past decade.^{7,8} A series of analytical applications such as the detection of protein, DNA, small biomolecules and organic pollutants are based on the ECL behaviours of those QDs.^{9–13} However, the weaker ECL intensity of QDs compared with that of conventional luminescent reagents such as luminal or ruthenium complexes, limited its application in the assay of the samples with low concentration owing to the low sensitivity of those QD-based sensors. Moreover, the ECL response of QDs with a high excited electrochemical potential was usually unstable. Therefore, it is urgent to find an effective way to enhance the ECL signal of QDs and improve their stability.

Because of their excellent electronic conductivity, high surface areas and good stability, carbon nanotubes were intro-

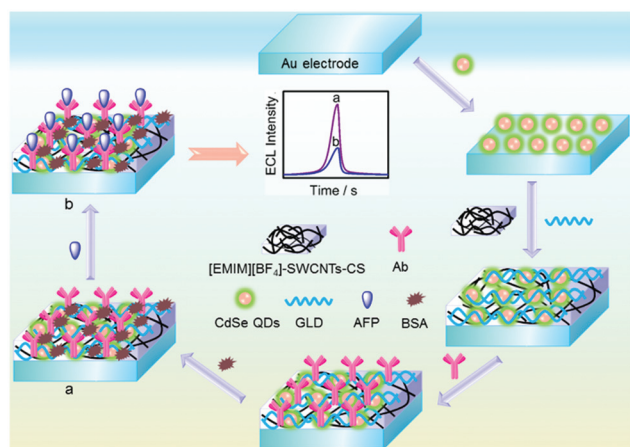
duced to enhance ECL response and improve the sensitivity for ECL detection in recent years.^{14–17} Room temperature ionic liquids (RTILs) have gained increasing interest in ECL analysis due to their wide potential window, extremely high ionic conductivity and less toxicity.^{18–20} Unfortunately, up to now, no efforts have been made to design ECL biosensors using carbon nanotubes and RTILs. In this work, it is reported that single-walled carbon nanotubes (SWCNTs) can be well dispersed in RTILs, which greatly simplified the assembly of SWCNTs on electrodes and provided an effective way for the construction of QD-based ECL biosensors. More significantly, the excellent conductivity of SWCNTs, and the extremely high ionic conductivity and wide potential window of RTILs in the as-prepared film lead to a synergic effect which dramatically enhances the ECL response of CdSe QDs and improves their stability.

In order to improve sensitivity in ECL biosensing, labelling techniques were usually applied to amplifying signals.^{21,22} However, the labelling process was time-consuming and needed professional operation, which made the detection procedure complex. In particular, the labelling process may lead to the denaturation of the biomolecules. In this work, a novel and universal strategy for label-free and highly sensitive electrochemiluminescence biosensing based on CdSe QDs/SWCNTs in RTILs was designed (Scheme 1). SWCNTs were well dispersed in RTILs which could form a homogeneous film on the electrode, greatly simplifying the assembly of SWCNTs. The synergic effect of SWCNTs and RTILs significantly improved the ECL response of CdSe QDs and their stability, resulting in the promotion of detection sensitivity. Using alpha-fetoprotein (AFP) as a model analyte, which is a kind of tumour marker and a reliable index of hepatocellular carcinoma (HCC),²³ the formed immunocomplex after immunoreaction increased the steric hindrances for K₂S₂O₈ to the surface of CdSe QDs and slowed down the electron-transfer speed in ECL reaction, leading to the decline of ECL intensity. By monitoring the variation of ECL intensity before and after the immunoreaction, a label-free signal-off ECL biosensor was proposed. This designed signal-off ECL biosensor exhibited good analytical performance for protein detection, which

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†Electronic supplementary information (ESI) available: Experimental details, the figure of ECL responses of CdSe QDs/SWCNTs-CS/GLD/Ab/BSA modified electrode to AFP with different concentrations and calibration curve, comparison of the different assay methods for AFP, and the figure of operational stability for the proposed biosensor. See DOI: 10.1039/c4an02129k



Scheme 1 Schematic illustration of the label-free ECL biosensing platform.

provided an alternative assay method for tumour markers in clinical diagnoses.

