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Ferrocenyl-terminated dendrimer as efficient quencher via electron and energy transfer for cathodic electrochemiluminescent bioanalysis

Shengyuan Deng, Jianping Lei, Ye Liu, Yin Huang and Huangxian Ju*

State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, P.R. China

Experimental

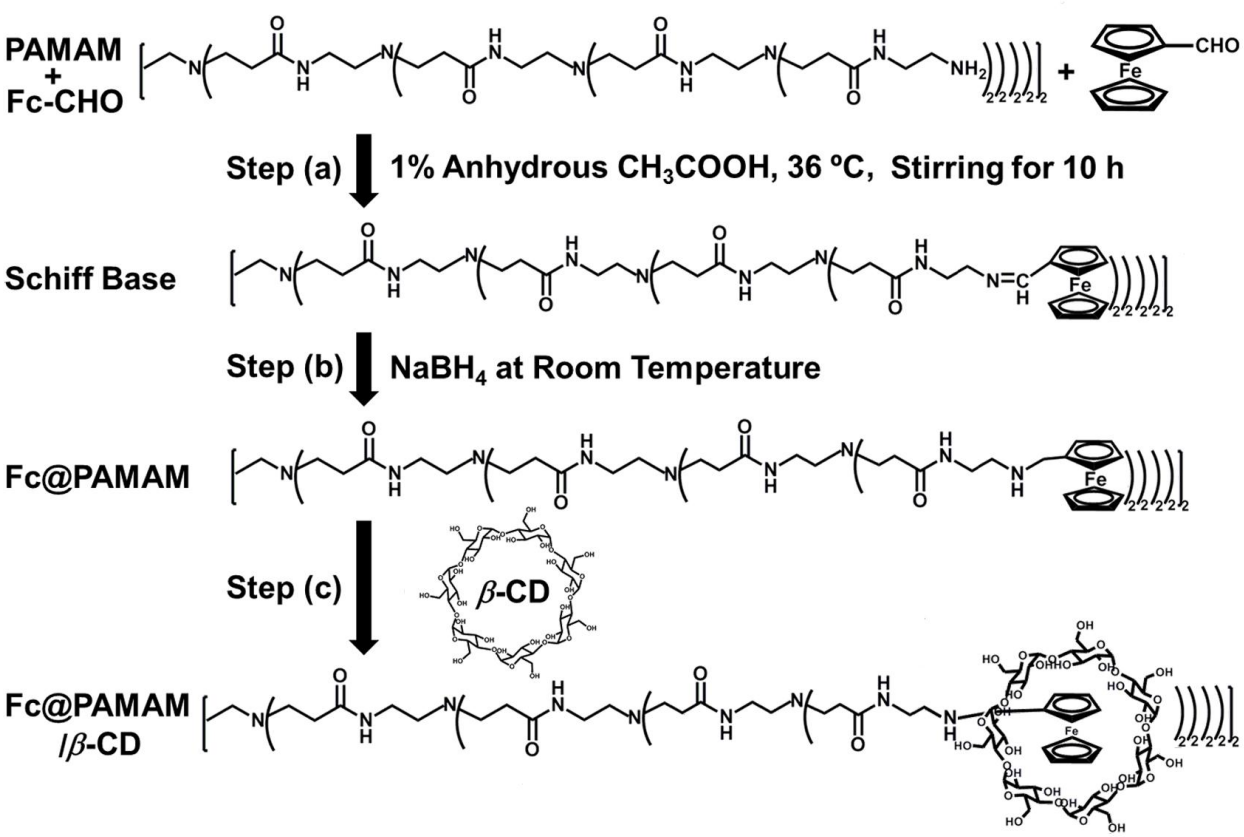
Materials and reagents. The *meso*-2,3-dimercaptosuccinic acid (DMSA)-stabilized CdTe quantum dots (QDs) were synthesized using an electrolysis method.[S1] The size and concentration of the QDs were estimated to be 1.3 nm and 10.3 μM, respectively. Ferrocenecarboxaldehyde (Fc-CHO), *β*-cyclodextrin (*β*-CD), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), bovine serum albumin (BSA) and polyamidoamine dendrimer (PAMAM, ethylenediamine core, generation 4.0) were purchased from Sigma-Aldrich Chemical Co., Ltd. (USA). Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) and DMSA were purchased from Alfa Aesar Co., Ltd. (Tianjin, China). Polyethylene glycol (PEG, M_W: 20000) was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Tellurium rod (4 mm in diameter) was purchased from Leshan Kayada Photoelectricity Co., China. Carcinoembryonic antigen (CEA) standard solution (1.0 mg mL^{-1}) was supplied by Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). Mouse monoclonal capture antibody (primary antibody, Ab_1, clone No. 27D6) and polyclonal signal antibody (secondary antibody, Ab_2, clone No. 28E4) were purchased from

