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Cite this: *Chem. Commun.*, 2011, **47**, 9879–9881

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COMMUNICATION

Electrochemiluminescence detection of near single DNA molecules by using quantum dots–dendrimer nanocomposites for signal amplification†

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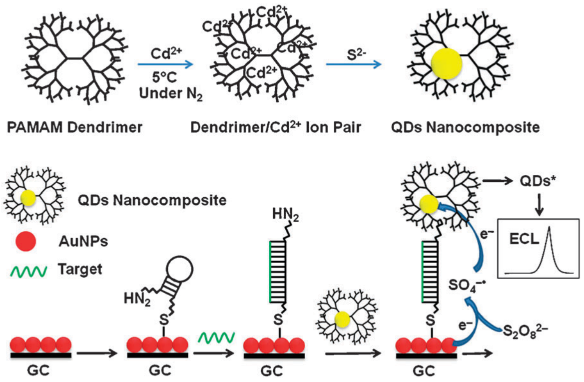
Received 16th June 2011, Accepted 25th July 2011
DOI: 10.1039/c1cc13592a

An ultrasensitive electrochemiluminescent biosensor was developed for detection of near single DNA molecules with a linear range of 7 orders of magnitude by combining the specific recognition of a molecular beacon with signal amplification of quantum dots–dendrimer nanocomposites.

The specific sequence detection of DNA has attracted considerable interest due to its broad applications in molecular diagnostics, genetic therapy and early screening of diseases.[1] Many analytical systems have been developed for the detection of DNA sequences by electrochemical,[2] optical,[3] or mass-sensitive techniques.[3b] Various highly sensitive approaches have been developed by amplifying the sample with a PCR technique,[4a] accelerating the reaction to produce a detectable signal with enzymatic cycles,[4b] amplifying the detectable signal with nanoparticles,[4c] and fluorescence signal amplification using organic dyes.[4d]

Recently, electrochemiluminescent (ECL) biosensing has received considerable attention owing to its high sensitivity, simplified optical setup, and good temporal and spatial controllability.[5] As a new kind of ECL emitters, quantum dots (QDs) show excellent advantages such as high fluorescence quantum yields,[6a] size or surface trap-control luminescence,[6b] and good stability against photobleaching. Particularly, QDs can generate efficient and stable ECL emission during the potential cycling,[7] and they can be conveniently joined to polymers to produce nanocomposites for preparation of signal probes.[8] For example, the nanocomposite of poly(amidoamine) (PAMAM) dendrimer and CdS QDs has been prepared on the electrode surface by an electrodeposition method, which shows 55-fold enhanced ECL compared with that of QDs film without a dendrimer.[9] Another nanocomposite has been prepared by adding QDs solution in the dendrimer NCs.[10a] These nanocomposites have been further functionalized with canavalin A or DNA for photoelectrochemical or ECL detection of cell concentration.[10] Here, we prepared the nanocomposite of CdS QDs–PAMAM dendrimer by one-pot synthesis in methanol solution. By covalently coupling the nanocomposite to the amino group of a DNA probe for signal amplification of ECL measurements, a new ultrahighly sensitive protocol for detection of near single DNA molecules was for the first time developed (Scheme 1).



Scheme 1 Schematic representation of ECL sensing platform for ultrasensitive DNA detection using QDs–dendrimer nanocomposite as label.

The ECL DNA biosensor was prepared by immobilizing a hairpin DNA molecular beacon (MB) probe with a thiol group at 5′-end and an amino group at 3′-end on an AuNPs modified glassy carbon electrode (GCE) *via* an Au–S bond. The formation of the Au–S bond led to good stability of the biosensor. After specific hybridization with the target DNA, the beacon loop was opened to expose the amino group, which was activated by glutaraldehyde and then covalently linked to the amino group on the surface of a PAMAM dendrimer. The assembled QDs–dendrimer nanocomposite generated strong and stable ECL in the presence of $S_2O_8^{2-}$ ion as coreactant. As a result, the ECL signal related to target DNA concentration.