Transmission electron microscopic image (the image was not shown) showed well-defined single QDs which confirmed the formation of CdSe QDs. Moreover, the size distribution was relatively uniform and the average size of individual CdSe QDs was estimated to be 2.2 nm which was consistent with our previous report.²⁴ The CdSe QDs with carboxylate containing groups which bore negative charge, could generate an electrostatic repellent interaction with each other. This phenomenon led to the narrow size distribution of CdSe QDs and its good dispersion, which was beneficial for fabricating ECL biosensor.

Ultraviolet-visible (UV-vis) absorption and photoluminescence (PL) emission spectra (the pictures are not exhibited) were further characterized the formation of CdSe QDs. CdSe QDs exhibited an obvious absorption peak at 430 nm and a strong PL emission peak at about 575 nm ($\lambda_{\text{ex}} = 450 \text{ nm}$), indicating the successful fabrication of CdSe QDs.²⁴

Comparing with the bare Au electrode (Fig. 1, curve a), the CdSe QDs/chitosan (CS) modified Au electrode exhibited an obvious ECL peak (Fig. 1, curve b), which suggested CS could form efficient polymer membranes for immobilizing CdSe QDs as an ECL active species on the electrode. After SWCNTs were assembled on the electrode, the ECL peak intensity further increased (Fig. 1, curve c). This phenomenon could mainly be attributed to the excellent electronic conductivity of SWCNTs which accelerated the electron-transfer speed in ECL reaction and the high surface areas of SWCNTs which could immobilize much more CdSe QDs on the electrode.^{14–16} However, after introducing 1-ethyl-3-methylimidazoliumtetrafluoroborate ([EMIM][BF₄]) which was a kind of RTIL, the ECL peak intensity was dramatically promoted (Fig. 1, curve d). The ECL peak intensity of the CdSe QDs/[EMIM][BF₄]-SWCNTs-CS modified electrode was 12.5-fold and 2.5-fold higher than that of CdSe QDs/CS (Fig. 1, curve b) and CdSe QDs/SWCNTs-CS (Fig. 1, curve c) modified electrodes, respectively. In particular, the amount of CdSe QDs was equal in these modified films.

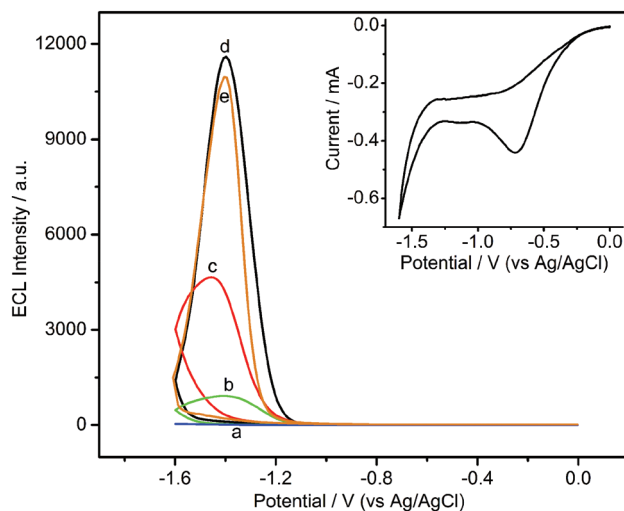


Fig. 1 ECL responses of bare Au electrode (a), CdSe QDs/CS (b), CdSe QDs/SWCNTs-CS (c), CdSe QDs/[EMIM][BF₄]-SWCNTs-CS (d) and CdSe QDs/[EMIM][BF₄]-SWCNTs-CS/GLD (e) modified Au electrodes in 0.1 mol L⁻¹ PBS (pH 7.4) containing 0.1 mol L⁻¹ K₂S₂O₈ and 0.1 mol L⁻¹ KCl between 0 and -1.6 V at 100 mV s⁻¹. The emission window was placed in front of the photomultiplier tube, which was biased at -800 V. Inset: CV of CdSe QDs/[EMIM][BF₄]-SWCNTs-CS modified Au electrode at 100 mV s⁻¹.

