A highly sensitive and selective "signal-on" electrochemiluminescent biosensor for mercury†

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A highly sensitive and selective electrochemiluminescent biosensor was designed for mercury(II) based on T–Hg^{2+}–T complex by using $Ru(bpy)_3^{2+}$-doped silica nanoparticles (Ru-SNPs) to label the oligonucleotides in order to improve the sensitivity.

Highly selective interaction of oligonucleotides with metal ions leads oligonucleotides to become novel tools for detection of the specific metal ions. It is reported that two multi-thymidine–thymidine (T–T) mismatches DNA can form strong and stable thymidine–Hg^{2+}–thymidine (T–Hg^{2+}–T) base pairs in the presence of Hg^{2+}.[1–4] The binding constant (K_b) of T–Hg^{2+}–T is about 4.14×10^6 L mol^{-1},[5] higher than that of A–T.[6] Specially, a T–T mismatch pair demonstrates extreme specificity toward Hg^{2+} even in the presence of other competitive metal ions, such as Pb^{2+}, Cu^{2+}, Zn^{2+}, and $Fe^{3+/2+}$, which frequently coexist with Hg^{2+} in the actual environmental samples. Selective detection of Hg^{2+} in the presence of these coexisting metal interferents is a critical issue to the application of most common sensors. According to this property, many Hg^{2+} sensors have been developed, most of them based on a fluorescent[7–12] or chromophoric method.[13–17] However, the detection limit of most of those methods is heavily limited and could not reach the standard of the U.S. Environmental Protection Agency (EPA), lower than 10 nM in drinking water, because of the low selectivity of fluorescent and chromophoric methods.

Electrochemiluminescence (ECL) is a chemiluminescent reaction of species undergoing electron-transfer reactions at an electrode surface. ECL sensors combine the advantages of both electrochemical and chemiluminescent sensors, such as high sensitivity, ease of control and using of simple equipment.[18–21] The most important ECL reagent is ruthenium complexes, especially tris(2,2′-bipyridine)ruthenium(II) [$Ru(bpy)_3^{2+}$], widely used as a label for immunoassays or DNA-probe assays, which could provide a highly sensitive assay. The chemical interaction of $Ru(bpy)_3^{2+}$ and DNA is that $Ru(bpy)_3^{2+}$ or derivatives of $Ru(bpy)_3^{2+}$ react with the functional groups being immobilized on the DNA. This method could improve stability, sensitivity of assay and save $Ru(bpy)_3^{2+}$ dosage. Dong *et al.*[22] designed $Ru(bpy)_3^{2+}$-doped silica–chitosan coating over the glassy carbon electrode, which could detect 2.8 nM of tripropylamine. Fang *et al.*[23] designed a DNA probe labelled with $Ru(bpy)_3^{2+}$-doped silica nanoparticles (Ru-SNPs) for detection of DNA hybridization with a linear response range of 2.0×10^{-13}–2.0×10^{-9} mol L^{-1}. These examples showed that ECL method has a high sensitivity for detection.

Barton *et al.*[24] found that when a base pair was mismatched, the transfer of electrons would be terminated. We could conceive two mismatched ssDNA modified on the electrode, one of which was labeled with ECL reagent, for which ECL intensity was weak due to the block of the electron-transfer. When two mismatched ssDNA switch to completely matched dsDNA, the block of the electron-transfer is eliminated, resulting in increasing of ECL intensity. Based on which, a "signal-on" DNA ECL sensor for Hg^{2+} can be designed, which combines the merits of the high selectivity of the T–Hg^{2+}–T pair with high sensitivity of ECL. At present, many sensors[7–9,15,16] for Hg^{2+} are "signal-off" ones, which can result in "false positive" results caused by external quenchers or other environmental factors.[25,26] At the same time, the signal intensity of the "signal-off" sensor is limited because the target can suppress only 100% of original signal,[27] which influences the detection limit. Therefore, a "signal-on" sensor, compared with a "signal-off" one, could overcome the above shortcomings.