Shuangliu Zhenglong Biochem. Lab (Chengdu, China). 0.1 M phosphate buffer salines (PBS) with various pHs were prepared by mixing the stock solutions of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 containing 0.1 M KNO_3 as the supporting electrolyte. 0.1 M pH 8.0 PBS was selected as the default detection solution unless being specified otherwise. The washing buffer was 0.05% (w/v) Tween-20 (PBST) in 0.01 M pH 7.4 PBS. The blocking solution was 0.01 M pH 7.4 PBS containing 0.5% (w/v) BSA. The clinical serum samples were fetched from Jiangsu Institute of Cancer Prevention and Cure. All other reagents were of analytical grade and used as received. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all assays. The N_2-saturated solution was prepared by bubbling highly pure N_2 into the solution for 30 min.

Apparatus. Photoluminescence (PL) and UV-vis absorption spectra were recorded on a RF-5301 PC fluorometer (Shimadzu Co., Japan) and a Shimadzu UV-3600 UV-Vis-NIR photospectrometer (Shimadzu Co., Japan), respectively. Attenuated total reflection Fourier transformation infrared (ATR-FTIR) spectra were obtained with a Vector 22 FTIR spectrometer (Bruker Optics, Germany). X-ray photoelectron spectral (XPS) experiments were operated on an ESCALAB 250 spectrometer (Thermo-VG Scientific Co., U.S.A.). Tapping mode atomic force microscopic (AFM) images were acquired under ambient conditions by directly casting sample dispersions onto freshly cleaved mica sheets using an Agilent 5500 AFM/SPM system (U.S.A.) with Picoscan v5.3.3 software. The occupied volumes of (accessible) solvent surface and Van de Waals surface were calculated with the dendrimer package of Materials Studio 5.0 (Accelrys Software Inc., U.S.A.). Cyclic voltammetric (CV) experiments were performed on a CHI 812B electrochemical workstation (Shanghai Chenhua Instruments Co., China), and electrochemiluminescent (ECL) measurements were carried out on a MPI-E multifunctional

electrochemical and chemiluminescent analytical system (Xi'an Remex Analytical Instrument Co., Ltd. China) with a modified glassy carbon electrode (GCE, 5 mm in diameter) as working, a platinum wire as auxiliary and a Ag/AgCl (saturated KCl) as reference electrodes. The ECL cell was self-made with three side necks and one middle neck. The reference and counter electrodes were placed into two side necks, while the modified GCE was put in the middle neck with its electrode surface downward approximating the optical window for recording the ECL signal. The ECL emission window was put in front of the photomultiplier tube (PMT, detection range from 300 to 650 nm) biased at −1000 V. Unless specifically mentioned, the scan rate was 100 mV s^{-1}. The reference levels of CEA in human serum samples were detected with an automated electrochemiluminescent analyzer (Elecsys 2010, Roche).

