Distance-dependent quenching and enhancing of electrochemiluminescence from a CdS:Mn nanocrystal film by Au nanoparticles for highly sensitive detection of DNA†

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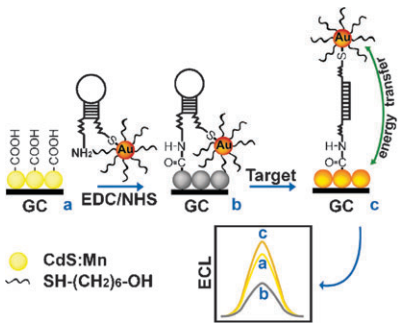
The quenching of electrochemiluminescence (ECL) from a CdS:Mn nanocrystal film by proximal Au nanoparticles was observed as a result of Förster energy transfer, while an enhancement of ECL takes place after hybridization with target DNA due to the energy transfer of ECL excited surface plasmon resonances in AuNPs to the CdS:Mn NCs at large separation, based on which an ultrasensitive and specific DNA biosensor was constructed.

The detection of DNA is of great importance to the diagnosis and treatment of genetic diseases, to the detection of infectious agents, drug discovery, or warning against bio-warfare agents.[1] Such bio-detection commonly relies on hybridization, and requires much attention to the achievement of both ultrasensitive and specific measurements. To fulfill these requirements, numerous DNA detection systems based on the hybridization between a DNA probe and its complementary target have been described. Transduction of hybridization of DNA in these systems can be measured electrochemically,[2] optically,[3] or using mass-sensitive devices.[4]

Of the diverse DNA detection techniques, electrogenerated chemiluminescence (ECL)-based bio-sensing has received considerable attention owing to its versatility, and simplified optical setup, good temporal and spatial control.[5] ECL signals could be obtained from the excited states of an ECL luminophore generated at the electrode surfaces during the electrochemical reaction. In the past decades, much effort has been made to develop ECL-active materials.[6] Since Bard *et al.* explored electrogenerated chemiluminescence properties of silicon semiconductor nanocrystals (NCs) in 2002,[7] the interest for the preparation and application of various semiconductor NCs with ECL activity has been growing.[8] Semiconductor NCs-based ECL is becoming an efficient approach for biological/chemical analysis.[9] For example, CdS NCs that generate strong ECL in the presence of coreactant could be used to develop ECL biosensors with increasing sensitivity by introduction of Au nanoparticles (NPs) for the transduction of biological binding events.[9a] Yet, comparing with great improvements in sensitivity caused by nanomaterials in optical bioassays such as surface-enhanced Raman scattering (SERS)[10] and surface-enhanced fluorescence (SEF),[11] new schemes taking advantage of unique optical properties of nanomaterials to achieve additional amplification are still highly desired for meeting the high sensitivity demands of electrochemiluminescent detection of nucleic acids.

Here we demonstrate a simple ECL sensing platform for highly sensitive and specific detection of DNA targets with CdS:Mn NCs as the ECL luminophores and Au NPs functioning as both ECL quencher and enhancer. The quenching of fluorescence by AuNPs has been exploited well to design DNA biosensors.[12] Such quenching is observed due to Förster resonance energy transfer (FRET) when the fluorophores and AuNPs are at close proximity.[13] AuNPs-enhanced Raman scattering, or surface plasmon resonance (SPR) have also brought about great advances in developing DNA bio-transducers.[14] Taking full advantage of these techniques tremendously lowers the detection limit of DNA targets. Likewise, AuNPs-enhanced fluorescence is a remarkable optical phenomenon. This enhancement is believed to take place at large separation owing to the interactions of the excited fluorophores with surface plasmon resonances in metals.[13] To sum up, the wide application of AuNPs in FRET, SERS, SPR and SEF techniques has made them excellent building blocks for constructing DNA biosensors. In this work, we report that the quenching of ECL was produced due to FRET between the CdS:Mn NC film and AuNPs which are labeled on hairpin-DNA probe linked on the NC film at close proximity. Upon the occurrence of the hybridization with target DNA, an ECL enhancement occurred owing to the interactions of the excited CdS:Mn NCs with ECL-induced SPR in AuNPs at large separation (Scheme 1). This combination of quenching with enhancement of ECL from the CdS:Mn NC



Scheme 1 DNA ECL sensing platform based on energy transfer between CdS:Mn NCs and AuNPs.

