Electronic Supplementary Information (ESI)

Label-free and highly sensitive electrochemiluminescent biosensing using quantum dots/carbon nanotubes in ionic liquid

Wenwen Tu,[ab] Xuelin Fang,[a] Jing Lou[a] and Zhihui Dai*[a]

[a]*Jiangsu Collaborative Innovation Center of Biomedical Functional Materials and Jiangsu Key Laboratory of Biofunctional Materials, School of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210023, P. R. China*

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

*Corresponding author. Fax: +86-25-85891051; Tel: +86-25-85891051; E-mail: daizhihuii@njnu.edu.cn

1. Experimental Section

1.1 Reagents and materials. Glutaraldehyde (GLD, 50%), hexaammineruthenium chloride (98%) and chitosan (CS) were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). Thioglycolic acid (TGA, 97%) was purchased from Alfa Aesar. Bovine serum albumin (BSA) was purchased from Fluka and used without further purification. 1-Ethyl-3-methylimidazoliumtetrafluoroborate ([EMIM][BF_4]) which was a kind of room temperature ionic liquid was purchased from J&K CHEMICAL LTD. Single-walled carbon nanotubes (SWCNTs, φ=10-20 nm) were purchased from Nanotech Port Co., Ltd. (Shenzhen, China). Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) was purchased from Shanghai Reagent Co. Ltd. Selenium metal powder (≥99.95%) was obtained from Shanghai Meixing Chemical Co. Ltd. Monoclonal anti-AFP antibody (Ab) was purchased from Boson Biotechnology Co. Ltd.

AFP standard solutions were supplied by Fujirebio Diagnostics AB (Göteborg, Sweden). Other chemicals were of analytical reagent grade. The washing solution was phosphate buffered saline (PBS) (0.01 mol L^{-1}, pH 7.4). PBS (0.01 mol L^{-1}, pH 7.4) containing BSA (1%, w/v) was used as blocking solution. Aqueous solutions were prepared with twice-distilled water. The pH value of PBS was 7.4, except where otherwise indicated. Blood samples from Jiangsu Hospital of Prevention and Cure (The Helsinki number was not needed in China) were centrifuged at 4000 rpm for 5 min to obtain the supernates as clinical serum samples.

1.2 Apparatus. Transmission electron microscope (TEM) image was taken on a JEOL-2100F apparatus at an accelerating voltage of 200 kV (JEOL, Japan). Photoluminescence (PL) spectrum was recorded on Cary Eclipse (Varian, USA). Ultraviolet-visible (UV-vis) absorption spectrum was obtained on Cary 60 spectrophotometer (Agilent, USA). Electrochemical impedance spectroscopy (EIS) was carried out at an open circuit potential with an Autolab potentiostat/galvanostat PGSTAT302N (Metrohm, Netherland) controlled by Nova 1.8 software with a three-electrode system in a KCl solution (0.1 M) containing a $K_3Fe(CN)_6/K_4Fe(CN)_6$ (5.0 mM, 1:1) mixture as a redox probe from 0.1 Hz-100 kHz with a signal amplitude of 10 mV. The electrochemiluminescence measurements were carried out on a MPIA multifunctional electrochemical analytical system (Xi'an Remex Electronic and Technological Co., China). ECL emission window was placed in front of the photomultiplier tube (PMT) (detection range from 300 to 650 nm) biased at -800 V in the process of detection. All experiments using a conventional three-electrode system with a modified Au electrode as working, a Pt wire as auxiliary, and an Ag/AgCl electrode as reference electrodes.

1.3 Synthesis of water-soluble TGA wrapped CdSe QDs. The water-soluble TGA

wrapped CdSe QDs were synthesized using a slightly modified procedure reported previously.[S1] After deoxygen deionized water (50 mL) containing $CdCl_2$ (2 mmol L^{-1}) was mixed with TGA (20 μL), NaOH solution (1 mol L^{-1}) was added to adjust its pH value to 10.0. The mixture was bubbled with highly pure N_2 for 40 min. Then freshly prepared NaHSe solution (0.7 mL, 70 mmol L^{-1}) was injected into the mixture to obtain a clear, light yellow solution after reflux at 100 °C for 4 h. The final molar ratio of Cd^{2+}/Se^{2+}/TGA for the preparation of the optimum QDs were 1:0.5:2.5. Finally, the solution was cooled under N_2 atmosphere and ultra-filtered at 5000 rpm for 5 min to remove unreacted reactants to obtain TGA-CdSe QDs. The obtained TGA-CdSe QDs was stored at 4 °C before use.

