Reusable Potassium Ion Biosensor based on Electrochemiluminescence Resonance Energy Transfer

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Experimental section

Reagents.

5'-NH_2-$(CH_2)_6$-TTTTTTTTTAAGGTTGGTGTGGTTGGAATTTTTTTT-$(CH_2)_6$-SH-3' (ssDNA 1) and 5'-CCAACCACACCA-3' (ssDNA 2, complementary with the middle part bases of ssDNA 1) were purchased from Shenggong Bioengineering Ltd. Company (Shanghai, China). $HAuCl_4$, bovine serum albumin (BSA), 1-methylimidazol, tri(2-carboxyethyl) phosphine hydrochloride (TCEP), 3-mercaptopropionic acid (MPA), N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO). 50 mM tris-HCl containing 15 mM H_2O_2 (pH 8.0) as a coreactant was used for ECL detection, and 50 mM Tris-HCl buffer containing 20 mM NaCl and 2 mM $MgCl_2$ (pH 7.4) 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 and ECL emission measurements were conducted on a MPI-A multifunctional electrochemical and chemiluminescent analytical system (Remax Electronic Instrument Limited Co., Xi'an, China) at room temperature. The spectral width of the photomultiplier tube (PMT) was 350-650 nm, and the voltage of the PMT was set at -800 V in the process of detection. Both electrochemical and ECL properties were investigated on a three-electrode system with a glassy carbon electrode (GCE, 3mm diameter) as working electrode, a Pt wire and saturated calomel electrode (SCE) served as the counter and reference electrodes. Transmission electron microscopy (TEM) was performed on a JEOL model 2000 instrument operating at 200 kV accelerating voltage. The UV-vis absorption spectra were obtained on a Shimadzu UV-3600 UV-vis-NIR photospectrometer (Shimadzu Co.).

Synthesis of CdS NCs. CdS NCs were prepared according to the literature[S1]. All glassware used in the synthesis procedures were immersed in freshly prepared aqua regia (HNO_3/HCl = 1:3) for 12 h, then washed with ultrapure water, and dried before use. Briefly, $Cd(NO_3)_2 \cdot 4H_2O$ (0.1683 g) was dissolved in 30 mL ultra-pure water, and heated to 70ºC under stirring, then a freshly prepared solution of Na_2S (0.5960 g) in 30 mL ultrapure water was slowly injected and instantly orange-yellow solution was obtained. The reaction was held at 70ºC 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 TEM and UV-vis spectrum. The final solution was rather stable for 2 months when stored in a refrigerator at 4 ℃.

Synthesis of Uncapped Au NPs. AuNPs were prepared through the reduction of $HAuCl_4$ by sodium borohydride ($NaBH_4$) based on the methods reported previously with small improvement[S2]. Briefly, 0.6 mL of ice cold 0.1 M $NaBH_4$ was added to 20 mL aqueous solution containing 2.5×10^{-4} M $HAuCl_4$ under stirring. The solution immediately turned to orange-red color, indicating the formation of gold nanoparticles, and was kept stirring in an ice bath for 10 min. Then, the solution was stirred at room temperature for another 3 h with the color changing from orange-red to wine red. The nearly monodispersed Au NPs were characterized by TEM. The average particle size measured from TEM was 5 ± 1 nm. The prepared AuNPs were stored in brown glass bottle at 4 ℃ for further use.

Preparation of CdS NCs film. The GCE was polished with successively finer grade sand papers and then polished to a mirror smoothness with aqueous slurries of alumina powders (average particle diameters: 0.3 and 0.05 μm Al_2O_3) on a polishing silk before the surface modification. Then the GCE was thoroughly rinsed with water and then sonicated in ethanol and ultrapure water in turn. CdS NC film was achieved by dropping 10 μL of CdS NC solution onto the pretreated surface of GCE and evaporated in air at room temperature. Then, the CdS NCs modified GCE was stored in 50 mM Tris-HCl buffer (pH 7.4) for characterization and further modification.

DNA hybridization. Briefly, ssDNA 1 was activated with 1.5 μL of 10 mM TCEP before use. 50 μL of 0.2 μM ssDNA 1 and 50 μL of 0.22 μM ssDNA 2 were mixed and incubated at 25 ºC for 1 h. The hybridized solution was stored for further use.

Fabrication of the ECL Biosensor. The beforehand prepared CdS NCs film modified GCE was immersed in 100 μL of 50 mM tris-HCl buffer (pH =7.4) containing 3 mM MPA for 1.5 h at 4 ºC for assembly of MPA. Then the GCE was rinsed thoroughly with water and tris-HCl buffer, and the terminal carboxylic acid groups of the MPA/CdS/GCE were activated by immersion in 1.0 mL of 0.10 M 1-methylimidazol-HCl aqueous solution (pH 7.4) containing 20 mg EDC and 10 mg NHS for 2 h at 4ºC. Then the electrode was rinsed with 50 mM Tris-HCl buffer (pH 7.4) to wash off the excess EDC and NHS. After that the resulting linker/MPA/CdS NCs/GCE was soaked in the hybridized DNA solution (100 μL) for 1h at 30ºC. Then the dsDNA/MPA/CdS NCs/GCE was washed thoroughly with 20 mM NaCl + 2 mM $MgCl_2$ + 50 mM tris-HCl buffer (pH 7.4) . Finally, the prepared electrode was soaked in the stable Au colloidal solution (150 μL) containing 20 mM NaCl and 2 mM $MgCl_2$ for 6 h at 37 ºC. Subsequently, the electrode was washed thoroughly with water for 5 min to remove the excess AuNPs, interference ions (Na^+, Mg^{2+}) and ssDNA 2. Then the prepared electrode was stored in 50 mM tris-HCl solution(pH 7.4) for K^+ detection.

Analytical Procedure. The Au NP/ss DNA 1/MPA/CdS NCs/GCE was incubated in 50 mM tris-HCl buffer (pH 8.0) containing different concentrations of K^+ at 25 ºC for 20 min. Then the ECL responses of the electrode were recorded in 50 mM tris-HCl (pH 8.0) containing 15 mM H_2O_2 as a coreactant and the corresponding concentrations of K^+. The voltage of the PMT was set at -800 V. ECL signals related to the K^+ concentrations could be measured.

Figure S-1. ECL spectrum of (a) CdS NCs film and (b) UV-vis absorption spectrum of Au NPs.

Figure S-2. (A) The effect of the immobilization time of Au NPs on the thiol modified DNA-electrode surface on the ECL quenching efficiency. 50 μL of 0.13 μM ssDNA 1, 50 μL of 0.15 μM ssDNA 2, and 10 mM KCl were used for this study. (B) The effect of the immobilization amount of DNA on the CdS NCs-electrode surface on the ECL quenching efficiency. The concentration of the ssDNA 1 ranged from 0.06-0.4 μM, meanwhile, the amount of ssDNA 2 made the corresponding changes to ensure the complete hybridization. The time of AuNPs immobilization was 6h, and 10mM KCl was used for this study.

Reference

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

S2. A. Gole, C. J. Murphy, *Chem. Mat.*, 2004, **16**, 3633-3640.