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film by AuNPs together in one assay provides great sensitivity for detection of DNA.

The new AuNPs-based amplified ECL protocol (Scheme 1) involves the modification of glassy carbon electrode (GCE, 3-mm diameter) by drop-coating of 5-nm 1.34 atom% Mn doped CdS NCs and assembly of 3-mercaptopropionic acid (MPA) (a), followed by linking of DNA hairpin probe/6-mercapto-1-hexanol (MCH)/5-nm-AuNPs to CdS:Mn film through *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) (b), and hybridization with target DNA (c). The as-prepared CdS:Mn film generated strong and stable ECL in the presence of co-reactant $S_2O_8^{2-}$ ions (see ESI†). In this CdS:Mn/$S_2O_8^{2-}$ system, ECL is produced upon concomitant reduction of CdS:Mn and $S_2O_8^{2-}$. The reduction of $S_2O_8^{2-}$ produces the strong oxidant $SO_4^{-\bullet}$, which then reacts with the reduced CdS:Mn to generate light. The proposed ECL process is as follows:[9a]

$$\text{CdS:Mn NCs} + ne^- \rightarrow n\text{CdS:Mn}^{-\bullet} \quad (1)$$

$$S_2O_8^{2-} + e^- \rightarrow SO_4^{2-} + SO_4^{-\bullet} \quad (2)$$

$$\text{CdS:Mn}^{-\bullet} + SO_4^{-\bullet} \rightarrow \text{CdS:Mn}^* + SO_4^{2-} \quad (3)$$

$$\text{CdS:Mn}^* \rightarrow h\nu + \text{CdS:Mn} \quad (4)$$

Therefore, the strong and stable ECL from CdS:Mn films is mainly attributed to the highly stable reduced form of CdS:Mn NCs as indicated by its cyclic voltammograms (Fig. 1(a)). Obviously, a pair of reduction and oxidation peaks appeared at *ca.* −1.25 V (C) and −0.96 V (A), respectively, which suggested that the reduced form of CdS:Mn is stable enough to undergo oxidation on scan reversal. Such a property of stability, we believe, largely originates from manganese doping, taking into account both the strong dependence of ECL emission on surface states[8a] and the large tendency of manganese impurities to stay on the surface.[15] The doping of manganese impurities in the CdS NC surface is further confirmed by the ECL spectrum of the CdS:Mn film (Fig. 1(b)). Two ECL peaks were observed in the ECL spectrum: a relatively strong peak at 584.5 nm is assigned to Mn^{2+}-related emission[16] and the other peak at *ca.* 660 nm results from CdS surface states.[8a] Nevertheless, only a broad ECL emission peaked at *ca.* 500 nm was obtained in the ECL spectrum of undoped CdS NCs film (see ESI†). Apparently, the ECL emission of the CdS:Mn film at 584.5 nm showed a considerable spectral overlap with surface plasmon absorption of Au NPs (inset in Fig. 1(b)). Based on such overlap as is necessary for efficient energy transfer in photoluminescence or chemiluminescence system,[17] the successful ECL energy transfer between CdS:Mn NCs and Au NPs was achieved.

The amplified signal of DNA hybridization associated with quenching and enhancement of ECL from the CdS:Mn NC film by Au NPs is demonstrated in Fig. 2A. When the DNA hairpin probe/MCH/AuNPs was brought close to the CdS:Mn NC film by EDC/NHS crosslinking, the ECL peak height (curve b) decreased by 25% compared with that of the CdS:Mn NC film before assembly (curve a). Excitingly, an enhancement of 55% in ECL peak height (curve c) was observed upon hybridization of 5.0 fM target DNA due to the unfolding of the DNA hairpin in comparison with that before assembly. It has been shown experimentally and theoretically that for close fluorophore–metal separation distances, dye molecules do not emit fluorescence photons because all the excitation energy is dissipated in the metal in Förster energy transfer processes.[13] In systems using semiconductor NCs as fluorophores and energy donors with Au NPs as the energy acceptors, likewise, the quenching of photoluminescence of NCs originates from excitation energy dissipation when NCs and Au NPs are at immediate proximity.[18] Herein, it is proposed that the quenching of ECL from CdS:Mn NCs by proximal 5 nm Au NPs is a result of Förster resonance energy transfer, that is to say some ECL excitation energy of CdS:Mn NCs is consumed in Au NPs. To clarify the origin of ECL enhancement after hybridization, the control experiment without the involvement of Au NPs was carried out. The results shown in Fig. 2B suggested that oligonucleotide itself did not bring about any significant enhancement in ECL peak height without the attachment of Au NPs. Furthermore, the observed small change in peak current of coreactant $S_2O_8^{2-}$ (inset in Fig. 2A) would not change the ECL peak height significantly (see ESI†). As summarized by Chandra Ray *et al.*, the origin of fluorescence enhancement near the

