CdS Quantum dots/ $Ru(bpy)_3^{2+}$ electrochemiluminescence resonance energy transfer system for sensitive cytosensing

Mei-Sheng Wu, Hai-Wei Shi, Jing-Juan Xu* and Hong-Yuan Chen

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

** Author for correspondence (Tel/Fax: +86-25-83587294; E-mail: xujj@nju.edu.cn)*

Experimental section

Apparatus. ECL signals were measured with a MPI-A multifunctional electrochemical and chemiluminescent analytical system (Remax Electronic Instrument Limited Co., Xi'an, China, 350 nm~650 nm) by a conventional three-electrode configuration at room temperature. Glassy carbon electrode (GCE, 3 mm diameter) was used as a working electrode; an SCE and a Pt wire were used as reference and auxiliary electrodes, respectively. The PMT was set at 700 V in the process of ECL detection. ECL spectrum was obtained by a series of optical filters (from 420 nm to 660 nm, spaced 20 nm, Omega Optical, Inc, US.). Nikon ECLIPSE 55i microscope mounted with Olympus DP71 cooled CCD camera was applied for chip imaging, results of which were analyzed by the Image- Pro Plus (IPP) 6.0 software.

Chemicals and solutions. N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC), 3-mercaptopropionic acid (MPA), N-hydroxysuccinimide (NHS), streptavidin (SA), 2-mercaptoethanol, biotin-NHS, $Ru(bpy)_3^{2+}$-NHS, Acridine Orange (AO) and Bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). Anti- β2 microglobulin antibody (β2 mAb) was purchased from Linc-Bio Science (China). All the other chemicals were of analytical grade. Ultra-pure water (Millipore, Bedford, MA) was used in the preparation of buffer solutions.

Preparation of CdS quantum dots The water-soluble CdS were synthesized as previously described[S1] with minor modifications. Briefly, $Cd(NO_3)_2 \cdot 4H_2O$ (0.1683 g) dissolved in 30 ml ultra-pure water, and heated to 70ºC under stirring, then added a freshly prepared Na_2S solution (0.5960 g) in 30 ml ultra-pure water. Instantly, orange- yellow solution was obtained. The reaction was held at 70ºC for 3 h with continuous refluxing. The final reaction precipitates were centrifuged and washed thoroughly with absolute ethanol two times, followed by washing with ultrapure water to remove a morsel of precipitates. The synthetic CdS NPs kept in a refrigerator at

4 ºC. The average size of the CdS QDs was about 5 nm, as indicated by transmission electron microscopy (TEM, Figure S1).

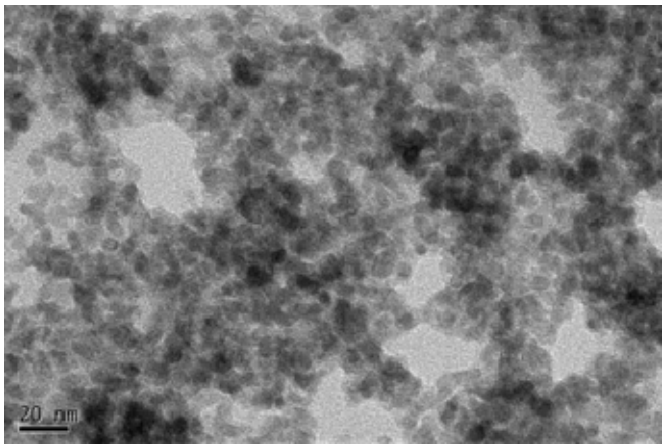

Figure S-1. TEM picture of CdS QDs.

Preparation of cell suspension Three cell lines, SMMC- 7721 cells (human hepatocellular carcinoma cell), K562 cells (human immortalised myelogenous leukaemia line) and MEF (Mouse Embryonic Fibroblast cells) were cultivated in DMEM cell culture medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS, GIBCO) and 1% streptomycin: penicillin (Amresco) at 37°C with 5% CO_2. In the cell labeling experiments, cells were collected and separated from the medium by centrifugation at 1500 rpm for 5 min and then washed twice with phosphate buffer solution (PBS) (0.1 M, pH 7.4). The sediment was resuspended in PBS to obtain a homogeneous cell suspension. Cells number was determined using a Petroff Hausser cell counter (USA).

Modification of SA with $Ru(bpy)_3^{2+}$ complex (Ru-SA) 1 mg SA was dissolved in 1mL of ultra-pure water. Then 100 μL of this solution was added in 810 μL of ultra-pure water and 90 μL of PBS (10x, 80 g of NaCl, 2.0 g of KCl, 14.4 g of Na_2HPO_4 and 2.4 g of KH_2PO_4 were dissolved in ultra- pure H_2O and then adjusted the volume to 1L, pH 7.4) and mixed with 1 mg Bis(2,2'-bipyridine)-4'-methyl-4-carboxybipyridine-ruthenium N-succinimidyl ester bis (hexafluorophosphate), which was first dissolved in 100 μL of DMSO. This mixture was incubated 24 hours at 4 °C. Then it was purified by dialysis in PBS (1x, 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 were dissolved in ultra- pure H_2O and then adjusted the volume to 1L, pH 7.4). Dialysis sacks 3.5 k molecular weight cut off was bought from Viskase (USA). This Ru-SA complex was stored in a refrigerator at 4 ºC.

Cell surface biotinylation and SA-Ru labeling The cells were incubated with biotin-NHS in a volume of 2 mL PBS for 30 min, then these biotinylated cells were incubated with Ru-SA for 1h at room temperature.