Herein, we prepared the Ru-SNPs for labeling of oligonucleotides. It was found that the ECL intensity of Ru-SNPs was much stronger than that of $Ru(bpy)_3^{2+}$ directly labeled to oligonucleotides because SNPs attached themselves to oligonucleotides containing more $Ru(bpy)_3^{2+}$. If combined with the merit of T–Hg^{2+}–T, it would match the requirement of the US EPA for mercury detection.

The functional Ru-SNPs were prepared according to the previous literature.[28,29] Preparation of the Ru-SNPs oligonucleotides probes is shown in Fig. 1A. $Ru(bpy)_3^{2+}$ is encapsulated in the silica network *via* the reverse microemulsion method, and the nucleation and growth of the silica are highly regulated in the water droplets.[30] The Ru-SNPs size was measured by transmission electron microscope (TEM). As shown in Fig. 1B the Ru-SNPs were extremely uniform in size, 55 ± 5 nm in diameter. At higher resolution (shown in the inset of Fig. 1B), Ru-SNPs have also visible dark dots, which are heavy metal ruthenium atom embedded inside the silica sphere. However, such dark dots could not be observed in the pure silica particles at the same TEM resolution. In the TEM it is found that some nanoparticles are aggregated together to form a network, which is due to the low polarity of the microemulsion water pool.

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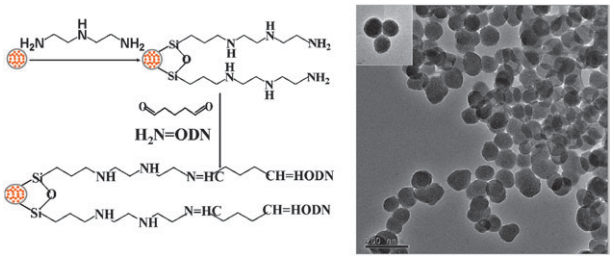

Fig. 1 (A) Preparation of Ru-SNPs oligonucleotides probes. (B) TEM image of Rubpy-doped silica nanoparticles at 50 000× magnification (inset: a region of 165 000× magnification).

When Hg^{2+} ions bind to thymine residues in DNA, the protons are released and Hg^{2+} can insert into two DNA T–T mismatches, which leads to the formation of a T–Hg^{2+}–T pair by covalent N–Hg bonds.[31] The structure of T–Hg^{2+}–T is shown in Fig. 2B. Switching the structure of DNA from mismatched dsDNA to a completely complementary one leads to elimination of the block of the electron-transfer and enhancement of ECL intensity. The principle of the Hg^{2+} DNA ECL sensor is shown in Fig. 2A. The sensor consists of oligonucleotides (1) with an alkanethiol moiety at the 5′-terminus, oligonucleotides (2) with amido at the 5′-terminus and functionalized Ru-SNPs. Oligonucleotides (1) and (2) are mismatched due to 10-mer T–T mismatch bases. Oligonucleotides (1), probe DNA, are immobilized onto the surface of the gold electrode *via* the thiol–Au interaction. The surface density of oligonucleotides (1) on the surface of the gold electrode (surface area of gold electrode, 3.14 mm^2) was about 5.7×10^{11} molecule/cm^2, as calculated by the method reported previously.[32] Oligonucleotides modified with luminophores can be obtained by interaction of functionalized Ru-SNPs and the amido at the 5′-terminus of oligonucleotides (2). By hybridization with oligonucleotides (1) and (2), the Ru-SNPs can be immobilized onto the surface of the gold electrode. Because of block of the electron-transfer between two mismatched oligonucleotides, ECL intensity is low. Addition of Hg^{2+} makes the previously non-complementary oligonucleotides switch to complementary dsDNA, which eliminates block of the electron-transfer, resulting in enhancement of ECL intensity. With the increasing of Hg^{2+} concentration, more T–T pairs switch to T–Hg^{2+}–T pairs, which results in increasing of ECL intensity, based on which a sensitive sensor for Hg^{2+} can be developed.

