-Supporting Information-

Potassium-doped Graphene Enhanced Electrochemiluminescence of SiO_2@CdS Nanocomposites for Sensitive Detection of TATA-binding protein

Jing Wang, Wei-Wei Zhao, Xiao-Rong Li, Jing-Juan Xu and Hong-Yuan Chen*

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

Tel/ Fax: +86-25-83597294; E-mail: xujj@nju.edu.cn

Experimental section

Materials.

Labeled DNA oligonucleotides were purchased from Shenggong Bioengineering Ltd. Company (Shanghai, China). The sequences of these oligonucleotides employed are given as following:

Amino modified DNA 1:

ss-DNA 1: 5'-NH_2-$(CH_2)_6$-AGGACTTTTATAGTGGAGGCCGCGCCGGGC-3'

biotin modified complementary DNA 2:

ss-DNA 2: biotin-GCCCGGCGCGGCCTCCAC***TATAAAAG***TCCT-3'

(Bold italic portion indicated the TATA binding protein binding site).

Amino modified bio bar code DNA:

bbc-DNA: 5'-NH_2-$(CH_2)_6$-ACGCTAGCTATGCTT-3'.

SiO_2 NPs (99.5%, 20±5 nm) was obtained from Aladdin reagent Inc (Shanghai, China). TATA binding protein (Constituents: 20% Glycerol, 20mM Tris HCl, 100mM Potassium chloride, 1mM DTT, 0.2mM EDTA, pH 8.0) was ordered from Abcam website. Reduction of graphene oxide (GR) was obtained from JCNano Co. Ltd. (NanJing), Potassium (K), phenanthrene (98%), 1, 2-dimethoxyethane (99.5%, 1, 2-DME) were purchased from Alfa Aesar. Streptavidin (essentially salt-free, lyophilized powder), bovine serum albumin (BSA), thrombin, alpha-fetoprotein (AFP), lysozyme, Poly(diallyldimethylammonium chloride) (PDDA), 3-aminopropyl-triethoxysilane (APTS), 1-methylimidazol, 3-mercaptopropionic acid (MPA), N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. 0.1 M Tris-HCl buffer containing 0.1 M NaCl and 5 mM $MgCl_2$ (pH 7.4), (after abbr. as $Tris^+$ buffer), was employed for hybridization and preparation of DNA stock solutions. All other reagents were of analytical grade and used as received. Millipore ultrapure water (resistivity≥18.2 MΩcm) was used throughout the experiment.

Apparatus.

The electrochemical measurements were recorded with CHI 660A electrochemical workstation (Shanghai CHI Instruments Co., China). The ECL emission measurements were conducted on a model MPI-A electrochemiluminescence analyzer (Xi'An Remax Electronic Science &Technology Co. Ltd., Xi'An, China) at room temperature, and the voltage of the PMT was set at -600 V in the process of detection. All experiments were carried out with a conventional three-electrode system. The working electrode was a 3 mm diameter glassy carbon electrode (GCE), Pt wire and SCE electrode served as the counter and reference electrodes, respectively.

The UV-vis absorption spectra were obtained on a Shimadzu UV-3600 UV-vis-NIR photospectrometer (Shimadzu Co.). Transmission electron microscopy (TEM) was performed with a JEOL model 2000 instrument operating at 200 kV accelerating voltage.

Preparation of the SiO_2@CdS/DNA composites.

5 nm CdS NCs were synthesized as the literature with some modification.[1] Briefly, $Cd(NO_3)_2 \cdot 4H_2O$ (0.1683 g) was dissolved in 30 mL ultra-pure water, and heated to 70 ºC under stirring, then injected into a freshly prepared solution of Na_2S (0.5960 g) in 30 mL ultra-pure water. Instantly, orange-yellow solution was obtained. The solution was held at 70 ºC for 3 h with continuous refluxing. The final reaction precipitates were centrifugated and washed thoroughly with absolute ethanol two times and ultrapure water two times. Then, the obtained precipitate was redispersed into water for centrifugation to collect the upper yellow solution of CdS NCs. The average size of synthesized CdS NCs was about 5 nm, as indicated by UV-vis spectrum and transmission electron microscopy (TEM).The final solution was rather stable for 2 months when stored in a refrigerator at 4 ºC.