Moreover, the ECL peak of the CdSe QDs/[EMIM][BF₄]-SWCNTs-CS modified electrode occurred at -1.39 V (Fig. 1, curve d) which was more positive than -1.46 V at the CdSe QDs/SWCNTs-CS modified electrode (Fig. 1, curve c), indicating that the presence of RTILs not only enhanced the ECL intensity of the CdSe QDs but also decreased the potential barriers of the ECL reaction. The good conductive performance of RTIL facilitated electron transportation and reduced surface diffusion capacitance.^{18,19} The excellent conductivity of SWCNTs, and the extremely high ionic conductivity and the wide potential window of RTILs in the as-prepared film lead to a synergic effect which significantly enhanced the ECL response of CdSe QDs. When glutaraldehyde (GLD) was coated on the surface of the modified films, the ECL response declined slightly (Fig. 1, curve e), owing to the insulation of GLD and the increased steric hindrance for the access of the coreactant to the electrode surface. Nevertheless, the ECL intensity was 94.5% of that without GLD (Fig. 1, curve d), which showed sufficient sensitivity for ECL determination. Based on the excellent ECL response of CdSe QDs/[EMIM][BF₄]-SWCNTs-CS/GLD films, they were advantageously used for developing an ECL biosensing platform.

The cyclic voltammogram (CV) of the CdSe QDs/[EMIM][BF₄]-SWCNTs-CS modified electrode displayed two cathodic peaks at -0.70 V and -1.13 V, respectively (Fig. 1, inset). The weak peak at -1.13 V was attributed to the reduction of CdSe QDs on the electrode, which might generate negatively charged radicals (CdSe^{-•}).²⁵ The strong peak at -0.70 V could result from the reduction of S₂O₈²⁻ to sulfate anion radicals (SO₄^{-•}).²⁶ Furthermore, the corresponding control experiments were performed. In the absence of CdSe QDs, the ECL

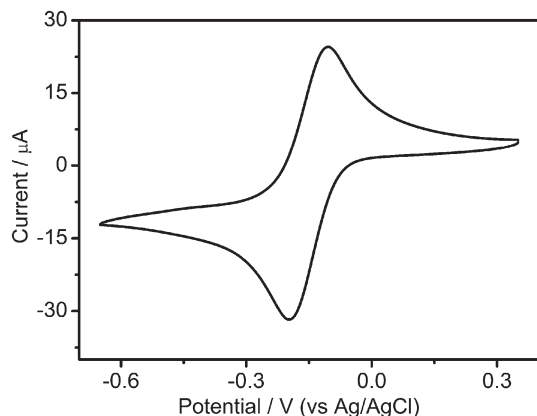


Fig. 2 CV of CdSe QDs/[EMIM][BF₄]-SWCNTs-CS/GLD modified Au electrode in 0.1 mol L⁻¹ KCl solution containing 5.0 mmol L⁻¹ hexaammineruthenium chloride at 100 mV s⁻¹.

response was hardly observed, implying that CdSe QDs was unique towards ECL active species in this system. SWCNTs and RTILs could not produce luminescence. On the other hand, without K₂S₂O₈, the ECL intensity of the CdSe QDs/[EMIM][BF₄]-SWCNTs-CS modified electrode was very weak, which verified K₂S₂O₈ as a coreactant was necessary for generating the ECL signal. According to the previous work,²⁵ the possible mechanism might be inferred that the produced CdSe^{•-} reacted with the formed SO₄^{•-} to generate excited state light-emitting species (CdSe*) and then it emitted light in the aqueous solution.

The CV of the CdSe QDs/[EMIM][BF₄]-SWCNTs-CS/GLD modified Au electrode exhibited a couple of stable, symmetrical and well-defined redox peaks due to the redox reaction of the [Ru(NH₃)₆]Cl₃/[Ru(NH₃)₆]Cl₂ couple (Fig. 2), indicating the presence of [EMIM][BF₄] and SWCNTs could prompt the electron transfer and improve the reversibility of electrode reaction. Moreover, electroactive area and heterogeneous electrons transfer constant were calculated to be 15.0 cm² and 9.26 s⁻¹, respectively.