1.4 Preparation of [EMIM][BF_4]-SWCNTs-CS. 1 mg CS were put into 10 mL 0.25% acetic acid solution and ultrasonicated to obtain a uniform CS solution. SWCNTs were not pretreated by acidification or oxidation. Then 1 mg pristine SWCNTs was added into 10 mL CS solution. The reaction mixture was sonicated for 20 min. Finally, 156 μL [EMIM][BF_4] was added into the mixture and stirred for 2 h to obtain [EMIM][BF_4]-SWCNTs-CS suspension.

1.5 Fabrication of the ECL biosensor. An Au disk electrode with 2 mm diameter was polished carefully with 1.0, 0.3, and 0.05 μm Al_2O_3 powder on fine abrasive paper, followed by successive sonication in ethanol and twice-distilled water. After it was cleaned and dried with nitrogen, 3 μL fresh TGA wrapped CdSe QDs solution was dropped on the electrode and dried under the air. Subsequently, 2 μL [EMIM][BF_4]-SWCNTs-CS suspension was applied to the electrode. After the [EMIM][BF_4]-SWCNTs-CS modified Au electrode was dried at room temperature, it was immersed into the GLD solution (2%) for 2 h. The activated CS membrane was then bound to anti-AFP antibody by dropping 3 μL Ab solution (1mg mL^{-1}, pH 7.4) and incubation at 4 °C for 16 h. Finally the electrode was

rinsed with a washing buffer and incubated with 30 μL BSA (2 wt %) blocking buffer for 1 h to block possible remaining active sites against nonspecific adsorption. After rinsing with 0.01 mol·L^{-1} PBS, the ECL biosensor was obtained.

1.6 Measurement procedure. To carry out ECL measurement, the biosensor was first incubated with 3 μL AFP solution for 45 min at 37 °C, followed by washing with 0.01 mol·L^{-1} PBS. The different electrodes were used for continuous incubation of AFP from low concentration to high value. The biosensor was then inserted in 0.1 mol L^{-1} PBS (pH 7.4) containing 0.1 mol L^{-1} $K_2S_2O_8$ and 0.1 mol L^{-1} KCl, and scanned from 0 V to -1.6 V to record the ECL response for immunoassay.

