Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information

Highly Enhanced Electrochemiluminescence of Novel Gold/Silica/CdSe-CdS Nanostructures for Ultrasensitive Immunoassay of Protein Tumor Marker

Gui-Fen Jie, Ping Liu and Shu-sheng Zhang *

Key Laboratory of Eco-chemical Engineering of Ministry of Education,College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, P. R. China

Chemicals. CEA, anti-CEA, and Bovine serum albumin (BSA, 96-99%) were purchased from Wuhan Boster Biological Technology Co. (Wuhan, China). 3-aminopropyl-triethoxysilane (APS) was obtained from Shanghai Bailingwei Biochemical Co. (Shanghai, China), and sodium silicate solution ($Na_2O(SiO_2)_{3-5}$, 27 wt% SiO_2) were purchased from Aldrich. Chloroauric acid ($HAuCl_4$), trisodium citrate was obtained from Shanghai Reagent Company (Shanghai, China). The human CEA samples were provided by the Medical School Hospital of Qingdao University (Qingdao, China). All other reagents were of analytical grade. Double distilled water was used for all experiments.

Instrumentation. The electrochemical measurements was carried out on a CHI 812 electrochemical working station (Shanghai CH Instruments Co., China) using a three-electrode system. The electrodes include an Au disk (4-mm-diameter) working electrode, a saturated calomel reference electrode (SCE), and a Pt counter electrode. The ECL emission was detected with a Model MPI-A Electrochemiluminescence Analyzer (Xi'An Remax Electronic Science & Technology Co. Ltd., Xi'An, China) at room temperature. The spectral width of the photomultiplier tube (PMT) was 200-800 nm and the voltage of the PMT was -400 V ~ -800 V in the detection process. Electrochemical impedance spectroscopy (EIS) was carried out with a CHI 660C electrochemical working station (Shanghai CH Instruments Co., China), using the same three-electrode system as that in the ECL detection. UV-visible spectra were carried out on a Cary 50 UV-vis-NIR spectrophotometer (Varian). Transmission electron microscopy (TEM) image was taken with a JEOL JSM-6700F instrument (Hitachi). Photoluminescence (PL) spectra were obtained on an RF-540 spectrophotometer (Shimadzu). Field-emission scanning electron microscopy (FESEM) was carried out using a JEOL JSM-6340 F instrument.

Synthesis of CdSe/CdS core/shell QDs. Mercaptoacetic acid-capped CdSe QDs (CdSe/TGA) were synthesized using a slightly modified procedure reported previously.[1] 0.46 g of $CdC1_2{\cdot}2.5H_2O$ was dissolved in 240 mL of water and 0.36 mL of TGA was added under stirring, followed by adjusting the pH to 11 with 0.1 M solution of NaOH. The solution was placed in a three-necked flask and deaerated by N_2 bubbling for 1 h. Under stirring, 12 mL of 0.1 M Na_2SeSO_3 was injected into this flask, and a clear light yellow solution of CdSe/TGA QDs was obtained by refluxing the reaction mixture at 100 □.

For overcoating CdSe QDs with CdS QDs, 0.116g of $CdCl_2{\cdot}$ 2.5 H_2O was dissolved in 120 mL

of water and 128 μL of TGA was added under stirring, followed by adjusting the pH to 11 with 0.1 M solution of NaOH. Then, the solution was added into the above CdSe colloid under stirring and N_2 bubbling, and 90 mL of 0.01 M Na_2S solution was slowly added dropwise. After refluxing for 1 h, CdSe@CdS colloid was obtained.

Synthesis of hybrid gold/SiO_2/CdSe-CdS-QD nanostructures.[2,3] The hybrid gold/silica/CdSe-CdS-QD nanoparticles were synthesized via a multistep procedure, which involved synthesis of gold nanoparticles, gold-particle surface activation, silica-shell deposition, modification of the silica surfaces with -NH_2 groups, and final self-assembly of the gold/SiO_2 particles onto CdSe-CdS-QD surfaces. The process was shown in scheme 1 (A).

A gold sol was prepared according to the standard sodium citrate reduction method.[4] This method produces a stable, deep-red dispersion of gold particles with an average diameter of around 16 nm. A freshly prepared aqueous solution of APS (0.24 mL, 1 mM) was added to 50 mL of gold sol under vigorous magnetic stirring. The mixture of APS and gold dispersion was allowed to stand for 15 min to ensure complete complexation of the amine groups with the gold surface. A solution of active silica was prepared by lowering the pH of a 0.54 wt% sodium silicate solution to 10-11. 1 mL of active silica was then added to 50 mL of the surface-modified gold sol, again under vigorous magnetic stirring for 15 minutes. The resulting dispersion (pH≈8.5) was then allowed to stand for at least one day, so that the active silica polymerizes onto the gold particle surface.