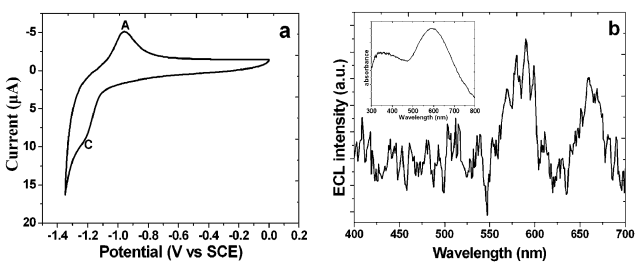

Fig. 1 (a) Cyclic voltammogram of an CdS:Mn NC film on GCE in deaerated 0.1 M pH 8.3 PBS. Scan rate, 50 mV s^{-1}. Scan range, 0 to −1.35 V. (b) ECL spectrum of the CdS:Mn NC film in 0.05 M $K_2S_2O_8$ + 0.1 M PBS (pH 8.3) obtained by stepping the potential between 0 and −1.5 V. Inset: UV-Vis absorption spectrum of MCH-capped Au NPs.

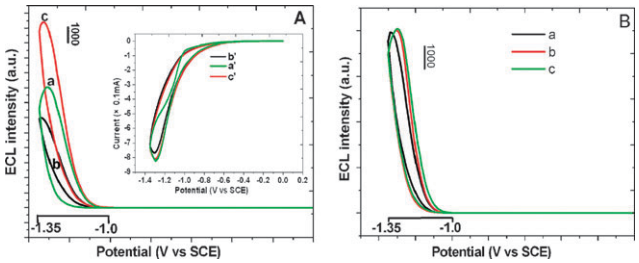

Fig. 2 (A) Cyclic ECL curves of (a) CdS:Mn NC film on GCE; (b) DNA hairpin/MCH/AuNPs functionalized CdS:Mn NC film on GCE; (c) functionalized CdS:Mn NC film on GCE in the presence of 5.0 fM complementary sequence for 1 h at room temperature. Inset: the corresponding cyclic voltammograms (CVs): a′ to a; b′ to b; c′ to c. (B) Cyclic ECL curves of (a) CdS:Mn NC film on GCE; (b) hairpin probe DNA/MPA/CdS:Mn NC film on GCE; (c) hairpin probe DNA/MPA/CdS:Mn NC film on GCE in the presence of 5.0 fM target DNA for 1 h at room temperature. ECL detection buffer: 0.1 M PBS (pH 8.3) containing 0.05 M $S_2O_8^{2-}$. Scan rate, 100 mV s^{-1}. Hybridization buffer: 0.1 M Tris-HCl + 0.1 M NaCl.

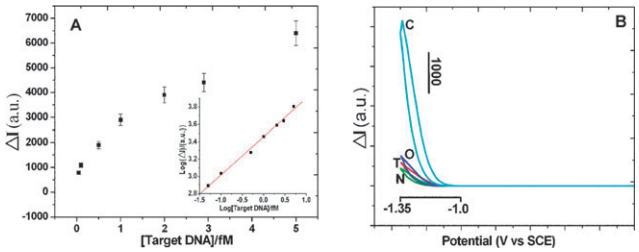

Fig. 3 (A) Relationship between the increment in ECL peak height before and after hybridization (ΔI) and target DNA concentration, three measurements for each point. Inset: logarithmic calibration curve for target DNA. (B) Comparison of hybridization events. C, 2.9 fM target DNA sequence; O, 2.9 fM one-base mismatch sequence; T, 2.9 fM three-base mismatch sequence; N, 2.9 fM non-complementary sequence. Hybridization conditions and ECL conditions are the same as those in Fig. 2.

