ChemComm

Cite this: *Chem. Commun.*, 2011, **47**, 4442–4444

www.rsc.org/chemcomm

COMMUNICATION

A novel electrochemiluminescent reagent of cyclometalated iridium complex-based DNA biosensor and its application in cancer cell detection†

Chunxiang Li, Juan Lin, Yingshu Guo and Shusheng Zhang*

Received 27th January 2011, Accepted 17th February 2011
DOI: 10.1039/c1cc10544b

An ultrasensitive biosensor employing a novel electrochemiluminescent (ECL) labeling reagent cyclometalated Ir complex has been reported, and successfully applied in cancer cell detection.

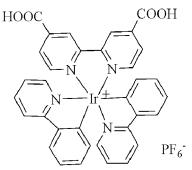
Although $Ru(bpy)_3^{2+}$/TPA system (TPA = tripropylamine) has played a crucial role in the development of electrochemiluminescence (ECL) and its applications,[1] much effort has been devoted to develop new ECL labels to improve the sensitivity. In fact, although many neutral Ir(III) complexes have been demonstrated to display high ECL efficiencies in organic solutions usually with a degree of efficiency much higher than $Ru(bpy)_3^{2+}$,[2] their applicative field has been restricted by their very poor water-solubility and lack of active groups for biological labeling. More recently, Chen *et al.*[3] proved that an ionic iridium(III) complex with an appended sugar also displayed a strong ECL signal, which prompted us to design ionic iridium(III) complexes and further apply it to construct a biosensor for cancer cell detection. In the present, a novel ECL reagent with carboxyl, $[(ppy)_2Ir(dcbpy)]^+ PF_6^-$ (shown in Scheme 1), was prepared. Its UV-vis absorption, fluorescence, and ECL characteristics were explored and probable mechanisms were speculated (discussed in detail in ESI†). Ultrasensitive biosensors based on the complex were fabricated for target DNA and cancer cell detection.

Using $[(ppy)_2Ir(dcbpy)]^+ PF_6^-$ as a label, a ECL nanoprobe was constructed that relied on AuNPs to amplify ECL signals and employed cysteamine as a bio-barcode (Scheme 2A). Based on the developed nanoprobe, a highly sensitive DNA biosensor was developed. As depicted in Scheme 2B, the strategy involved thiolfunctionalized capture DNA-1 self-assembled on a Au electrode and hybridized with one end of the target DNA-1 sequence, the other end of which was recognized with a signal probe DNA-1 labeled with AuNPs. The amplified signal by AuNPs for the detection of target DNA was obtained. The concentration of target DNA was quantified by the ECL intensity. As shown in Fig. S6 in ESI,† the ECL intensities increased with the increase of concentration of the target DNA. The fitting equation is $\Delta I_{ECL} = 4.57C + 46.88$ (ΔI_{ECL} is the ECL intensity subtracted the blank; C is the concentration of target DNA, 10^{-15} M; $N = 15$, $R = 0.9991$), and a broad linear range covers nearly three orders of magnitude from 2.0×10^{-15} to 1.0×10^{-12} M. The theoretical limit (3σ) of detection was calculated to be 8.6×10^{-16} M, which is 2 orders of magnitude lower than a reported assay[4] (100 fM) labeled with a Ru complex employing the same AuNPs amplification and 5 orders of magnitude lower than that labeled with Ru complex (9×10^{-11} M) without amplification.[5] To investigate the accuracy of the assay, a relative standard deviation (RSD) was determined by measuring the ECL signal of 2.0×10^{-14} M target DNA with eleven replicates, and yielded a RSD of 7.8%, indicating a good reproducibility.

At the same time, a control experiment was carried out using conventional $[(bpy)_2Ru(dcbpy)]^{2+} 2PF_6^-$) as a label. The results are shown in Fig. S10 in ESI,† the linear range is from 8.0×10^{-13} to 10^{-11} M, and the detection limit of 3.2×10^{-13} M target DNA was deduced using 3σ. From comparison, it was clearly seen that the sensitivity of the strategy is increased 400 times by replacing the traditional Ru label. We believe that the extraordinarily high sensitivity is attributed to high ECL efficiency and the relative stability of the Ir complex.

To further reveal the difference in sensitivity of the biosensor between employing an Ir complex and a Ru complex label, the ECL responses of the biosensor with the same concentration of target DNA are depicted in Fig. 1. It can be found that, in the presence of the same concentration of target DNA, the ECL signal labeled with Ir complex is about 200 times higher than that based on Ru complex.