Preparation of Fc-incorporated PAMAM framework (Fc@PAMAM). Surface primary amine of NH_2-terminated PAMAM was partially modified with ferrocenyl group through the amine-aldehyde reaction with the Schiff base as intermediate (Scheme S1, steps *a* and *b*).[S2] Typically, Fc-CHO (7.5 mg) in 3.75 mL methanol was added dropwise to 0.25 mL of 10% (*w*/*v*) dendrimer solution containing 16% anhydrous CH_3COOH as the catalyst. The mixture was mildly vortexed for 10 h at 36 ºC, which showed a color change from orange to dark brown. Free Fc-CHO and excessive acid were removed by ultrafiltration (molecular weight cut-off: 30 kDa) at 14000 *rcf* for 10 min. The concentrated product was collected at 2000 *rcf* for 5 min, and then redispersed and purified by thrice washing with methanol to obtain 5 mL solution. Subsequently, 1 mL of 20 mg $NaBH_4$ was quickly added into the solution with vigorous vortex at room temperature for 2 h to reduce the imine-forming double-bond. The ultrafiltration and condensation process was repeated in succession for 4-5 times. Finally, the product was dried at 40 ºC overnight in vacuum to vaporize the residual methanol.



Scheme S1 Synthesis of Fc@PAMAM organometallic framework complex (steps *a* and *b*) and host-guest intramolecular incorporation of Fc@PAMAM into *β*-CD (step *c*).

Inclusion of Fc group by *β*-CD. 500 μL of 1 mM *β*-CD dissolved in 0.1 M NaOH was mixed with 500 μL of 100 μM Fc@PAMAM and mildly vortexed at ambient condition for 1 h followed by ultrafiltration at 2000 *rcf* for 10 min and washed thrice with ultrapure water. After host-guest interaction of Fc@PAMAM with *β*-CD (Scheme S1, step *c*), the resulting solution was cast on GCE for electrochemical investigation.

Preparation of Fc@PAMAM-labeled Ab_2 (Ab_2-Fc@PAMAM). 100 μM Fc@PAMAM in 500 μL of 10 mM pH 6.5 MES was mixed with 10 μL of 10 μg mL^{-1} Ab_2, 100 mM EDC and 400 mM NHS by mild vortex for 5 min. The suspension was centrifuged at 8000 *rcf* for 10 min by decanting the white floccus and supernatant and twice washed with PBST and ultrapure water to

obtain Ab_2-Fc@PAMAM, which was stored at 4 ºC and diluted to 200 μL with ultrapure water prior to use.

Preparation and measurement procedure of ECL immunosensor. A GCE was polished to mirror using 0.3 and 0.05 μm alumina slurry (Beuhler) followed by sonication in ethanol and water. After the electrode was rinsed with water and allowed to dry in N_2 atmosphere, 20 μL of DMSA-CdTe QDs solution was dropped onto the surface. After dried in air at room temperature, 20 μL of 2.0 mg mL^{-1} PEG aqueous solution was dropped onto the QDs film. The electrode was incubated in a 100% moisture-saturated environment at room temperature overnight to passively adsorb PEG polymer. Following a slow wash with stream of ultrapure water, 20 μL of 50 μg mL^{-1} Ab_1 in 10 mM pH 7.4 PBS was dropped on the PEG/QDs modified GCE and incubated for 2 h at 36 ºC. Then, the electrode was washed with water and incubated with 20 μL of 0.5% BSA (w/v) in 10 mM pH 7.4 PBS for 1 h to block possible remaining active sites against nonspecific adsorption. Finally, the immunosensor was rinsed with water and stored at 4 ºC prior to use.

For sandwich-type immunoassay, the immunosensor was incubated with 20 μL of CEA standard solution or serum sample for 40 min at 36 °C. After a washing step, 20 μL of Ab_2-Fc@PAMAM was cast upon the immunosensor for 120-min incubation at 36 °C, followed by washing again. Finally, the ECL signal was detected in air-saturated 0.1 M pH 9.0 PBS containing 0.1 M KNO_3.