Fig. 3A shows the ECL intensity of the sensor for Hg^{2+} under the different concentrations of Hg^{2+} in Tris-HCl (pH 7.6) containing 5.0×10^{-3} mol L^{-1} 2-(dibutylamino)ethanol (DBAE) by using cyclic voltammetry (CV) as the scan mode. It was found that the ECL intensity was increased with increasing of the concentration of Hg^{2+}, and the ECL intensity had a linear relationship with the logarithm of Hg^{2+} concentration in the range of 5.0×10^{-9}–5.0×10^{-7} mol L^{-1} with a regression coefficient R of 0.9961 (see Fig. 3B). The detection limit for Hg^{2+} is 2.3×10^{-9} mol L^{-1} (defined as S/N = 3), which is lower than that of fluorescent[7–12] and chromophoric sensors.[13–17]

The stability of the ECL signal of the sensor in the presence and absence of Hg^{2+}, respectively, has also been investigated,

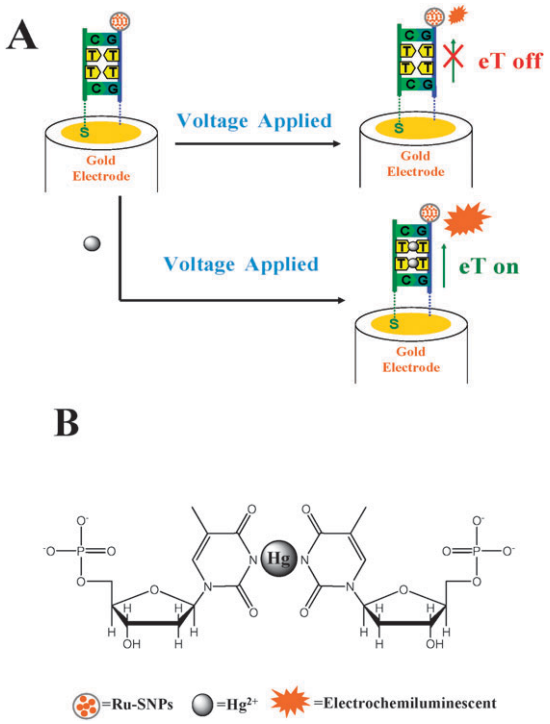

Fig. 2 (A) Principle of the ECL Hg^{2+} DNA sensor. (B) Configuration of the T–Hg^{2+}–T pair.

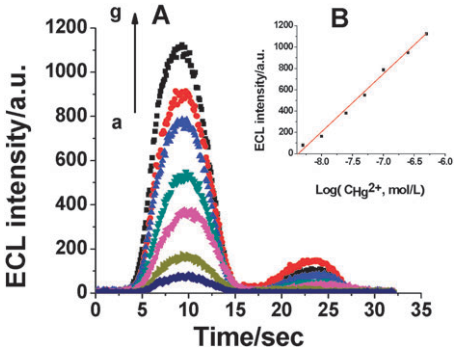

Fig. 3 (A) ECL intensity curves from the modified ECL sensors with different Hg^{2+} concentrations under CV scanning: (a) 5.0×10^{-9}; (b) 1.0×10^{-8}; (c) 2.5×10^{-8}; (d) 5.0×10^{-8}; (e) 1.0×10^{-7}; (f) 2.5×10^{-7}; (g) 5.0×10^{-7} mol L^{-1} in 10 mM Tris-HCl (pH 7.6) containing 5.0×10^{-3} mol L^{-1} DBAE at the scan rate of 50 mV s^{-1}, potential range 0.8–1.6 V. (B) Calibration curves between ECL intensity and the logarithm of Hg^{2+} concentration in Tris-HCl (pH 7.6).

and the results show that the relative standard deviation (RSD) of the ECL response (n = 5) was 2.6 and 3.0%, respectively. ECL intensity decreases only 5% for the detection of 15 repeated analyses, which indicates that the DNA sensor has good stability. In addition we also found that the sensor still retained its ECL activity and intensity in Tris-HCl buffer solution after the sensor had been stored at 4 °C for four weeks.