After using the Fe(CN)₆^{3-/4-} redox couple as the electrochemical probe, electrochemical impedance spectroscopy (EIS) was an effective method for monitoring the changes in the assembly process (Fig. 3). Due to its poor conductivity, CdSe QDs could inhibit the electron transfer, leading to increasing electron transfer resistance (*R*_{et}) for the redox probe (curve b), compared with the bare Au electrode (curve a). When CdSe QDs/[EMIM][BF₄]-SWCNTs-CS was modified on an Au electrode, *R*_{et} increased a little (curve c). Although SWCNTs have excellent conductivity and RTILs possess high ionic conductivity in the as-prepared film, the badly conductive CS which formed polymer membranes greatly blocked the transfer between Fe(CN)₆^{3-/4-} redox couple and the electrode. Upon the addition of GLD, the semicircle domain further increased (curve d), owing to the insulation of GLD. Subsequently, monoclonal anti-AFP antibody (Ab) was added to the surface of the CdSe QDs/[EMIM][BF₄]-SWCNTs-CS/GLD modified electrode, *R*_{et} increased (curve e), indicating that Ab had been successfully

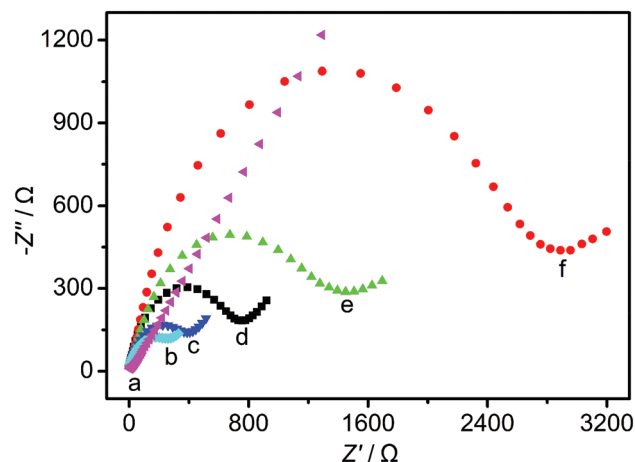


Fig. 3 EIS of bare (a), CdSe QDs (b), CdSe QDs/[EMIM][BF₄]-SWCNTs-CS (c), CdSe QDs/[EMIM][BF₄]-SWCNTs-CS/GLD (d), CdSe QDs/[EMIM][BF₄]-SWCNTs-CS/GLD/Ab (e) and CdSe QDs/[EMIM][BF₄]-SWCNTs-CS/GLD/Ab/BSA (f) modified Au electrodes in 0.1 mol L⁻¹ KCl solution containing 5.0 mmol L⁻¹ K₃Fe(CN)₆/K₄Fe(CN)₆.

grafted onto the electrode surface which hindered the redox couple probe transfer. After bovine serum albumin (BSA) blocking, *R*_{et} promoted dramatically (curve f). Proteins of insulative properties would obstruct the diffusion of the redox probe to the electrode, elevating the resistance. The stepwise increase of electron transfer resistance verified the successful fabrication of the ECL biosensing interface.

Under the optimal conditions in 0.1 mol L⁻¹ PBS (pH 7.4) containing 0.1 mol L⁻¹ K₂S₂O₈ and 0.1 mol L⁻¹ KCl with an incubation time of 45 min and the incubation temperature of 37 °C. Fig. 4 displays the ECL intensity of the biosensor before (a) and after (b–k) incubating in AFP solution with different concentrations. In the presence of AFP, the ECL intensity was lower than that in the absence of AFP, and the ECL intensity