nanostructured metal can be understood as arising from two contributions.[19] First, by concentrating the incident light into local electromagnetic 'hot spots', nanostructured surfaces can lead to increased absorption of incident light by fluorophores. Second, metal nano-structures can alter radiative and non-radiative decay rates of nearby fluorophores, altering both fluorescence lifetime and quantum yield.[19] Thus, for CdS:Mn NCs/AuNP separation larger than 10 nm after hybridization using a DNA hairpin with 24-base loop and 5-bp stem (1 nm for 3-base), it is suggested that the ECL enhancement by Au NPs is attributed to energy transfer of the ECL-excited surface plasmon resonance in AuNPs to CdS:Mn NCs, resulting in an alteration in radiative and non-radiative decay rates of nearby excited CdS:Mn NCs. This was further supported by control experiments using a shorter DNA hairpin with 16-base loop and 5-bp stem. A 20% enhancement was obtained, indicating such ECL enhancement by Au NPs is a distance-dependent plasmon-exciton energy transfer. (Figure not shown)

Fig. 3A displays the relationship between the increase in ECL peak height before and after hybridization (ΔI) and target DNA concentrations (50 aM–5.0 fM). The resulting logarithmic calibration curve of ΔI *vs.* target DNA concentration has good linearity in the range over 50 aM to 5.0 fM ($R = 0.996$, shown as inset in Fig. 3A). The favorable response of 50 aM target DNA indicates a remarkably low detection limit (S/N = 3), *i.e.*, 2100 copies in 70 μL sample. Binding specificity is undoubtedly another crucial measure of the utility of the biosensor. The much smaller increases in ECL peak heights before and after hybridization observed in control experiments for 2.9 fM one-base mismatch, three-base mismatch and non-complementary sequences than that related to 2.9 fM target DNA hybridization reflects the high selectivity associated with hairpin structure of the DNA probe (shown in Fig. 3B).

In summary, we have demonstrated for the first time a sensitive DNA ECL sensor based on both the quenching and enhancement of ECL from CdS:Mn NCs by Au NPs in one assay. Such energy transfer in ECL systems opens a new way for transduction of biological recognition events. Additionally, the doping of manganese impurities in the semiconductor nanoparticle surface also indicates new opportunities for developing ECL chromophores with desired emission spectra. Further improvements in the sensitivity are expected through adjusting DNA hairpin probes with suitable length to increase the enhancement efficiency.

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Notes and references

1 (*a*) A. Sassolas, B. D. Leca-Bouvier and L. J. Blum, *Chem. Rev.*, 2008, **108**, 109–139; (*b*) D. Kato, N. Sekioka, A. Ueda, R. Kurita, S. Hirono, K. Suzuki and O. Niwa, *Angew. Chem.*, 2008, **120**, 6783–6786.

2 (*a*) Y. Xiao, X. Qu, K. W. Plaxco and A. J. Heeger, *J. Am. Chem. Soc.*, 2007, **129**, 11896–11897; (*b*) G. Liu, Y. Wan, V. Gau, J. Zhang, L. Wang, S. Song and C. Fan, *J. Am. Chem. Soc.*, 2008, **130**, 6820–6825.

3 (*a*) K. Lee, J.-M. Rouillard, T. Pham, E. Gulari and J. Kim, *Angew. Chem., Int. Ed.*, 2007, **46**, 4667–4670; (*b*) K. Faulds, F. McKenzie, W. E. Smith and D. Graham, *Angew. Chem., Int. Ed.*, 2007, **46**, 1829–1831; (*c*) L.-Q. Chu, R. Förch and W. Knoll, *Angew. Chem., Int. Ed.*, 2007, **46**, 4944–4947; (*d*) S. Zhang, H. Zhong and C. Ding, *Anal. Chem.*, 2008, **80**, 7206–7212; (*e*) Y. K. Jung, T. W. Kim, J. Kim, J.-M. Kim and H. G. Park, *Adv. Funct. Mater.*, 2008, **18**, 701–708; (*f*) H. A. Al Attar and A. P. Monkman, *Adv. Funct. Mater.*, 2008, **18**, 2498–2509.