The photoluminescence (PL) spectra of the QDs–dendrimer nanocomposite suspensions synthesized with PAMAM G4 and G5 in methanol showed an emission peak at 495 nm (ex. at 380 nm) (Fig. S1 in ESI†). Thus the nanocomposites prepared using two generations of the dendrimers displayed similar fluorescence properties.[11] Their maximum absorption peaks occurred at 326 and 279 nm, respectively (Fig. S1 in ESI†), exhibiting the average particle sizes of QDs to be 1.66 and 1.30 nm.[12] The calculated sizes were consistent with those observed from the TEM images (Fig. S2 in ESI†). The smaller size of QDs in the PAMAM G5 dendrimer was due to the greater dendrimer size with a crowded periphery and more

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† Electronic supplementary information (ESI) available: Experimental details and optimal conditions. See DOI: 10.1039/c1cc13592a

functional groups.[12] From the extinction coefficient of QDs, the molar concentrations of the QDs in PAMAM G4 and G5 dendrimers were 15.7 and 51.0 μM, respectively.[13] From the final G4 and G5 dendrimer concentrations of 50 μM, each QD was stabilized by about three G4 or one G5 dendrimer. Both PAMAM G4 and G5 dendrimers showed two FT-IR absorption peaks with a slight difference in peak positions (Fig. S3 in ESI†). The absorption peaks for the PAMAM G4 dendrimer were at 1640 and 1555 cm^{-1}, while those for PAMAM G5 were at 1642 and 1550 cm^{-1}. These peaks were readily assigned to C═O stretching (amide I) and N–H bending/C–N stretching (amide II) vibrations of the dendrimer interior, respectively. The broad FT-IR absorption band of these dendrimers at 3440 cm^{-1} was assigned to the N–H stretching mode of dendrimer surface amine groups.[14] After QDs–dendrimer nanocomposites were formed, the peak for the amide I red shifted, while the peak for the amide II blue shifted. These shifts clearly indicated that the two interior functional groups were sensitive to the inclusion of the QDs in the dendrimer. In addition, the blue shift of the N–H stretching absorption band by 10 cm^{-1} showed the interaction of some dendrimer surface amine groups with the QDs, indicating some QDs were attached on the dendrimer surface.

At 10 aM DNA target, the CdS QDs–dendrimer modified electrode in 0.1 M pH 7.4 PBS containing 0.1 M KCl and $S_2O_8^{2-}$ as a coreactant showed much higher ECL response than using 0.1 M H_2O_2 as coreactant (Fig. S4 in ESI†). This electrode also showed the ECL emission in air-saturated 0.1 M pH 7.4 PBS containing 0.1 M KCl, which decreased dramatically when the solution was bubbled with high-purity nitrogen (Fig. S4c, ESI†),[15a] indicating that O_2 could be electrochemically reduced to produce H_2O_2 coreactant, and bubbling greatly decreased their concentrations. Considering the more sensitive response in the presence of $S_2O_8^{2-}$, this work used 0.1 M $S_2O_8^{2-}$ as coreactant. The possible ECL mechanism should be similar to that reported previously.[15b] Briefly, during the cathodic scan, the QDs in nanocomposites could be electrochemically reduced to produce the radical $QDs^{\bullet-}$, which then reacted with the reduced product of $S_2O_8^{2-}$ to generate a strong ECL signal. Although the solution of $S_2O_8^{2-}$ also showed ECL emission at bare GCE, AuNPs/GCE, MB/AuNPs/GCE and DNA-MB/AuNPs/GCE obtained at 100 pM DNA, which was similar to the previous observation and was produced from the excited reduction product of $S_2O_8^{2-}$ coreactant,[16] the ECL intensities of 52, 70, 75 and 78 a.u. were much lower than that of 950 a.u. at the QDs–dendrimer nanocomposite modified electrode at the same DNA concentration (Fig. 1). Furthermore, this solution only showed an ECL response of 18 a.u. at G4 dendrimer/DNA-MB/AuNPs/GCE (Fig. 1, curve a), which was much lower than that of 82 a.u. at QDs–G4 dendrimer/DNA-MB/AuNPs/GCE obtained at 10^{-18} M DNA (Fig. 2A, curve b). The difference of the ECL signals at QDs–G4 dendrimer/DNA-MB/AuNPs/GCE and DNA-MB/AuNPs/GCE in the presence of 10^{-18} M target DNA was larger than three times the standard deviation of 0.90 a.u. for the ECL emission from DNA-MB/AuNPs/GCE, suggesting the high sensitivity of this designed system.