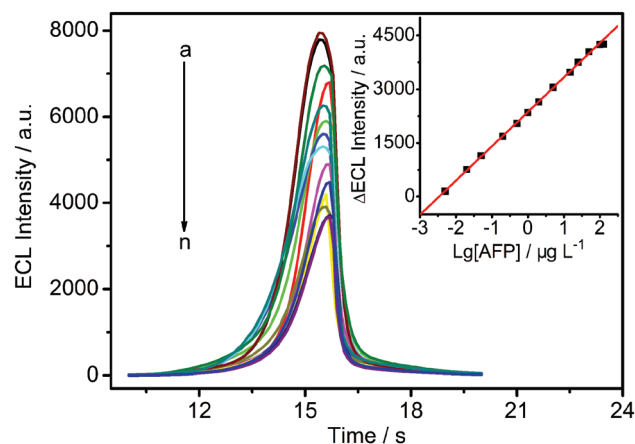


Fig. 4 (A) ECL responses of the biosensor to 0 (a), 0.005 (b), 0.02 (c), 0.05 (d), 0.2 (e), 0.5 (f), 1 (g), 2 (h), 5 (i), 15 (j), 25 (k), 50 (l), 100 (m) and 125 (n) μg L⁻¹ AFP between 0 and -1.6 V at 100 mV s⁻¹. The emission window was placed in front of the photomultiplier tube, which was biased at -800 V. Inset: linear calibration curve.

decreased stepwise with increasing concentrations of AFP. The formed immunocomplex after immunoreaction increased the steric hindrances for $K_2S_2O_8$ to the surface of CdSe QDs and slowed down the electron-transfer speed in ECL reaction, leading to the decline of ECL intensity. Based on the variation of ECL intensity before and after the immunoreaction, this label-free signal-off ECL biosensor was achieved (Scheme 1). The calibration curve was constructed by plotting ΔECL intensity (ΔECL intensity = $I_0 - I$, when the AFP concentration was zero, the ECL intensity was recorded as I_0 ; I was the ECL intensity of AFP with different concentrations.) against $\lg C$ (C was different concentrations of AFP). A good linear relationship between the ΔECL intensity and the logarithm value of AFP concentration ranging from $0.005 \mu\text{g L}^{-1}$ to $100 \mu\text{g L}^{-1}$ was observed (Fig. 4, inset). The detection limit was estimated to be $0.003 \mu\text{g L}^{-1}$ at a signal-to-noise ratio of 3σ (where σ was the relative standard deviation of 10 parallel measurements when AFP concentration was zero). Since the normal level of AFP in human serum was about $20 \mu\text{g L}^{-1}$,²³ the sensitivity and linear range of the designed method were enough for clinical application. As a control experiment, the calibration curve was performed with a non-ionic liquid modification electrode (Fig. S1 in ESI†). The linear range was $0.2 \mu\text{g L}^{-1}$ to $40 \mu\text{g L}^{-1}$ with a detection limit of $0.1 \mu\text{g L}^{-1}$ at 3σ (where σ was the relative standard deviation of 10 parallel measurements when AFP concentration was zero) (Fig. S1, inset in ESI†). Obviously, this linear range was much narrower and the detection limit was quite higher than that of the proposed biosensor with ionic liquid (Fig. 4), which indicated that the presence of RTILs facilitated electron transportation and remarkably enhanced the ECL intensity of the CdSe QDs, leading to dramatically promoting sensitivity and broadening the linear range for ECL detection of AFP. Furthermore, the linear range was much wider and the detection limit was quite lower than those of some previously reported methods^{27–33} (Table S1 in ESI†). Therefore, the proposed ECL biosensor exhibited promising application in the assay of tumour marker with wide concentration range and low detection limit.

The parallel measurements of the relative standard deviation (RSD) for one electrode modified eight times and eight independent electrodes with the incubation solution containing $10 \mu\text{g L}^{-1}$ AFP were 1.47% and 5.36%, respectively, which suggested that the ECL biosensor possessed good fabrication reproducibility. Upon successive scans for 12 cycles (Fig. S2 in ESI†), the ECL intensity tended to level off with RSD of 2.90% at the AFP concentration of $5 \mu\text{g L}^{-1}$, indicating satisfactory operational stability of the ECL biosensor. The synergic effect of SWCNTs and RTILs improved the stability of CdSe QDs. When the ECL biosensor was not in use, it was stored in 0.01 mol L^{-1} PBS at 4°C and was measured every few days. There was no evident change in the ECL response to $10 \mu\text{g L}^{-1}$ AFP after 30 days of storage. This implied that the structure of the CdSe QDs/[EMIM][BF₄]-SWCNTs-CS/GLD films were very efficient for retaining the activity of protein and preventing it to break away from the biosensor.