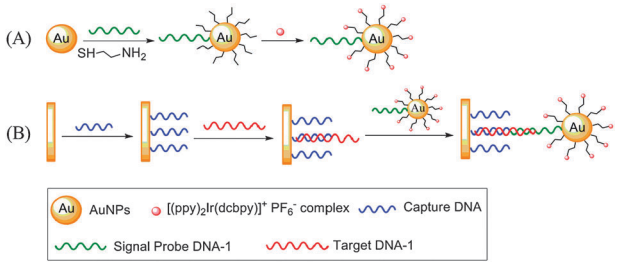
Recently, circular amplifications based on DNA polymerase and endonuclease were confirmed to be effective means for improving sensitivity.[6] Encouraged by the above experiment



Scheme 1 The structure of the $[(ppy)_2Ir(dcbpy)]^+ PF_6^-$ complex included in this study.

State Key Laboratory Base of Eco-chemical Engineering, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, P. R. China. E-mail: shushzhang@126.com; Fax: +86 532 84022750; Tel: +86 532 84022750

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1cc10544b



Scheme 2 Process of ECL nanoprobe preparation (A) and the determination of target DNA (B).

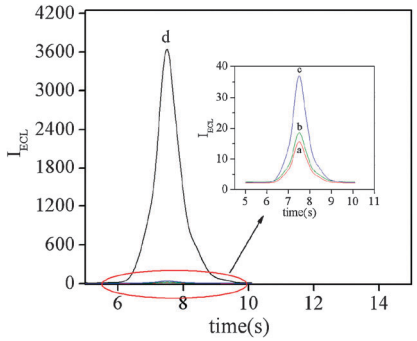
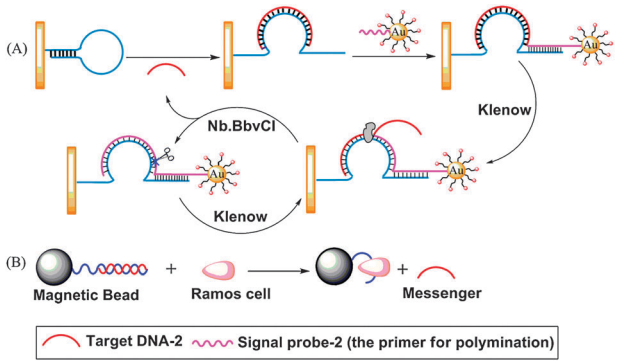


Fig. 1 The comparison of ECL signals of Scheme 2B for a blank sample (without target DNA) based on Ru complex (a), blank sample based on Ir complex (b), target DNA detection employing Ru complex (c), and target DNA detection based on Ir complex (d). The target DNA concentration is 8.0×10^{-13} M.

results and to demonstrate the generality of Ir complex as a luminescent reagent, the above developed nanoprobe was designed to construct a protocol based on strand-displacement DNA polymerization and nicking endonuclease assisted cycle (shown in Scheme 3A). The system consisted of a hairpin probe with an endonuclease recognition site that was immobilized on a Au electrode, an ECL nanoprobe that was attached with primer sequences, Klenow fragment *exo*- (denoted as "Klenow" for short) for DNA polymerization, dNTP mixture and nicking endonuclease. The loop part of the hairpin probe was complementary to the target sequence, when in the presence of target DNA, the stem part of the hairpin was opened, to which the nanoprobe was attached through the signal probe-2, which is the primer for DNA strand polymerization. The polymerization



Scheme 3 Schematic representation of strategy for DNA detection based on DNA polymerization and nicking endonuclease assisted circular amplification process (A) and cancer cell detection (B).

was thus initiated, in which the new strand prolonged from the primer and then replaced and released the target sequence. Meanwhile, the strand segment with cleavage site was cleaved and triggered new strand polymerization. Since the polymerization and cleave can be recycled for multiple rounds, hundreds of target DNA were released and triggered other hairpin probes. Finally amplification was accomplished.