The ECL intensity increased with the increasing pH up to 7.4 due to the changes in both the electron injection rate for formation of $CdS^{\bullet-}$ and the reduction rate of $S_2O_8^{2-}$. The decrease of ECL intensity in basic solutions was assigned to the consumption of $SO_4^{\bullet-}$ by the scavenging reaction with OH^-.[17] Based on pH optimization (Fig. S5 in ESI†), the ECL measurements were performed in pH 7.4 PBS containing

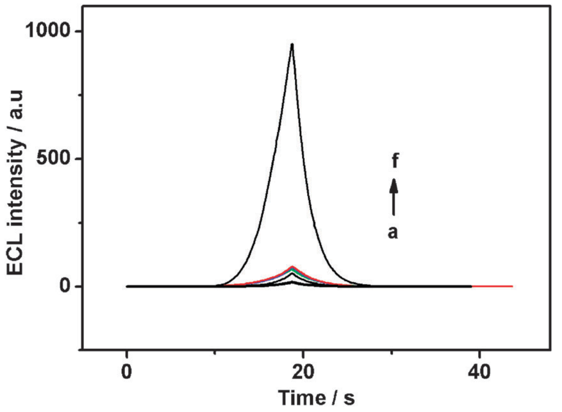

Fig. 1 ECL responses of G4 dendrimer/DNA-MB/AuNPs/GCE (a), bare GCE (b), AuNPs/GCE (c), MB/AuNPs/GCE (d), DNA-MB/AuNPs/GCE (e) and QDs–G4 dendrimer/DNA-MB/AuNPs/GCE (f) in 0.1 M pH 7.4 PBS containing 0.1 M KCl and 0.1 M $S_2O_8^{2-}$ at 100 mV s^{-1}.

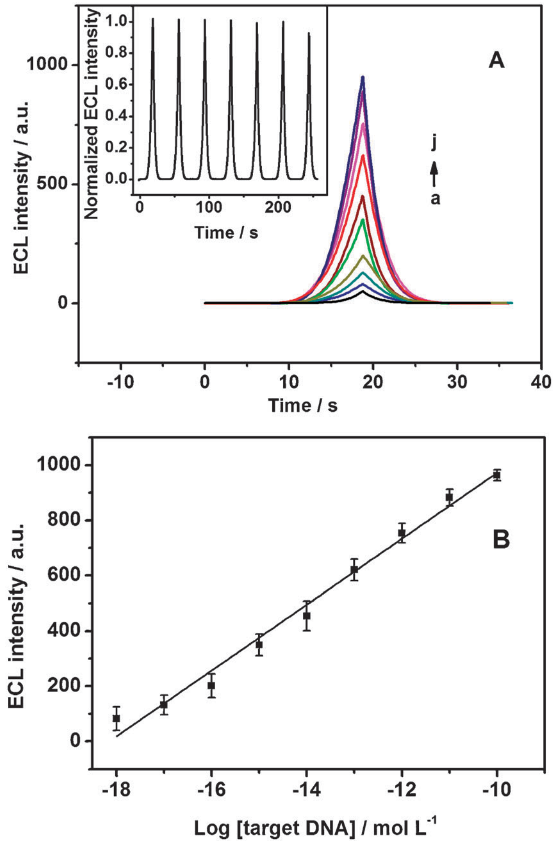

Fig. 2 (A) ECL responses of DNA biosensor using QDs–G4 dendrimer nanocomposite ECL label at target DNA concentrations of 0.0, 1.0, 10, 100 aM, 1.0, 10, 100 fM, and 1.0, 10, 100 pM from a to j, and (B) calibration curve. The error bars are standard deviations of four repeated measurements. Inset in A: successive scans between 0.2 and −1.7 V for 7 cycles. The detection conditions are the same as in Fig. 1.