The silica-coated nanoparticles were treated with (3-aminopropyl)-trimethoxysilane (APS, a silane coupling agent), which reacted with the surface silanol groups to produce silica surfaces functionalized with -NH_2 groups. Finally, the surface-modified nanoparticles were mixed with CdSe-CdS QDs in the volume ratio of 1:1 under shaking, and allowed to stand for at least one day. Because of strong binding interactions between the -COOH of the CdSe-CdS QDs and the -NH_2, the gold/SiO_2 NPs were self-assembled onto the surface of the CdSe-CdS QDs, resulting in the targeted hybrid gold/silica/CdSe-CdS-QD superstructures.

Preparation of the ECL Immunosensor. A gold disk electrode with 4 mm diameter was polished carefully with 1.0, 0.3 and 0.05 μm α-Al_2O_3 powder on fine abrasive paper and washed ultrasonically with water. Before modification, the bare electrode was scanned in 0.5 M H_2SO_4 between -0.2 and 1.5 V until a reproducible cyclic voltammogram (CV) was obtained. After the

electrode was rinsed thoroughly with doubly distilled water and allowed to dry, 10 μL of gold/SiO_2/CdSe-CdS-QD solution was dropped on the electrode and dried in the air, followed by cross-linking 3% APS on the nanostructures/electrode. Then, 10 μL of gold NPs was assembled on the electrode, followed by incubating the electrode in 0.1 mg mL^{-1} antibody solution (20 mM PBS, pH 6.0) at 4 $^{\circ}$C for at least 12 h. Finally, it was rinsed with pH 7.4 PBS and incubated in 20 μL of 2 wt% BSA at 37 $^{\circ}$C for 1 h to block nonspecific binding sites. Scheme 1 (B) outlines the fabricating procedures for the ECL immunosensor.

Characterization of the hybrid Gold/SiO_2/CdSe-CdS-QDs nanostructures.

PL spectra. Figure 1 shows the photoluminescence (PL) of the CdSe-CdS-QDs in aqueous solution. The PL emission peak at 567 nm (λ_{ex} = 440 nm) indicates the consequence of quantum confinement.[5]

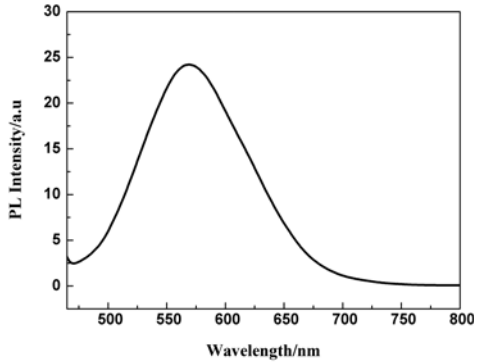

Figure S1. Photoluminescence spectra of the CdSe-CdS-QDs in aqueous solution.

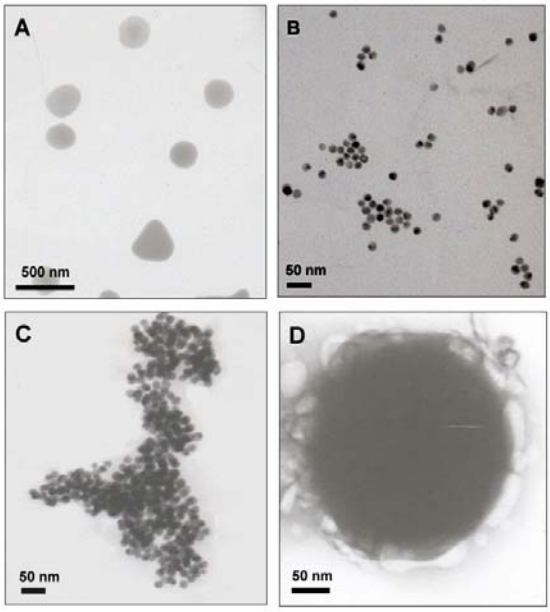

Figure S2. TEM images of synthesized CdSe-CdS-QDs (A), gold NPs, (C) gold/SiO_2 core/shell NPs, and (D) an enlarged image of a single gold/SiO_2/CdSe-CdS particle.