The resulting calibration curve of Scheme 3A is shown in Fig. 2. The ECL intensities increased with an increase in the target DNA concentrations from 2.0×10^{-16} to 1.0×10^{-14} M with a 2-order polynomial curve of $\Delta I_{ECL} = -0.07C^2 + 13.88C + 13.76$ (ΔI_{ECL} is the ECL intensity subtracted the blank; C is the concentration of target DNA, 10^{-16} M; $N = 11$, $R = 0.994$), and the detection limit of 8.2×10^{-17} M could be estimated by using 3σ ruler, which is one of the most sensitive methods for detection and analysis of DNA to the best of our knowledge. To investigate the accuracy of the assay, RSD was determined by measuring the ECL signal of 4.0×10^{-15} M target DNA with eleven replicates, and its values was 8.5%, indicating a good reproducibility of the assay.

Also, a simultaneous control experiment was carried out by labeling with Ru complex. The acquired results are shown in Fig. S11 in ESI.† The dynamic range was from 3×10^{-14} to 1×10^{-12}, and the detection limit of 9.7×10^{-15} M was calculated according to 3σ. It is obvious that the sensitivity of the proposed protocol is improved 2 orders of magnitude by replacing conventional Ru complex.

In order to demonstrate the feasibility of the strategy (Scheme 3A) in practical application, a design for the determination of cancer cells was achieved based on the high specificity and affinity of cell aptamers for the recognition of unique molecular signatures on the surface of cells.[7] As Scheme 3B shows, briefly, amino groups-modified aptamers were attached onto the surface of carboxylated magnetic beads (MBs) to fabricate conjugates MBs-aptamer. Then, MBs-aptamer were hybridized with the messenger DNA sequence (its sequence is the same as target DNA-2 and partially complementary to the aptamer) to form the duplex. In the presence of Ramos cells, the MBs-aptamer bound to the target cells and resulted in the disassembly of the original duplex and release of messenger DNA sequence. The released DNA sequences were then introduced into the cycle amplification system to trigger

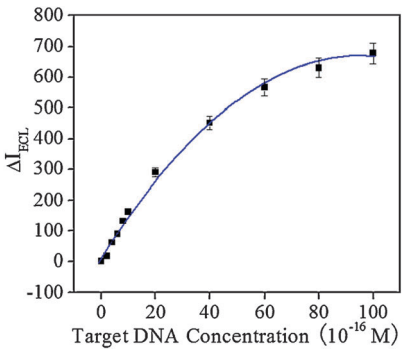


Fig. 2 The calibration curve of ECL intensities *vs.* the concentration of target DNA from 2.0×10^{-16} to 1.0×10^{-14} M (Scheme 3A) based on DNA polymerization and nicking endonuclease assisted circular amplification process.

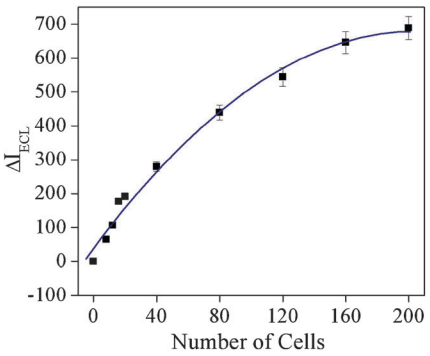


Fig. 3 The calibration curve for Ramos cell determination (Scheme 3) from 8 to 200 cells target cells. The blank was subtracted for each value.

circular polymerizations. The following processes are the same for Scheme 3A.

The cell detection results are shown in Fig. 3, the ECL signals of the system increased with the increase of Ramos cells number ranging from 8 to 200 cells. The correlation equation was $\Delta I = -0.015C^2 + 6.26C + 39.41$ (ΔI is the ECL intensity subtracted the blank; C is the number of target cells, $n = 10$, $R = 0.989$). A detection limit of 6 cells can be estimated using 3σ, which was significantly improved compared to reported techniques for cancer cell detection.[8] A series of eleven repetitive measurements of 40 target cells were used for estimating the precision and value of RSD as 8.5%.

The control experiment based on the detection system with Ru complex as luminescence labeling reagent was performed and the calibration curve is illustrated in Fig. S12 in ESI,† which reveals a quantitative relationship with the number of Ramos cell ranging from 120 to 2000 cells, and the detection limit of 87 cells is estimated (3σ). It is clearly seen from comparison that the sensitivity of the proposed system is increased approximately 15 times. It is worth mentioning that almost single cell detection is realized by introducing a luminescence reagent with excellent ECL efficiency by only a simple method. Additionally, the ultrahigh sensitivity could also be attributed to effective signal amplification of AuNPs, polymerase and endonuclease assisted cycle and high activity to binding with the targets of the aptamers.[9]

To assess the specificity of the method for the detection of Ramos cells, experiments were conducted on HeLa and CEM cells. The experimental results demonstrated that the proposed system for Ramos cells exhibited a good specificity originating from the specific binding between target and aptamer (depicted in Fig. S7 in ESI†).