0.1 M KCl and 0.1 M $K_2S_2O_8$. Fig. 2A shows the ECL signals of the QDs–G4 dendrimer nanocomposite attached biosensor at different concentrations of the target DNA. The ECL signal increased with the increasing target DNA concentration in the range of 5.0 aM–0.1 nM. Upon successive scans between 0.2 and −1.7 V for 7 cycles, acceptable stability and repeatability of the ECL emission from the sensing layer could be observed (inset in Fig. 2A). The plot of ECL intensity *versus* the logarithm of the target DNA concentration using the QDs–G4 dendrimer nanocomposite as an ECL label showed a good linear relationship in a concentration range of seven orders of magnitude (Fig. 2B). The *R* value for the linear regression was 0.9958. The limit of detection (LOD) was 2.5 aM, corresponding to 15 DNA molecules in a volume of 10 μL. Thus a near single molecule detection could be obtained. Quite remarkably, the LOD was about 20 times lower than the lowest LOD of DNA based on ECL detection.[18] The comparison of analytical performance of the proposed method with those reported previously for ECL detection of DNA is given in Table S1 in ESI.† Interestingly, although QDs–G5 dendrimer/DNA-MB/AuNPs/GCE showed slightly higher ECL intensity than QDs–G4 dendrimer/DNA-MB/AuNPs/GCE at the same DNA concentration (Fig. 3A) due to the higher content of QDs in the G5 dendrimer, the biosensor using the QDs–G5 dendrimer nanocomposite as ECL label showed a lower sensitivity of 80 a.u. decade^{-1} and a more narrow linear range of 10 aM to 1.0 f M (Fig. 3A) than those of 125 a.u. decade^{-1} and 5.0 aM to 0.1 nM using the QDs–G4 dendrimer nanocomposite as ECL label (Fig. 2B), possibly due to the steric hindrance of the PAMAM G5 dendrimer with larger size.[13] The biosensor showed good specificity for sequence detection of the target DNA oligonucleotide. After single-base mismatched and three-base mismatched DNA oligonucleotide solutions at the concentration of 1 f M were dropped on MB/AuNPs/GCEs for hybridization and then labelled with the QDs–G4 dendrimer nanocomposite, respectively, the biosensors showed much weaker ECL signals than that hybridized with complementary DNA at the same concentration (Fig. 3B).

In conclusion, this work has demonstrated that the ECL technology can be used to develop ultrasensitive methods for near single molecule detection. The QDs–dendrimer nanocomposite as the ECL emitter provides such a novel possibility. The developed protocol integrates the specificity of an MB for molecular recognition to target analyte, the high loading of QDs by the dendrimer for ECL signal amplification, and the advantages of AuNPs for immobilizing the MB on the electrode surface and accelerating the electron transfer. The proposed biosensor shows a good specificity for monitoring the DNA hybridization and a forceful ability for near single molecule detection of target DNA with a very wide concentration of at least 7 orders of magnitude. This strategy possesses promising potential in application to near single molecule detection of other kinds of biomolecules.

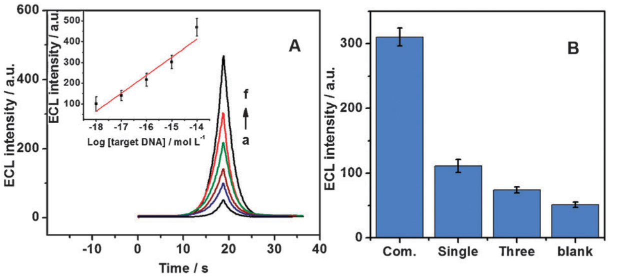

Fig. 3 (A) ECL responses of DNA biosensor using QDs–G5 dendrimer nanocomposite as ECL label at target DNA concentrations of 0.0, 0.001, 0.01, 0.1, 1.0 and 10.0 fM from a to f. Inset: plot of ECL intensity *vs.* logarithm value of target concentration. The detection conditions are the same as in Fig. 1; (B) ECL signals using QDs–G4 dendrimer nanocomposite for detection of 1 f M target DNA, single-base mismatch sequence and three-base mismatch sequence.

This work was financially supported by National Basic Research Program of China (2010CB732400), the National Natural Science Foundation of China (20821063, 20875044).

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