AFM characterization

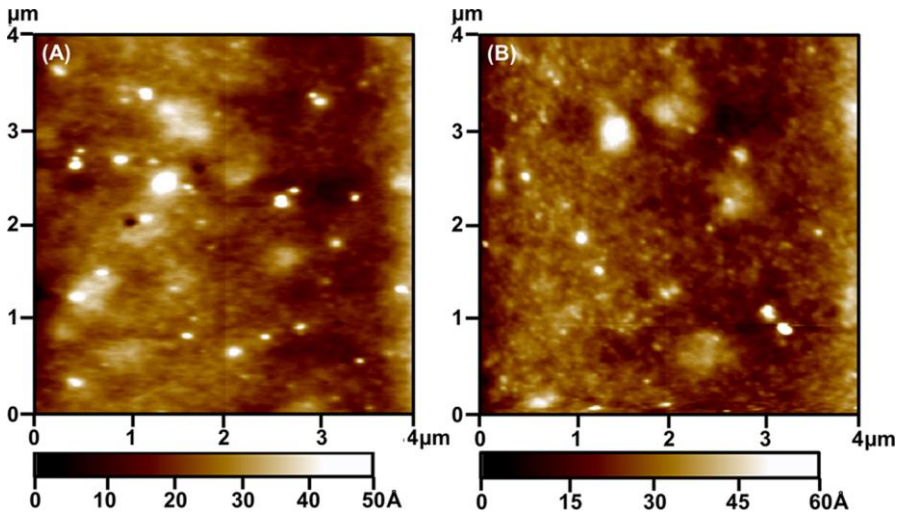


Fig. S1 AFM topographic images of (A) PAMAM and (B) Fc@PAMAM.

CV characterization of Fc@PAMAM modified GCE

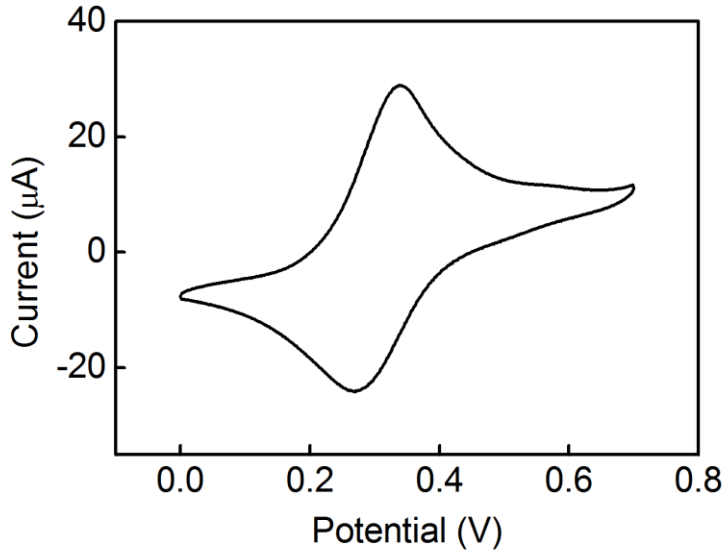


Fig. S2 CV plot of Fc@PAMAM modified GCE in 0.1 M pH 8.0 PBS.

Quenching mechanism of Fc@PAMAM on cathodic QDs-based ECL

Similar to the mechanism for quenching effect of Fc on the $Ru(bpy)_3^{2+}$-based ECL system,[S3] the quenching effect of Fc@PAMAM on the cathodic ECL at QDs modified electrode could be partly attributed to the energy transfer from excited QDs (QD*) to the cyclopentadiene of Fc in Fc@PAMAM. At the same time, during the cathodic scan, the reduced QDs ($QD^{\bullet -}$) could transfer electron to the oxidation product of Fc@PAMAM, which prohibited the formation of QD*, and thus quenched the ECL emission. The electron transfer process could be expressed as follows:

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad \text{(S1)}\ [S4]$$

$$QD + e^- \rightarrow QD^{\bullet -} \quad \text{(S2)}\ [S4]$$

$$QD^{\bullet -} + H_2O_2 \rightarrow QD + OH^{\bullet} + OH^- \quad \text{(S3)}\ [S5]$$

$$OH^{\bullet} + QD \rightarrow QD^{\bullet +} + OH^- \quad \text{(S4)}\ [S6]$$

$$OH^{\bullet} + \text{Fc@PAMAM} \rightarrow Fc^+\text{@PAMAM} + OH^- \quad \text{(S5)}\ [S7]$$

$$QD^{\bullet -} + Fc^+\text{@PAMAM} \rightarrow \text{Fc@PAMAM} + QD \quad \text{(S6)}$$

The Fc@PAMAM not only consumed $OH^{\bullet}$ (Eq. S5), which was an important species for the formation of hole-injected QDs, its oxidation product could also act as an efficient electron scavenger to oxidize the electron-injected QDs ($QD^{\bullet -}$) (Eq. S6), leading to great decrease of excited states (QD*). Thus the quenching effect of Fc@PAMAM on the cathodic QDs-based ECL contained both energy and electron transfer. The dual quenching effect greatly improved the sensitivity for bioanalysis.