Table 1 Comparison of the assay results of clinical serum samples using the proposed and reference methods

Serum samples	Proposed method ($\mu\text{g L}^{-1}$)	Reference method ($\mu\text{g L}^{-1}$)	Relative error (%)
1	3.30	3.26	1.23
2	10.01	10.72	−6.62
3	83.64	83.75	−0.13

The selectivity of the ECL biosensor was investigated by using another two kinds of cancer markers including carcino-embryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9). Compared with the ECL response obtained from the pure $10 \mu\text{g L}^{-1}$ AFP, the response of the mixture containing $10 \mu\text{g L}^{-1}$ AFP, $500 \mu\text{g L}^{-1}$ CA19-9 and $500 \mu\text{g L}^{-1}$ CEA showed no obvious difference (RSD = 4.90%), indicating that CA19-9 and CEA cause the negligible interference in the AFP assay. Therefore, the biosensor had a good selectivity for ECL detection of AFP.

This ECL biosensor was employed to detect AFP content in clinical serum samples. The assay results of clinical serum samples, compared with the reference values obtained by commercial turbidimetric immunoassay, showed an acceptable agreement, with relative deviation less than 6.62% (Table 1), indicating acceptable accuracy of the proposed ECL biosensor for clinical samples assay. These results confirmed the reliability and promising application of the designed method in clinical diagnoses.

Conclusions

In summary, a novel and universal strategy for label-free and highly sensitive ECL biosensing based on CdSe QDs/SWCNTs in RTILs was designed. The excellent conductivity of SWCNTs, and the extremely high ionic conductivity and wide potential window of RTILs in the as-prepared film lead to a synergic effect which significantly enhanced the ECL response of CdSe QDs and improved their stability. Using AFP as a model analyte, the formed immunocomplex after immunoreaction increased the steric hindrances for coreactant to the surface of CdSe QDs and slowed down the electron-transfer speed in ECL reaction, leading to the decline of ECL intensity. By monitoring the variation of ECL intensity before and after the immunoreaction, a label-free signal-off ECL biosensor was proposed. The stepwise increase of electron transfer resistance verified the successful fabrication of the ECL biosensing interface. This designed signal-off ECL biosensor exhibited good analytical performance such as wide linear concentration wide, low detection limit, good reproducibility, satisfactory stability and high selectivity. Furthermore, the proposed biosensor was successfully applied to the assay of clinical serum samples, which not only extended the application field of QD-based ECL sensor but also provided a promising and alternative assay method for tumour markers in clinical diagnoses.

