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Electrochemiluminescence detection of near single DNA molecule with quantum dots-dendrimer nanocomposite for signal amplification

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Experimental

Materials and reagents. Generation four and five poly (amidoamine) (PAMAM G4 & G5, with ethylene diamine core) dendrimers, cadmium chloride, sodium sulfide, methanol were purchased from Sigma-Aldrich company (USA) without further purification. Chloroauric acid ($HAuCl_4$), trisodium citrate, glutaraldehyde (GLD) and $K_2S_2O_8$ were obtained from Shanghai Reagent Company (Shanghai, China). Gold nanoparticles (AuNPs) were prepared by citrate reduction of $HAuCl_4$ in aqueous solution. From the absorption peak at 520 nm, the average diameter of the gold nanoparticles was calculated to be 12 nm.

DNA oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Their sequences are given as follows:

Hairpin DNA molecular beacon probe (MB, 17-base loop and 5-bp stem): 5'-SH-$(CH_2)_6$-TGGAGATCAGTCTGATAAGCTACTCCA-$(CH_2)_6$-NH_2-3'.

Target: 5'-TAGCTTATCAGACTGAT-3'.

One-base mismatch: 5'-TAGCTTAT**A**AGACTGAT-3'.

Three-base mismatch: 5'-TAG**G**TTAT**A**AGAC**A**GAT-3'.

0.1 M phosphate buffer solution (PBS) with pH 7.4 was prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4. The electrolyte was PBS containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl. All reagents were used as received. Doubly distilled water was used throughout the experiments.

Apparatus. The electrochemical and ECL measurements were performed with a model MPI-A electrochemiluminescence analyzer (Xi'An Remax Electronic Science & Technology Co. Ltd., Xi'An, China) at room temperature using a three-electrode system, which contained a 5-mm-diameter GCE as working electrode, a saturated Ag/AgCl reference electrode, and a Pt counter electrode. The spectral width of the photomultiplier tube (PMT) was 200-800 nm, and the voltage of the PMT was set at 600 V in the detection process. The cyclic scan was performed in the potential range from 0.2 to –1.7 V.

UV–vis absorption spectra were recorded on UV-3600 UV–vis-NIR photospectrometer (Shimadzu Co., Japan). Photoluminescence (PL) spectra were obtained on a Jasco FP 820 fluorometer (Jasco Co.). The transmission electron microscopic (TEM) images of the QDs nanocomposites were obtained on a TEM (Jeol JEM-200CX) operating at an acceleration voltage of 100 kV. The suspensions of PAMAM G4 and G5 dendrimer-QDs nanocomposites were dropped on carbon-coated copper grids and allowed to dry at room temperature before TEM imaging. Each spot corresponded to an individual nanoparticle (only the inorganic cores have enough contrast to be visualized by TEM). Fourier transform-infrared (FT-IR) spectra were recorded on a Nicolet 400 FT-IR spectrometer (Madison, WI).

Preparation of QDs nanocomposites. PAMAM/CdS nanocomposite was synthesised by modifying a previously reported procedure.[S1] Briefly, 5×10^{-7} mol of PAMAM dendrimer G4 or G5 containing 64 or 128 terminal amino groups was first dissolved in 1 ml of methanol. After 7 ml of methanol was added in the solution, 1 ml of methanol solution of cadmium chloride was added into PAMAM dendrimer solution with a Cd^{2+}/dendrimer mole ratio of 10:1 and vigorous stirring under nitrogen at 5 ^{o}C for about 1 h, during which Cd^{2+} coordinated with the amine and amide groups in PAMAM dendrimer. Finally, equimolar sodium sulfide was added to this system dropwise under stirring to obtain CdS QDs nanocomposites. The final volume of the formed nanocomposite suspension was 10 ml. The obtained QDs nanocomposite suspensions were stored in a refrigerator at 5 °C for future use. The nanocomposites with PAMAM G4 were stable for one month, while those with PAMAM G5 could last longer.