4 F. Patolsky, A. Lichtenstein and I. Willner, *J. Am. Chem. Soc.*, 2001, **123**, 5194–5205.

5 (*a*) W. Miao and A. J. Bard, *Anal. Chem.*, 2003, **75**, 5825–5834; (*b*) W. Miao and A. J. Bard, *Anal. Chem.*, 2004, **76**, 7109–7113; (*c*) Y. Li, H. Qi, F. Fang and C. Zhang, *Talanta*, 2007, **72**, 1704–1709.

6 M. M. Richter, *Chem. Rev.*, 2004, **104**, 3003–3036.

7 Z. Ding, B. M. Quinn, S. K. Haram, L. E. Pell, B. A. Korgel and A. J. Bard, *Science*, 2002, **296**, 1293–1296.

8 (*a*) N. Myung, Y. Bae and A. J. Bard, *Nano Lett.*, 2003, **3**, 1053–1055; (*b*) S.-N. Ding, J.-J. Xu and H.-Y. Chen, *Chem. Commun.*, 2006, 3631–3633.

9 (*a*) G. Jie, B. Liu, H. Pan, J.-J. Zhu and H.-Y. Chen, *Anal. Chem.*, 2007, **79**, 5574–5581; (*b*) X. Liu, H. Jiang, J. Lei and H. Ju, *Anal. Chem.*, 2007, **79**, 8055–8060.

10 Y. Wei, C. Cao, R. Jin and C. A. Mirkin, *Science*, 2002, **297**, 1536–1539.

11 K. Aslan, J. Huang, G. M. Wilson and C. D. Geddes, *J. Am. Chem. Soc.*, 2006, **128**, 4206–4207.

12 (*a*) D. J. Maxwell, J. R. Taylor and S. Nie, *J. Am. Chem. Soc.*, 2002, **124**, 9606–9612; (*b*) R. Gill, I. Willner, I. Shweky and U. Banin, *J. Phys. Chem. B*, 2005, **109**, 23715–23719.

13 T. Neumann, M. L. Johansson, D. Kambhampati and W. Knoll, *Adv. Funct. Mater.*, 2002, **12**, 575–586.

14 (*a*) L. He, M. D. Musick, S. R. Nicewarner, F. G. Salinas, S. J. Benkovic, M. J. Natan and C. D. Keating, *J. Am. Chem. Soc.*, 2000, **122**, 9071–9077; (*b*) R. J. Stokes, A. Macaskill, P. J. Lundahl, W. E. Smith, K. Faulds and D. Graham, *Small*, 2007, **3**, 1593–1601.

15 M. Shim and P. Guyot-Sionnest, *Nature*, 2000, **407**, 981–983.

16 A. Nag, S. Sapra, C. Nagamani, A. Sharma, N. Pradhan, S. V. Bhat and D. D. Sarma, *Chem. Mater.*, 2007, **19**, 3252–3259.

17 (*a*) K. Becker, J. M. Lupton, J. Müller, A. L. Rogach, D. V. Talapin, H. Weller and J. Feldmann, *J. Nat. Mater.*, 2006, **5**, 777–781; (*b*) X. Huang, L. Li, H. Qian, C. Dong and J. Ren, *Angew. Chem., Int. Ed.*, 2006, **45**, 5140–5143.

18 (*a*) T. Pons, I. L. Medintz, K. E. Sapsford, S. Higashiya, A. F. Grimes, D. S. English and H. Mattoussi, *Nano Lett.*, 2007, **7**, 3157–3164; (*b*) B. Tang, L. Cao, K. Xu, L. Zhuo, J. Ge, Q. Li and L. Yu, *Chem.–Eur. J.*, 2008, **14**, 3637–3644.

19 S. S. R. Dasary, U. S. Rai, H. Yu, Y. Anjaneyulu, M. Dubey and P. Chandra Ray, *Chem. Phys. Lett.*, 2008, **460**, 187–190.