In order to further confirm the feasibility of the system for the detection of cancer cells in biological fluids, the system was used to detect Ramos cells in real human blood treated with anticoagulant. By optimizing, 6% of human blood in volume was used (see Fig. S8 in ESI†). The resulting calibration curve is shown in Fig. S9 in ESI.† The fitting equation is $\Delta I = -0.01C^2 + 6.05C + 21.80$ (ΔI is the ECL intensity subtracted the blank; C is the number of target cells, $n = 10$, $R = 0.991$) with a broad dynamic range from 8 to 200 cells, which is in agreement with the pure target cell samples. All these results suggest the potentiality of the proposed system for cancer detection in real clinical samples.

In summary, the study has presented a new luminescence labeling reagent for ECL, and it has been successfully applied to construct an ultrasensitive biosensor. More importantly, based on the reagent, the sensitivity of the biosensor for target DNA detection is improved more than 200 times compared to that employing the well-known ECL reagent Ru complex. As proven by our research, this assay allows us to determine cancer cells down to 8 cells and exhibits a significant specificity for Ramos cells, even in real human blood samples. It is expected that the easily synthetic, air-stable and very efficient phosphorescent emitter can be used in more fields for biological assays and clinical diagnoses.

The work was supported by the National Basic Research Program of China (2010CB732404), the National Nature Science Foundation of China (21025523), and the Basic Research Project of Qingdao (10-3-4-4-5-jch).

Notes and references

1 (*a*) M. Shen, J. Rodríguez-López, J. Huang, Q. Liu, X.-H. Zhu and A. J. Bard, *J. Am. Chem. Soc.*, 2010, DOI: 10.1021/ja105282u; (*b*) C. Wu, H.-F. Chen, K.-T. Wong and M. E. Thompson, *J. Am. Chem. Soc.*, 2010, **132**, 3133; (*c*) W. Miao, *Chem. Rev.*, 2008, **108**, 2506.
2 (*a*) D. Bruce and M. M. Richter, *Anal. Chem.*, 2002, **74**, 1340; (*b*) B. D. Muegge and M. M. Richter, *Anal. Chem.*, 2004, **76**, 73; (*c*) J. I. Kim, I.-S. Shin, H. Kim and J.-K. Lee, *J. Am. Chem. Soc.*, 2005, **127**, 1614.
3 M.-J. Li, P. C. Jiao, M. Lin, W. W. He, G.-N. Chen and X. Chen, *Analyst*, 2011, **136**, 205.
4 R. X. Duan, X. M. Zhou and D. Xing, *Anal. Chem.*, 2010, **82**, 3099.
5 J. Zhang, H. Qi, Y. Li, J. Yang, Q. Gao and C. X. Zhang, *Anal. Chem.*, 2008, **80**, 2888.
6 (*a*) B. Shlyahovsky, D. Li, Y. Weizmann, R. Nowanski, M. Kotler and I. J. Willner, *J. Am. Chem. Soc.*, 2007, **129**, 3814; (*b*) F. Wang, J. Elbaz, C. Teller and I. Willner, *Angew. Chem., Int. Ed.*, 2010, **49**, 1; (*c*) J. Liu and Y. Lu, *Curr. Opin. Biotechnol.*, 2006, **17**, 580; (*d*) C. Scheibe, A. Bujotzek, J. Dernedde, M. Weber and O. Seitz, *Chem. Sci.*, 2011, DOI: 10.1039/c0sc00565g.
7 Z. Xiao, D. Shangguan, Z. Cao, X. Fang and W. Tan, *Chem.–Eur. J.*, 2008, **14**, 1769.
8 (*a*) C. D. Medley, J. E. Smith, Z. Y. Tang, W. S. Bamrungsap and W. Tan, *Anal. Chem.*, 2008, **80**, 1067; (*b*) X. Chen, M.-C. Estévez, Z. Zhu, Y. F. Huang and Y. L. Chen, *Anal. Chem.*, 2009, **81**, 7009.
9 Z. W. Tang, D. H. Shangguan, K. M. Wang, H. Shi, K. Sefah, P. Mallik-ratchy and W. H. Tan, *Anal. Chem.*, 2007, **79**, 4900.