The ECL behavior of the hybrid gold/SiO_2/CdSe-CdS-QDs nanocomposites is very similar to that of other semiconductor NCs, such as Si NCs[6] or CdSe NCs[7]. Consequently, the ECL mechanism of the nanocomposites was suggested to involve the formation of excited-state R^* via electron-transfer process. In this case, upon the potential scan with an initial negative direction, the CdSe-CdS-QDs immobilized on the electrode were reduced to nanocrystalline species ($QD^{-\bullet}$) [7], while the coreactant $S_2O_8^{2-}$ was reduced to the strong oxidant $SO_4^{-\bullet}$. Then $SO_4^{-\bullet}$ could react with the negatively charged $QD^{-\bullet}$ through electron transfer, which produced the excited state (QD^*) to emit light. Compared with the pure CdSe-CdS-QDs, on the one hand, the large numbers of gold NPs coated on the QDs in the hybrid nanocomposites could greatly accelerate the electron transfer in ECL reaction; On the other hand, the superstructure had more open pores and larger surface area, allowing faster diffusion of $K_2S_2O_8$ and electron transfer in ECL reaction, thus the ECL signal of the hybrid gold/SiO_2/CdSe-CdS-QDs nanocomposites was greatly improved. The possible ECL mechanisms are as follows.[7]

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

(3) $QD^{-\bullet}+SO_4^{-\bullet}\rightarrow QD^*+SO_4^{2-}$ (4) $QD^* \rightarrow QD + h\nu$

ECL characterization of the ECL immunosensor.

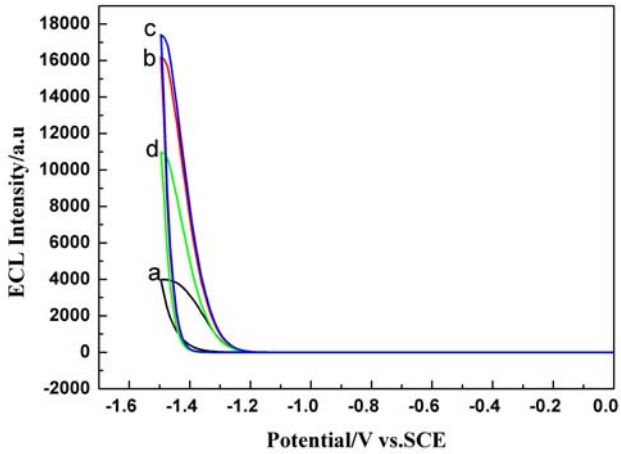

Figure S3. ECL–potential curves of (a) gold/SiO_2/CdSe-CdS-QDs/electrode, (b) (a)+APS, (c) (b)+gold NPs, (d) (c)+Ab modified Au electrodes in 0.1 M PBS (pH 7.4) containing 0.1 M KCl and 0.05 M $K_2S_2O_8$.

Optimization of Experimental Conditions.

Figure S4 shows the effect of pH of the detection solution on the ECL responses of the immunosensor in the presence of 0.0016 ng mL^{-1} CEA. It could be seen that the ECL increased

with the increment of pH value from 6.0 to 7.4 and then decreased from 7.4 to 9.0. Since the maximum pH value at 7.4 was optimal for the biological systems, so the ECL detection was performed in pH 7.4 PBS containing 0.1 M KCl and 0.05 M $K_2S_2O_8$.

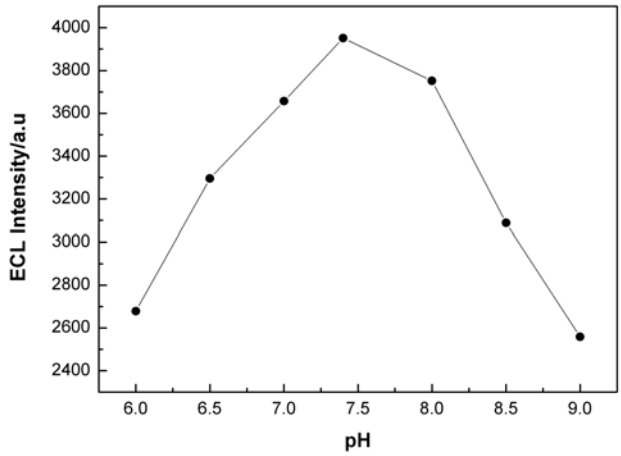

Figure S4. Effect of pH on the ECL intensity of the immunosensor in 0.0016 ng mL^{-1} CEA under optimal conditions.