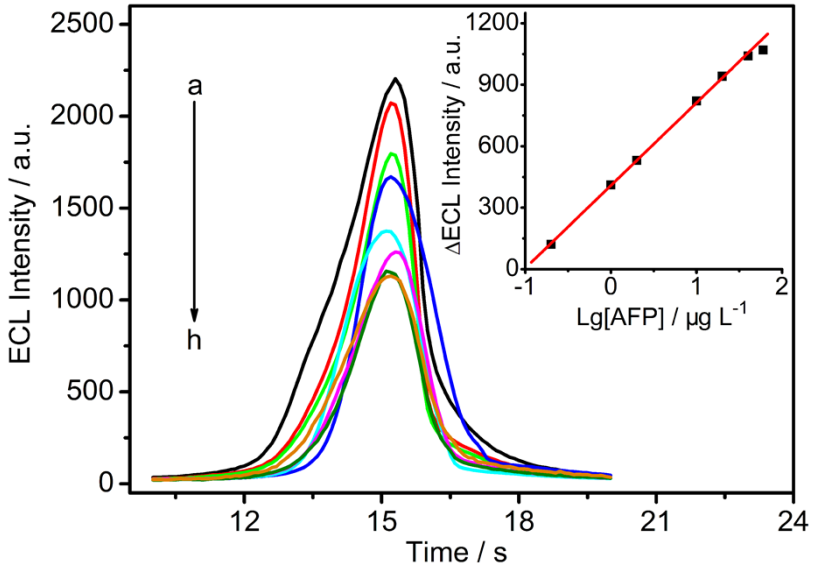

Fig. S1 (A) ECL responses of the CdSe QDs/SWCNTs-CS/GLD/Ab/BSA modified Au electrode to 0 (a), 0.2 (b), 1 (c), 2 (d), 10 (e), 20 (f), 40 (g) and 60 (h) μg L^{-1} AFP in 0.1 mol L^{-1} PBS (pH 7.4) containing 0.1 mol L^{-1} $K_2S_2O_8$ and 0.1 mol L^{-1} KCl between 0 and -1.6 V at 100 mV s^{-1}. The emission window was placed in front of the photomultiplier tube, which was biased at -800 V. Inset: linear calibration curve.

Table S1 Comparison of the performance of the proposed strategy with the previous reported researches.

Method	Linear range (μg L^{-1})	Detection limit (μg L^{-1})	Refs.
Fluorescence	0.1-750	0.05	S2
Electrochemistry	0.1-300	0.03	S3
Resonance light scattering	0.10-50	0.04	S4
Surface plasmon resonance	1.0-200.0	0.65	S5
Chemiluminescence	1.0-80	0.89	S6
Photoelectrochemistry	0.05-50	0.04	S7
Colorimetry	0.5-1000	0.06	S8
Electrochemiluminescence	0.005-100	0.003	This work

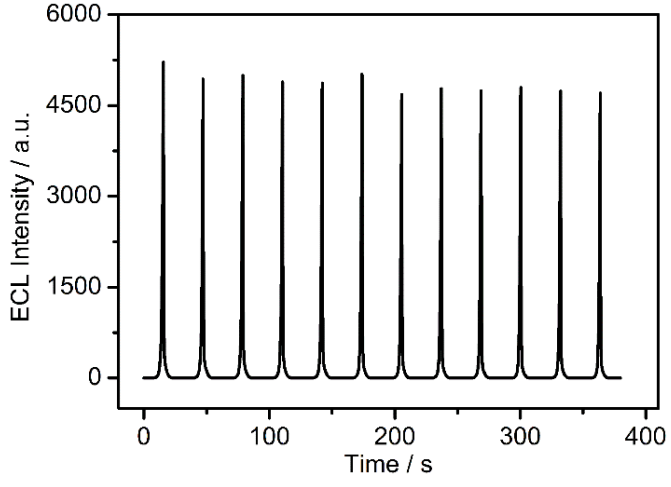

Fig. S2 ECL emissions of the biosensor towards 5 μg L^{-1} AFP in PBS (pH 7.4) containing 0.1 mol L^{-1} KCl and 0.1 M $K_2S_2O_8$ under continuous cyclic potential scan between 0 and -1.6 V for 12 cycles at 100 mV s^{-1}. The emission window was placed in front of the photomultiplier tube, which was biased at -800 V.

References

S1 H. Jiang and H. X. Ju, *Anal. Chem.*, 2007, **79**, 6690.

S2 J. Y. Hou, T. C. Liu, Z. Q. Ren, M. J. Chen, G. F. Lin and Y. S. Wu, *Analyst*, 2013, **138**, 3697.

S3 K. J. Huang, J. Li, Y. Y. Wu and Y. M. Liu, *Bioelectrochemistry*, 2013, **90**, 18.

S4 Z. G. Chen, Y. L. Lei and X. Chen, *Microchim. Acta*, 2012, **179**, 241.

S5 R. P. Liang, G. H. Yao, L. X. Fan and J. D. Qiu, *Anal. Chim. Acta*, 2012, **737**, 22.

S6 Z. J. Yang, H. Liu, C. Zong, F. Yan and H. X. Ju, *Anal. Chem.*, 2009, **81**, 5484.

S7 G.-L. Wang, J.-J. Xu, H.-Y. Chen and S.-Z. Fu, *Biosens. Bioelectron.*, 2009, **25**, 791.

S8 X. Xiang, L. Chen, C. L. Zhang, M. Luo, X. H. Ji and Z. K. He, *Analyst*, 2012, **137**, 5586.