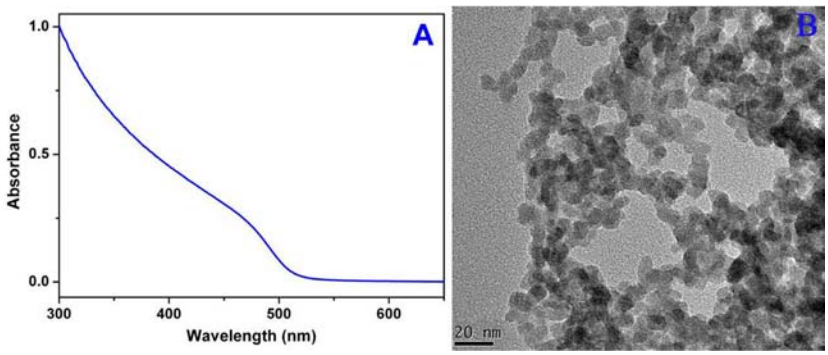

Figure S-1. A: UV-Vis absorption spectrum of CdS NCs; B: TEM picture of CdS NCs.

For the preparation of SiO_2@CdS nanocomposites,[2] 0.02 g of SiO_2 NPs were first dispersed in 2 mL of ethanol and treated with 0.4 mL of APTS. After stirring for 6 h, the suspension was centrifuged and washed with ethanol repeatedly for four times, and the amino-functionalized SiO_2 NPs were obtained. Then, the amino-functionalized NPs were dispersed in 1 mL of the terminal carboxylic acid group activated CdS NCs, which derived from the reaction in 1.0 mL of 0.1 M 1-methylimidazol aqueous solution (pH 7.4) containing 20 mg EDC and 10 mg NHS for 2 h at 4 ºC. The mixed suspension was stirred at 4 ºC for 12 h. Unbound CdS NCs were removed by successive centrifugation and washing with water several times. Finally, the as-prepared SiO_2@CdS nanocomposites, which had the same color as CdS NCs itself, were obtained and dispersed in water.

To generate SiO_2@CdS/DNA composites, the ssDNA 1 and bbc-DNA modified with amino at their 5'-end were used, and with the molar ratio of 1:10. Briefly, 100 μL 1 μM ssDNA 1 and 1 mL 1 μM bbc-DNA were added to 200 μL SiO_2@CdS nanocomposites, after shaking gently for 12 h, they covalently attached through the binding reaction of amino group and activated carboxylic acid group. Then, 100 μL 1 μM biotin modified ssDNA 2 was added into for incubating with the ssDNA 1 to form the DNA duplex. After 2 h reaction, the mixture was centrifuged at 10000 rpm for 30 min to remove the redundant oligonucleotides. Finally, the SiO_2@CdS/DNA composites were kept in 0.1 M Tris-HCl buffer containing 0.5 M NaCl and 5 mM $MgCl_2$ (pH 7.4) at 4 ºC before use.

Synthesis of Potassium-Doped Graphene with Streptavidin Composites.

K-doped graphene was synthesized according to our previous work, and the details were as following.[3] The phenanthrene/K solution complex was made by reacting K (200 wt% to graphene) with 0.2 M of phenanthrene (98%) in 20 mL of 1, 2-DME (99.5%) solution. Then, the GR (20 mg) were added to the phenanthrene/K solution complex for the K-doping into graphene by π-stacking interaction between the graphene and phenanthrene. Finally, the reaction was conducted for 48 h with stirring using a magnetic bar at 500 rpm at room temperature. The resulting product was thoroughly washed several times with ethanol, and then dried at 60 °C under air atmosphere.