Fabrication and ECL detection of biosensor. A GCE was polished with 1.0 and 0.05 μm alumina, respectively. The GCE was sonicated and thoroughly washed with water and finally dried in air. 20 μL of 4 nM AuNPs solution was cast on the pretreated GCE and dried in air at room temperature; as a result, a stable AuNPs modified GCE was obtained via physical adsorption due to the unique properties of AuNPs. 10 μL MB solution was then spread on the AuNPs film for 2-h incubation. Following a rinsing step with pH 7.4 PBS, 10 μL target DNA solution was dropped onto the electrode for 4-h incubation at 37 ^{o}C to form double-stranded DNA structure. Afterwards, the electrode was rinsed again, and 10 μL of glutaraldehyde was added to link amine group of MB by incubation at 37 ^{o}C for 1 h followed with a washing step. 10 μL of as-synthesized QDs-dendrimer nanocomposite solution was then dropped onto the electrode surface for 1-h incubation at 37 ^{o}C. Before measurement, the biosensor was washed with PBS to remove physically absorbed QDs nanocomposites. The covalently bound QDs-

dendrimer nanocomposite produced strong ECL emission for DNA detection in 0.1 M PBS (pH 7.4) containing 0.1 M $K_2S_2O_8$ and 0.1 M KCl by scanning the potential from 0.2 to -1.7 V at 100 mV s^{-1}.

PL and absorption spectra of QDs nanocomposites and gold nanoparticles

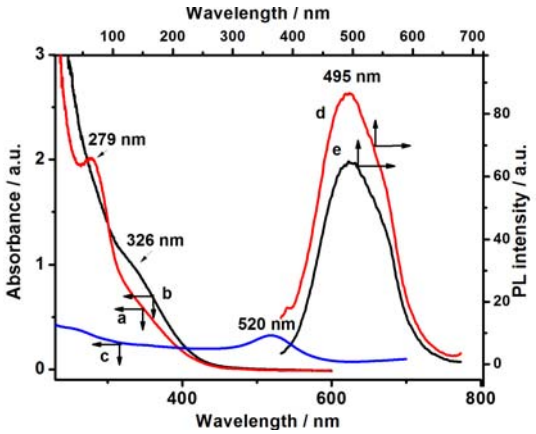

Fig. S1 UV-Vis absorption (a,b) and PL (d,e) spectra of QDs-PAMAM G5 (a,d) and G4 (b,e) dendrimer nanocomposite (c) UV-Vis absorption of gold nanoparticles.

TEM images

CdS QDs-G5 dendrimer nanocomosite showed smaller size than QDs-G4 dendrimer nanocomposite (Fig. S2).

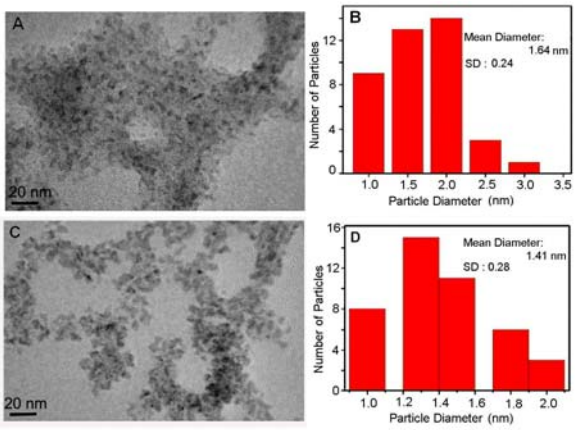

Fig. S2 TEM images and size distribution histograms of QDs-PAMAM G4 (A, B) and G5 (C, D) dendrimer nanocomposite.

FT-IR spectra of free PAMAM G4 and G5 and their related QDs nanocomposites

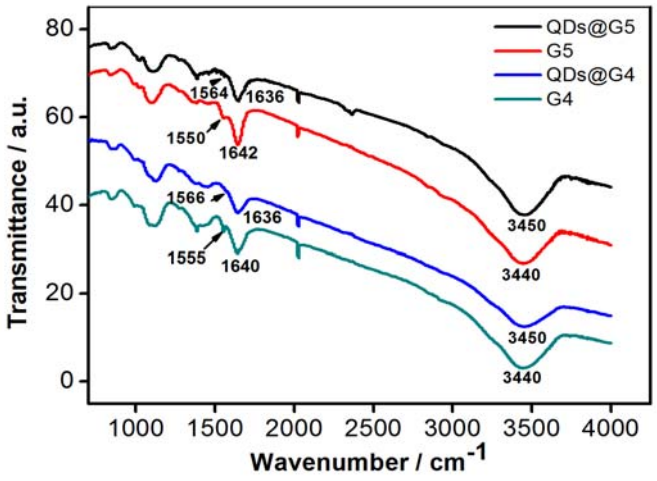

Fig. S3 FT-IR spectra of free PAMAM G4 and G5 and their related QDs nanocomposites.