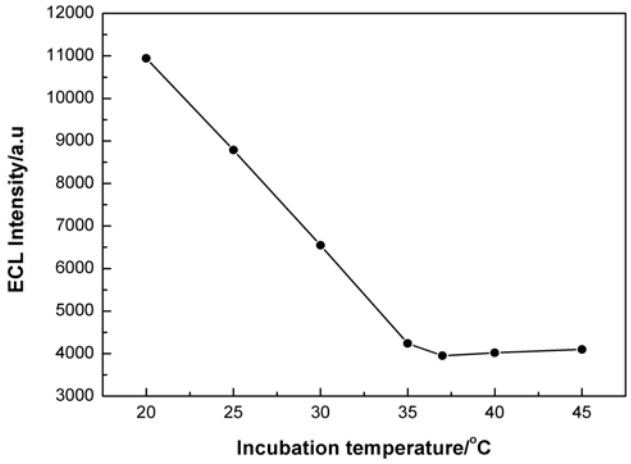

Figure S5. Effect of incubation temperature on ECL response of the immunosensor in 0.0016 ng mL^{-1} CEA under optimal conditions.

The time and temperature of the antigen-antibody reaction also greatly affected the analytical performance of the proposed immunosensor. As shown in Figure S5, the ECL intensity of the immunosensor changed with the incubation temperature in the range from 20 °C to 45 °C, and reached a minimum value at 37 °C, indicating the maximum immunoreaction occurred at the temperature. At this temperature, the ECL response of the immunosensor decreased with the increase of incubation time and leveled off after 50 min (Figure S6), suggesting that the immunoreaction reached an equilibrium state. Therefore, 37 °C and 50 min were selected as the

incubation temperature and time for the determination of CEA in this study.

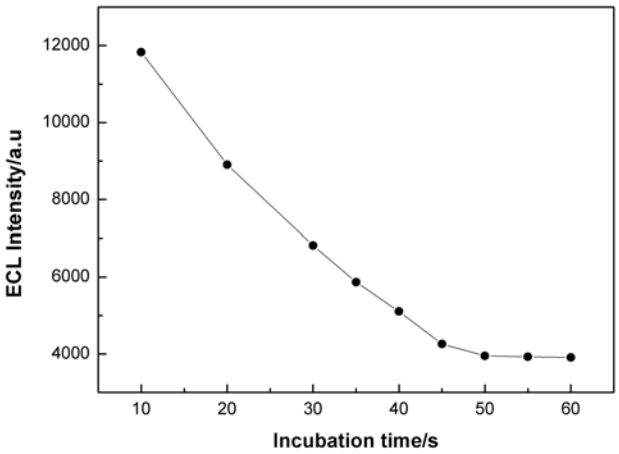

Figure S6. Effects of incubation time on ECL response of the immunosensor toward 0.0016 ng mL^{-1} CEA under optimal conditions.

Fig. S7 shows the ECL emission of the immunosensor under consecutive potential scans from 0 to -1.5 V for 19 cycles. The ECL signals are high and stable, suggesting that the sensor is suitable for ECL detection.

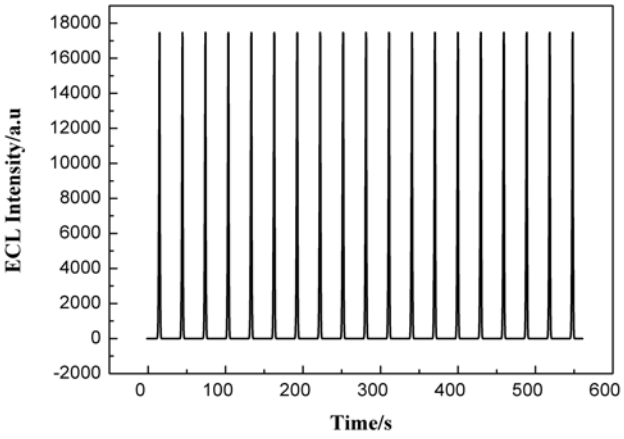

Figure S7. ECL emission from the immunosensor in pH 7.4 PBS containing 0.1 M KCl and 0.05 M $K_2S_2O_8$ under continuous cyclic voltammetry for 19 cycles. Scan rate: 100 mV s^{-1}. The voltage of the PMT was set at -600 V.

Specificity, stability, reproducibility, and regeneration of the ECL immunosensor. To investigate the specificity of the immunosensor, we mixed 0.04 ng mL^{-1} CEA, 4 ng mL^{-1} human IgG and 4 ng mL^{-1} BSA, and then detected the ECL response of the mixture. Compared with the ECL response of the ECL immunosensor in 0.04 ng mL^{-1} pure CEA, no significant difference (R.S.D=6.2%) was observed, indicating that both the human IgG and BSA could not cause the

observable interference. The results suggest that the ECL immunosensor displays good selectivity for CEA detection.

After the ECL immunosensor was stored in pH 7.4 PBS at 4 ℃ over 20 days, it was used to detect the same CEA concentration, the results did not show an obvious change, demonstrating that the immunosensor had good stability.