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Notes and references

- 1 S. F. Liu, X. Zhang, Y. M. Yu and G. Z. Zou, *Anal. Chem.*, 2014, **86**, 2784.
- 2 Y. M. Long, L. Bao, J. Y. Zhao, Z. L. Zhang and D. W. Pang, *Anal. Chem.*, 2014, **86**, 7224.
- 3 Y. Cheng, Y. Huang, J. P. Lei, L. Zhang and H. X. Ju, *Anal. Chem.*, 2014, **86**, 5158.
- 4 W. J. Miao, *Chem. Rev.*, 2008, **108**, 2506.
- 5 Y. Kim and J. Kim, *Anal. Chem.*, 2014, **86**, 1654.
- 6 Z. H. Chen, Y. Liu, Y. Z. Wang, X. Zhao and J. H. Li, *Anal. Chem.*, 2013, **85**, 4431.
- 7 H. R. Zhang, M. S. Wu, J. J. Xu and H. Y. Chen, *Anal. Chem.*, 2014, **86**, 3834.
- 8 P. Zhao, L. F. Zhou, Z. Nie, X. H. Xu, W. Li, Y. Huang, K. Y. He and S. Z. Yao, *Anal. Chem.*, 2013, **85**, 6279.
- 9 S. N. Ding, B. H. Gao, D. Shan, Y. M. Sun and S. Cosnier, *Chem. – Eur. J.*, 2012, **18**, 1595.
- 10 L. L. Li, K. P. Liu, G. H. Yang, C. M. Wang, J. R. Zhang and J. J. Zhu, *Adv. Funct. Mater.*, 2011, **21**, 869.
- 11 L. Zhang, Y. Cheng, J. P. Lei, Y. T. Liu, Q. Hao and H. X. Ju, *Anal. Chem.*, 2013, **85**, 8001.
- 12 J. Ji, L. He, Y. Y. Shen, P. P. Hu, X. H. Li, L. P. Jiang, J. R. Zhang, L. L. Li and J. J. Zhu, *Anal. Chem.*, 2014, **86**, 3284.
- 13 S. L. Yang, J. S. Liang, S. L. Luo, C. B. Liu and Y. H. Tang, *Anal. Chem.*, 2013, **85**, 7720.
- 14 X. F. Tang, D. Zhao, J. C. He, F. W. Li, J. X. Peng and M. N. Zhang, *Anal. Chem.*, 2013, **85**, 1711.
- 15 W. P. Deng, F. Liu, S. G. Ge, J. H. Yu, M. Yan and X. R. Song, *Analyst*, 2014, **139**, 1713.
- 16 N. P. Sardesai, J. C. Barron and J. F. Rusling, *Anal. Chem.*, 2011, **83**, 6698.
- 17 S. Y. Deng, Z. T. Hou, J. P. Lei, D. J. Lin, Z. Hu, F. Yan and H. X. Ju, *Chem. Commun.*, 2011, **47**, 12107.
- 18 L. C. Chen, Y. J. Zhang, S. Y. Ren, D. J. Huang, C. Zhou, Y. W. Chi and G. N. Chen, *Analyst*, 2013, **138**, 7006.
- 19 L. C. Chen, D. J. Huang, Y. J. Zhang, T. Q. Dong, C. Zhou, S. Y. Ren, Y. W. Chi and G. N. Chen, *Analyst*, 2012, **137**, 3514.
- 20 L. C. Chen, D. J. Huang, S. Y. Ren, Y. W. Chi and G. N. Chen, *Anal. Chem.*, 2011, **83**, 6862.
- 21 D. J. Lin, J. Wu, F. Yan, S. Y. Deng and H. X. Ju, *Anal. Chem.*, 2011, **83**, 5214.
- 22 X. Liu, Y. Y. Zhang, J. P. Lei, Y. D. Xue, L. X. Cheng and H. X. Ju, *Anal. Chem.*, 2010, **82**, 7351.
- 23 S. F. Chou, W. L. Hsu, J. M. Hwang and C. Y. Chen, *Clin. Chem.*, 2002, **48**, 913.
- 24 Q. Liu, M. Han, J. C. Bao, X. Q. Jiang and Z. H. Dai, *Analyst*, 2011, **136**, 5197.
- 25 G. F. Jie, L. Wang and S. S. Zhang, *Chem. – Eur. J.*, 2011, **17**, 641.
- 26 L. Yuan, X. Hua, Y. F. Wu, X. H. Pan and S. Q. Liu, *Anal. Chem.*, 2011, **83**, 6800.
- 27 J. Y. Hou, T. C. Liu, Z. Q. Ren, M. J. Chen, G. F. Lin and Y. S. Wu, *Analyst*, 2013, **138**, 3697.
- 28 K. J. Huang, J. Li, Y. Y. Wu and Y. M. Liu, *Bioelectrochemistry*, 2013, **90**, 18.
- 29 Z. G. Chen, Y. L. Lei and X. Chen, *Microchim. Acta*, 2012, **179**, 241.
- 30 R. P. Liang, G. H. Yao, L. X. Fan and J. D. Qiu, *Anal. Chim. Acta*, 2012, **737**, 22.
- 31 Z. J. Yang, H. Liu, C. Zong, F. Yan and H. X. Ju, *Anal. Chem.*, 2009, **81**, 5484.
- 32 G. L. Wang, J. J. Xu, H. Y. Chen and S. Z. Fu, *Biosens. Bioelectron.*, 2009, **25**, 791.
- 33 X. Xiang, L. Chen, C. L. Zhang, M. Luo, X. H. Ji and Z. K. He, *Analyst*, 2012, **137**, 5586.