The selectivity of the structure of T–T to other interferent ions, such as Pb^{2+}, Cu^{2+}, Ag^{+}, Zn^{2+}, Ni^{2+}, Fe^{3+} and Cd^{2+}, was investigated under the optimum conditions. The

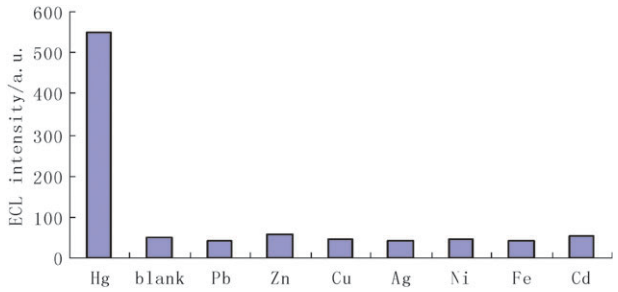

Fig. 4 ECL intensity on the modified gold electrodes after reaction with various divalent metal ions (Hg^{2+} concentration at 5.0×10^{-8} mol L^{-1} and other competing ions concentration at 10^{-6} mol L^{-1}).

concentrations of these competing ions were 10^{-6} mol L^{-1}, 50 times that of Hg^{2+}. Fig. 4 shows the ECL intensity of the sensor to these metal ions possibly coexisting with Hg^{2+}. It was observed that in the presence of other ions, ECL intensity of the sensor was very low, which does not interfere with the assay of Hg^{2+}. It is also proved that these metal ions could not insert into a T–T base pair to form a stable structure such as T–Hg^{2+}–T, and T–T base pair is only selective to Hg^{2+}. The conclusion coincides with the results of the other methods.[7–17]

The proposed method has been applied to evaluate Hg^{2+} in waste water samples. The results showed the average concentration of Hg^{2+} detected in the samples was 7.8×10^{-9} mol L^{-1} with an RSD of 3.2% ($n = 5$), and it had good accordance with the results detected by the atomic absorption method (8.0×10^{-9} mol L^{-1}).

In summary, a high sensitivity and selectivity ECL DNA sensor for mercury(II) is proposed. This method exploits Hg^{2+} inserted into two DNA T–T mismatches to form a stable T–Hg^{2+}–T base pair. Ru-SNPs interact with oligonucleotide (2) with amido at the 3′-terminus, which hybridizes with a non-complementary oligonucleotide (1) immobilized on the gold electrodes by self-assembly of a terminal thiol moiety. In the absence of Hg^{2+}, ECL intensity of the sensor is low because of the mismatch of two ssDNA. However, when Hg^{2+} is present the mismatch T–T switches to stable formation of T–Hg^{2+}–T base pair, which results in the previous dsDNA mismatches switching to completely match dsDNA and obviously enhances ECL intensity. ECL intensity has a relationship with the concentration of Hg^{2+}. At the same time, the ECL DNA sensor is extremely selective to Hg^{2+}, in the presence of high concentrations of other metal ions possibly coexisting with Hg^{2+}, the disturbance from these ions could be negligible.