Effect of coreactant

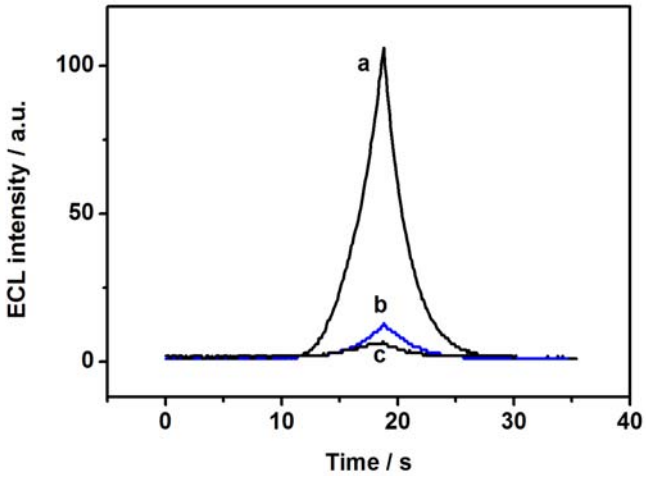

Fig. S4 ECL responses of QDs-G4 dendrimer nanocomposite-based biosensor to 10 aM DNA target in 0.1 M pH 7.4 PBS containing 0.1 M KCl in present of (a) 0.1 M $K_2S_2O_8$, (b) 0.1 M H_2O_2, and (c) O_2-free 0.1 M pH 7.4 PBS at 100 mV s^{-1}.

pH effect

With the increasing pH, the ECL intensity increased first and reached a maximum ECL response at pH 7.4 (Fig. S5) and then decrease in higher basic solution. Thus, pH 7.4 was chosen for ECL measurements.

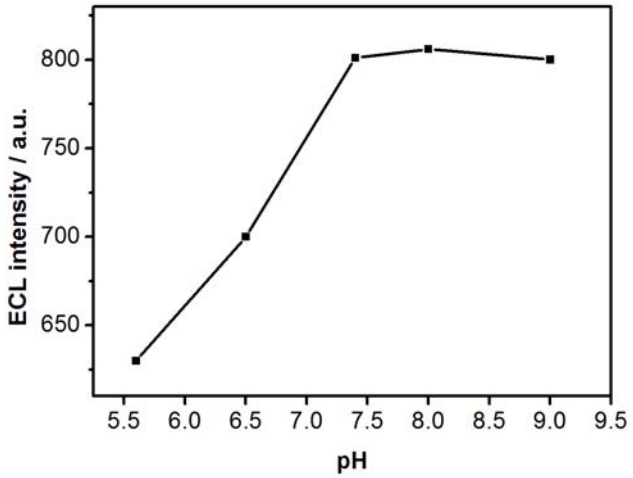

Fig. S5 Effect of pH of detection solution on ECL intensity of QDs-G4 dendrimer nanocomposite.

Table S1. Analytical performance of electrochemiluminescence methods for DNA detection

ECL label	**linear range**	**LOD**	**ref.**
$Ru(bpy)_3^{2+}$ label	4.0 – 54.0 nM	90 pM	S2
avidin-modified QDs	5.0 nM – 5.0 μM	10 pM.	S3
CdS:Mn nanocrystals	50.0 aM – 5.0 fM	50 aM	S4
$Ru(bpy)_3^{2+}$ label	1.0 – 100.0 pM	100 fM	S5
$Ru(bpy)_3$-$[B(C_6F_5)_4]_2$ label	1.0 fM – 10.0 nM	5 fM	S6
CdS QDs nanocomposite	5.0 aM – 0.1 nM	2.5 aM	This work

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