5% PDDA (50 μL) solution was added to 0.2 mg mL^{-1} K-doped graphene dispersion (1 mL) with the mild sonication for 40 min. Since the presence of PDDA, the K-doped graphene with streptavidin composites was obtained *via* the electrostatic interaction between negatively charged streptavidin and positively charged K-doped graphene. Detailedly, 20 μg mL^{-1} streptavidin incubated with the PDDA modified K-doped graphene for 2 h at 4 ºC, and centrifuged to remove the unbound substance. Finally, K-doped graphene with streptavidin composites was stored in $Tris^+$ buffer at 4 ºC before use.

Fabrication of the ECL Biosensor.

The GCE was pretreated before modification by polishing its surface with successively finer grades sand papers and then polished to a mirror smoothness with aqueous slurries of alumina powders (average particle diameters: 0.3 μm and 0.05 μm Al_2O_3) on a polishing silk. The GCE was thoroughly rinsed with water and then sonicated in ethanol and ultrapure water in turn. A suspension of K-doped graphene with streptavidin composites (5 μL) was applied to the GCE and dried at room temperature. Then the modified GCE was immersed in the SiO_2@CdS/DNA composites solution to anchor them for an incubation of 60 min at 37 ºC followed by washing with $Tris^+$ buffer. The electrode was then blocked with 2 wt% BSA blocking solution for 2 h at 4 °C to block non-specific binding sites and washed with the $Tris^+$ buffer thoroughly. Finally, the assembled electrodes were allowed for 40 min incubation at 37 ºC in the target protein solution consisting of different concentration TBP. Thereafter, the electrodes were rinsed and then introduced for the respective ECL measurements.

Comparison Experiment.

The GCE was immersed 3mM MPA for 3 h to modify the carboxylic acid group on the surface, following by the activation of EDC and NHS. Then the treated GCE was dipped in the 5% PDDA solution for 40 min, after that

the negatively charged streptavidin was assembled on the GCE *via* the electrostatic interaction. Finally, the pure CdS/DNA and SiO_2@CdS/DNA composites were incubated on the modify GCE respectively by the interaction of biotion and streptavidin.

Characterization of constructed ECL biosensor.

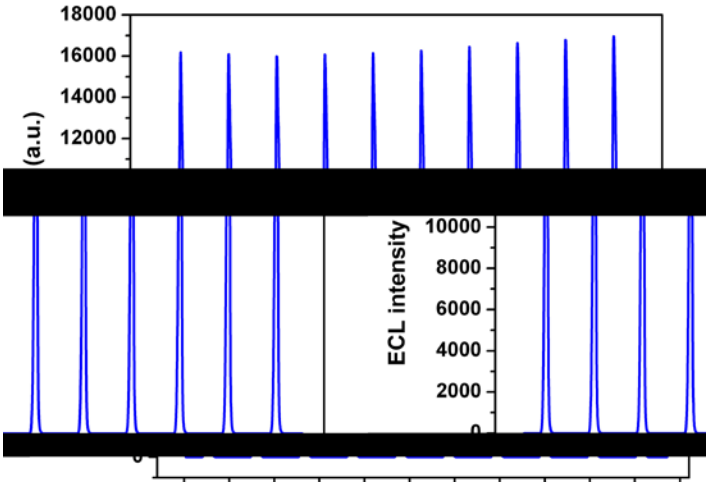

Figure S-2. ECL emission from the K-doped graphene with streptavidin modified GCE anchoring SiO_2@CdS/DNA composites in 0.1M PBS (pH 7.4) containing 0.05M $S_2O_8^{2-}$ under continuous cyclic potential scan for 10 cycles. Scan rate, 100 mVs^{-1}.

References.

1. Y. Shan, J.-J. Xu and H.-Y. Chen, *Chem Commun*, 2009, **47**, 905.
2. J. Qian, C. Zhang, X. Cao and S. Liu, *Anal. Chem.*, 2010, **82**, 6422.
3. X.-R. Li, F.-Y. Kong, J. Liu, J.-J. Xu and H.-Y. Chen, *Adv. Funct. Mater.*, 2012, 10.1002/adfm.201103025.