The reproducibility of the immunosensor was estimated by detecting 0.4 ng mL^{-1} CEA with three immunosensors made at the same electrode. Three measurements from the batch resulted in a relative standard deviation of 7.5%, indicating good reproducibility of the fabrication protocol.

Regeneration is of interest to immunoanalysis. After the immunosensor was used to detect CEA, the electrode was treated with 0.2 M glycine-hydrochloric acid buffer solution (pH 3.0) to remove the Ab-Ag molecules. It had repeated four times consecutive measurements and a relative standard deviation (R.S.D.) of 9.5% was obtained. The results demonstrated that the immunosensor could be regenerated and used again.

Table S1. Comparison of the Sensitivity Data of the Developed Immunosensor with Those of Other CEA Immunoassays

immunoassay method	linear range	detection limit	ref
chemiluminescent multiplex immunoassay	1.0-70 ng mL^{-1}	0.65 ng mL^{-1}	8
immunofluorescence assay	0.5-1000 pmol L^{-1}	1.31 pmol L^{-1}	9
Amperometric immunoassay	0.01-160 ng mL^{-1}	5.0 pg mL^{-1}	10
piezoelectric immunoassay	1.5-30μg mL^{-1}	1.5 μg mL^{-1}	11
chemiluminescence enzyme immunoassay	2-162 ng mL^{-1}	0.69 ng mL^{-1}	12
Potentiometric immunoassay	1.5-200 ng mL^{-1}	0.5 ng mL^{-1}	13

ESEIA immunoassay	0.01-200 ng mL^{-1}	1.5 pg mL^{-1}	14
ECL Immunosensor	0.32-10000 pg mL^{-1}	0.064 pg mL^{-1}	This work

Table S2. Comparison of CEA Determinations on Serum Samples by the ECL Immunosensor and Standard ELISA, Recovery for adding normal serum of 50 pg mL^{-1}, and RSD (%) for five replicate Measurements by our method

Serum samples	1	2	3	4	5
Our method (pg/mL)	0.68	4.75	23.6	72.6	669.8
ELISA (pg/mL)	0.65	4.56	22.4	70.1	639.2
Relative deviation	5.1%	4.2%	5.4%	3.6%	4.8%
Recovery (%) for adding normal serum of 50 pg mL^{-1}	94.2	96.8	97.4	104.6	108.7
RSD (%) for five replicate measurements	7.4	6.5	5.8	6.1	5.3

References:

(1) N. Gaponik, D. V. Talapin, A. L. Rogach, K. Hoppe, E. V. Shevchenko, A. Kornowski, A. Eychmu¨ller, H. Weller, *J. Phys. Chem. B*, 2002, **106**, 7177.

(2) N. Liu, B. S. Prall, V. I. Klimov, *J. Am. Chem. Soc*., 2006, **128**, 15362.

(3) L. M. Liz-Marzan, M. Giersig, P. Mulvaney, *Langmuir*, 1996, **12**, 4329.

(4) X. Ji, X. Song, J. Li, Y. Bai, W.Yang, X. Peng, *J. Am. Chem. Soc.*, 2007, **129**, 13939.

(5) W. T. Chen, T. T.Yang, Y. J. Hsu, *Chem. Mater.*, 2008**, 20***,* 7204.

(6) Ding, Z.; Quinn, B. M.; Haram, S. K.; Pell, L. E.; Korgel, B. A.; Bard, A. J. *Science* **2002**, *296*, 1293–1297.

(7) Myung, N.; Ding, Z. F.; Bard, A. J. *Nano. Lett.* **2002**, *2*, 1315–1319.

(8) Z. Fu, Z.Yang, J. Tang, H. Liu, F. Yan, H. Ju, *Anal. Chem*., 2007, **79**, 7376.

(9) N. Honda, U. Lindberg, P. Persson, S. Hoffmann, H. Takei, *Clin. Chem*., 2005, **51**, 1955.

(10) D. P. Tang, R.Yuan, Y. Chai, *Anal. Chem*., 2008, **80**, 1582.

(11) B. Zhang, X. Zhang, H.Yan, S. Xu, D. Tang, W. Fu, *Biosens. Bioelectron*., 2007, **23**, 19.

(12) W. Dungchai, W. Siangproh, J. Lin, O. Chailapakul, S. Lin, X. Ying, *Anal. Bioanal. Chem*., 2007, **387**, 1965.

(13) D. P. Tang, R. Yuan, Y. Chai, *J. Phys. Chem. B*, 2006, **110**, 11640.

(14) D. Tang, J. Ren, *Anal. Chem*., 2008, **80**, 8064.