Optimization of detection conditions

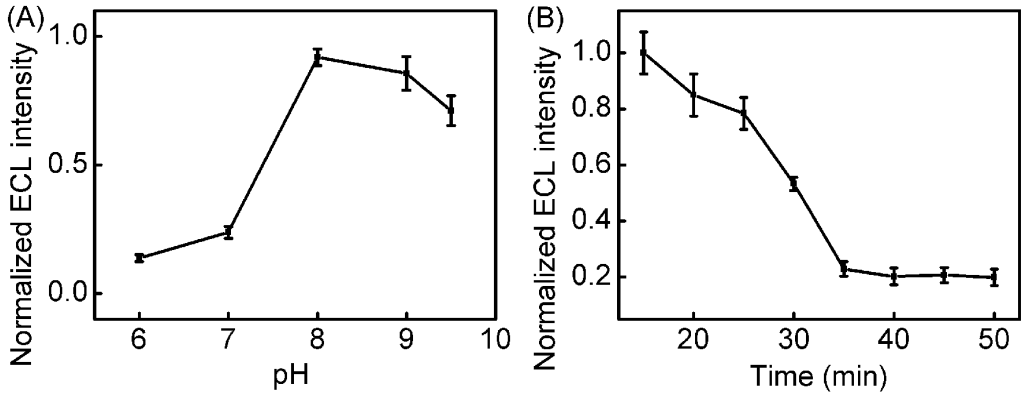


Fig. S3 Effects of (A) buffer pH and (B) incubation time of CEA on ECL intensity.

0.1 M pH 8.0 PBS and the incubation time of 35 min for CEA capture are the optimal detection conditions.

Reproducibility, stability and sample detection

The intra-assay and inter-assay precisions of the ECL immunosensor were examined by detecting the ECL emission at 0.5 ng mL^{-1} CEA. The relative standard deviation (RSD) for five measurements of CEA with the same immunosensor was 8.9%, while the RSD for five parallel measurements with five immunosensors was 11.4%, indicating good precision of the immunoassay method and acceptable fabrication reproducibility of the immunosensors. Eight measurements of ECL emission upon continuous cyclic scans of the ECL immunosensor at 0.5 ng mL^{-1} CEA showed coincident signal with RSD of 3.5%, indicating acceptable reliability and stability of the detection signal.

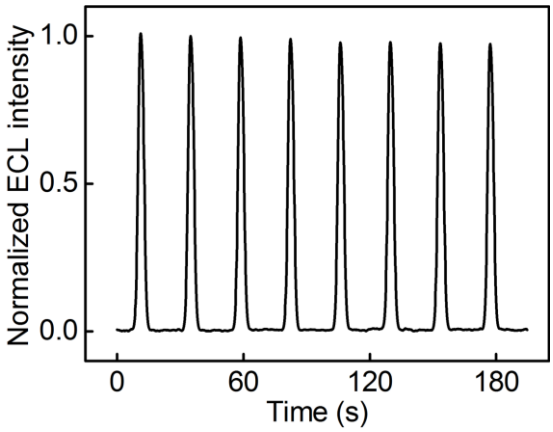


Fig. S4 Continuous cyclic scans of the immunosensor in air-saturated detection solution after incubation with 0.5 ng mL^{-1} of CEA and then Ab_2-Fc@PAMAM.

Table S1. Assay results of CEA (ng mL^{-1}) in clinical serum samples using the proposed and reference methods.

Sample No.	1	2	3
Proposed method	3.19	2.44	2.31
Reference method	3.55	2.62	2.09
Relative error (%)	−10.1	−6.9	10.5

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