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Notes and references

1 G. H. Clever, C. Kaul and T. Carell, *Angew. Chem., Int. Ed.*, 2007, **46**, 6226–6236.
2 S. Katz, *J. Am. Chem. Soc.*, 1952, **74**, 2238–2245.
3 Y. Miyake, H. Togashi, M. Tashiro, H. Yamaguchi, S. Oda, M. Kudo, Y. Tanaka, Y. Kondo, R. Sawa, T. Fujimoto, T. Machinami and A. Ono, *J. Am. Chem. Soc.*, 2006, **128**, 2172–2173.
4 Y. Tanaka, S. Oda, H. Yamaguchi, Y. Kondo, C. Kojima and A. Ono, *J. Am. Chem. Soc.*, 2007, **129**, 244–245.
5 Y. Li, Y. Jiang and X. P. Yan, *Anal. Chem.*, 2006, **78**, 6115–6120.
6 Z. Q. Zhu, Y. Y. Su, J. Li, D. Li, J. Zhang, S. P. Song, Y. Zhao, G. X. Li and C. H. Fan, *Anal. Chem.*, 2009, **81**, 7660–7666.
7 A. Ono and H. Togashi, *Angew. Chem., Int. Ed.*, 2004, **43**, 4300–4302.
8 L. Q. Guo, H. Hu, R. Q. Sun and G. N. Chen, *Talanta*, 2009, **79**, 775–779.
9 S. J. Liu, H. G. Nie, J. H. Jiang, G. L. Shen and R. Q. Yu, *Anal. Chem.*, 2009, **81**, 5724–5730.
10 C. K. Chiang, C. C. Huang, C. W. Liu and H. T. Chang, *Anal. Chem.*, 2008, **80**, 3716–3721.
11 B. C. Ye and B. C. Yin, *Angew. Chem., Int. Ed.*, 2008, **47**, 8386–8389.
12 J. Wang and B. Liu, *Chem. Commun.*, 2008, 4759–4761.
13 J. S. Lee, M. S. Han and C. A. Mirkin, *Angew. Chem., Int. Ed.*, 2007, **46**, 4093–4096.
14 X. Xue, F. Wang and X. Liu, *J. Am. Chem. Soc.*, 2008, **130**, 3244–3245.
15 C. W. Liu, Y. T. Hsieh, C. C. Huang, Z. H. Lin and H. T. Chang, *Chem. Commun.*, 2008, 2242–2244.
16 S. He, D. Li, C. Zhu, S. Song, L. Wang, Y. Long and C. Fan, *Chem. Commun.*, 2008, 4885–4887.
17 D. Li, A. Wieckowska and I. Willner, *Angew. Chem., Int. Ed.*, 2008, **47**, 3927–3931.
18 X. B. Yin, S. J. Dong and E. K. Wang, *TrAC, Trends Anal. Chem.*, 2004, **23**, 432–436.
19 M. M. Richter, *Chem. Rev.*, 2004, **104**, 3003–3036.
20 H. Wei and E. K. Wang, *TrAC, Trends Anal. Chem.*, 2008, **27**, 447–459.
21 W. J. Miao, *Chem. Rev.*, 2008, **108**, 2506–2553.
22 L. H. Zhang and S. J. Dong, *Anal. Chem.*, 2006, **78**, 5119–5123.
23 Z. Chang, J. M. Zhou, K. Zhao, N. N. Zhu, P. G. He and Y. Z. Fang, *Electrochim. Acta*, 2006, **52**, 575–580.
24 S. O. Kelley and J. K. Barton, *Chem. Biol.*, 1998, **5**, 413–425.
25 A. Sassolas, B. D. Leca-Bouvier and L. J. Blum, *Chem. Rev.*, 2008, **108**, 109–139.
26 J. Wang, *Chem.–Eur. J.*, 1999, **5**, 1681–1685.
27 Y. Xiao, X. G. Qu, K. W. Plaxco and A. J. Heeger, *J. Am. Chem. Soc.*, 2007, **129**, 11896–11897.
28 S. Santra, P. Zhang, K. M. Wang, R. Tapec and W. Tan, *Anal. Chem.*, 2001, **73**, 4988–4993.
29 N. N. Zhu, H. Cai, P. G. He and Y. Z. Fang, *Anal. Chim. Acta*, 2003, **481**, 181–189.
30 J. Schmidt, C. Guesdon and R. J. Schomacker, *J. Nanopart. Res.*, 1999, **1**, 267–276.
31 T. Yamane and N. Davidson, *J. Am. Chem. Soc.*, 1961, **83**, 2599–2607.
32 A. B. Steel, T. M. Herne and M. J. Tarlov, *Anal. Chem.*, 1998, **70**, 4670–4677.