Preparation of ECL cytosensor GCE was polished carefully with abrasive paper, 0.05 μm Al_2O_3 powder and then washed ultrasonically with water and allowed to dry at room temperature. 10 μL CdS QDs was dropped on the electrode and dried in air at room temperature, then this CdS QDs modified electrode was stored in 0.1 M Tris- HCl buffer (pH= 7.4) containing 3 mM MPA for 5 h at 4ºC. After rinsed thoroughly with ultra-pure water and Tris- HCl buffer, the terminal carboxylic acid groups of the CdS surface were activated to conjugate amine compounds of β2 mAb by adding EDC and sulfo-NHS [S2]. Briefly, CdS film was activated by immersing it in 2 ml of 0.1 M borate buffer containing 2 mg EDC and 0.5 mg sulfo-NHS for 30 min at room temperature with continuous gentle mixing. Then the activation reaction was quenched by the addition of 1.4 μL (20 mM) 2-mercaptoethanol for 5 min incubating at room temperature. The activated CdS QDs film was incubated in β2 mAb (1mg/ mL) at room temperature for 1 h, and then the reaction was quenched by adding 0.1 M Tris buffer. This electrode was immersed in borate buffer and then blocked the unoccupied sites with 5 % BSA solution.

ECL measurements After 20 min incubation of Ru-SA labeled cells with β2 mAb/ CdS QDs modified GCE at room temperature, the ECL spectra were recorded with MPI-A multifunctional electrochemical and chemiluminescent analytical system by optical filter from 440 nm to 660 nm. For quantitative measurement, the ECL signals were recorded at the maximum emission wavelength of $Ru(bpy)_3^{2+}$.

Impedance measurements Impedance measurements were performed in the presence of 2.5 mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe in PBS (containing 0.1 M KCl, pH 7.4) at the frequency range from 10^{-1} to 10^6 Hz at the potential of 220 mV.

Specific binding affinity assay To study the specific binding affinity between β2 mAb and β2 M on SMMC- 7721 cells surface, we fabricated β2 mAb/ CdS QDs modified ITO glass electrodes and seeded the Ru-SA labeled SMMC- 7721 cells on these modified electrodes. After each indicated time interval of incubation, we rinsed the cells with sterile PBS several times, and stained with AO for 2 min, then washed with sterile PBS and these electrodes were imaged under an inversion fluorescence microscope (Leica DMIRE 2).

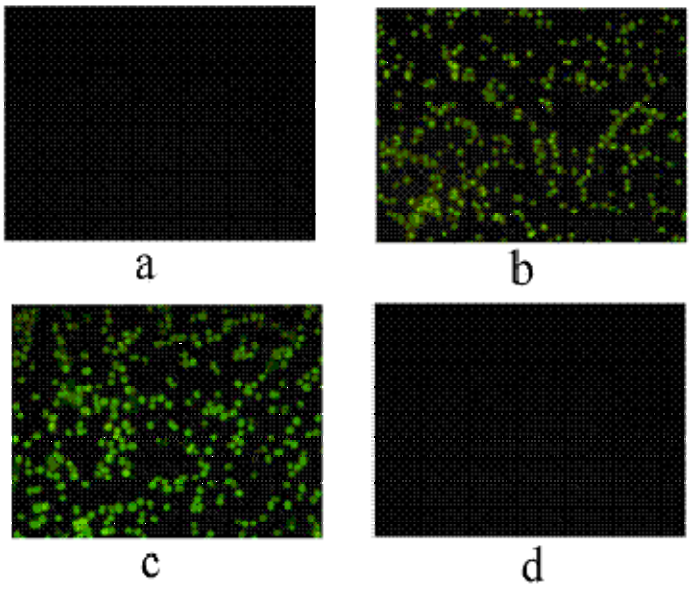

Figure S-2. Fluorescence microscopic images of AO-stained SMMC-7721 cells captured on β2 mAb/ CdS QDs modified ITO electrode after incubation for 0 min (a), 5 min (b), 10 min (c) and on CdS QDs modified ITO electrode after incubation for 10 min (d).

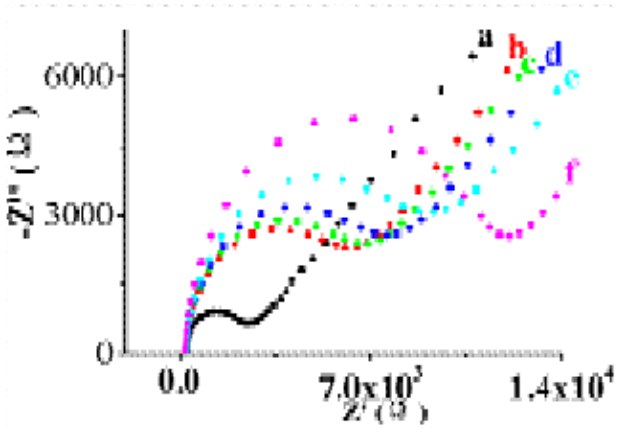

Figure S-3. Electrochemical impedance spectra (Nyquist plots) of the β2 mAb/ CdS QDs modified GCE (a), subsequently conjugated with different concentration of Ru-SA labeled SMMC- 7721 cells (from b to f), cells concentration: 10^1 cell/ mL (b), 10^2 cell/ mL (c), 10^3 cell/ mL (d), 10^4 cell/ mL (e), 10^5 cell/ mL (f). The incubation time was 10 min.

Reference

S1. Y. Shan, J. J Xu and H. Y. Chen. *Chem. Commun,* 2009, 905-907.

S2. J. H. Wang, T. C. Liu, Y. C. Cao, X. F. Hua, H. Q. Wang, H. L. Zhang, X. Q. Li and Y. D. Zhao. *Colloids and Surface A: Physicochem Eng Aspects,* 2007, **302**, 168-173.