Supplementary Information

Electrochemiluminescence Quenching by CdTe Quantum Dots through Energy Scavenging for Ultrasensitive Detection of Antigen

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Experimental section

Materials. Mouse IgG (Ag) and goat anti-mouse IgG (Ab) were obtained from Boster Biological Technology, Ltd. (Wuhan, China). Bovine serum albumin (BSA), 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). Tellurium powder (99.99%), $CdCl_2$•$2.5H_2O$ (99.0%) and $NaBH_4$ (96%) were obtained from Tianjin Chemical Reagent Plant (Tianjin, China). Other chemicals were of analytical reagent grade and used as received. 0.1 M phosphate buffer solution (PBS, pH 8.3) containing 0.05 M $K_2S_2O_8$ was used for ECL detection, and 0.01 M PBS (pH 7.4) for the preparation of the Ag, Ab and BSA stock solutions. All aqueous solutions were prepared using ultra-pure water (Milli-Q, Millipore).

Apparatus. The cyclic ECL curves were carried out on a MPI-E multifunctional electrochemical and chemiluminescent analytical system (Remax Electronic Instrument Limited Co., Xi'an, China) by cyclic potential scan from 0 V to -1.35 V in 0.1 M pH 8.3 PBS containing 0.05 M $K_2S_2O_8$ as a coreactant. A glassy carbon electrode (GCE, 3 mm diameter), a Pt wire and a saturated calomel electrode (SCE) served as the working, counter and reference electrodes, respectively. The observation window was placed in front of the photomultiplier tube biased at 500 V. Transmission electron microscopy (TEM) and selected area electron diffraction (SAED) were performed with 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.). Photoluminescence spectra were recorded on a RF-5301PC spectrophotometer (Shimadzu Co).

Synthesis of CdS:Mn NCs. Briefly, 0.1683 g of $Cd(NO_3)_2$•$4H_2O$ and 0.0134 g of $Mn(CH_3COO)_2$•$4H_2O$ were successively dissolved in 30 ml ultra-pure water. The reactant mixture was heated to 70ºC under stirring, to which a freshly prepared solution of Na_2S in 30 ml ultra-pure water was added and instantly orange-yellow precipitates were obtained. The reaction was held at 70ºC for 3 h with continuous refluxing. The final products of precipitates were centrifuged and washed thoroughly with absolute ethanol two times, followed by washing with ultra-pure water to get rid of any Mn^{2+} and other ions remaining outside the clusters. The average size of the CdS:Mn NCs was about 5 nm. In control experiment, the 5 nm undoped CdS NCs were synthesized by the same method except that the $Mn(CH_3COO)_2$•$4H_2O$ was not added.

Synthesis and activation of CdTe QDs. Briefly, 0.069g of $CdCl_2$•$2.5H_2O$ was dissolved in 25 mL of water, and 55 μL of MPA was added followed by deaeration with N_2 for 30min. Next, oxygen-free NaHTe solution, which was freshly prepared from 0.016 g tellurium powder and 0.3 g $NaBH_4$ in 25 mL of water at 60℃, was injected into the above solution under vigorous stirring. Herein the molar ratio of Cd^{2+}/MPA/HTe^- was fixed at 1:2:0.41. The solution was then refluxed at 100℃ for 3h. The reaction mixture was purified by precipitation in absolute ethanol. Finally, the desired CdTe QDs were obtained.

For the activation of carboxylic acid group on the surface of CdTe QDs, 1.0 mg CdTe QDs was dispersed in 1 mL 0.1 M 1-methylimidazol aqueous solution (pH 7.4) containing 25 mg EDC and 12 mg NHS and activated for 1.5 h at room temperature. The activated CdTe QDs were separated by centrifugation and washed with water and 0.01M PBS buffer (pH 7.4) alternatively for several times followed by redispersion in 1mL 0.01M PBS buffer (pH 7.4). The activated CdTe QDs were denoted A-CdTe QDs. For control experiment, the CdTe QDs were dispersed in 1 mL 0.1 M 1-methylimidazol aqueous solution (pH 7.4) containing 25 mg EDC and activated for 1.5 h at room temperature. Influence of free NHS on photoluminescence of CdTe QDs was also studied by dispersing 1.0 mg CdTe QDs in 1 mL 0.1 M 1-methylimidazol aqueous solution (pH 7.4) containing 12 mg NHS for 1.5 h at room temperature.

Preparation of colloidal Ab/CdTe Conjugates. 100 μL 1 mg•mL^{-1} A-CdTe QDs in 0.01 M PBS (pH 7.4) was mixed with 150 μL 50 μg•mL^{-1} Ab, followed by incubation at 4℃ for 12 h. For the

blocking of non-specific binding sites of A-CdTe QDs, 100 μL 2 wt% BSA was added into the A-CdTe-Ab conjugates solution and incubated at 4℃ for another 4 h. The resulting conjugates were separated by centrifugation and redispersed in 350 μL 0.01M PBS buffer (pH 7.4). The A-CdTe-Ab-BSA conjugates are denoted Ab/CdTe.

Preparation of ECL immunosensor. For the CdS:Mn NCs thin-film preparation, the obtained NCs powders were ultrasonically dispersed in water to obtain a colloidal CdS:Mn NCs solution of 1mg•mL^{-1}, which was kept in a refrigerator at 4 ºC. 10 μL of CdS:Mn NCs solution was drop-cast on the cleaned GCE and dried in air at room temperature, as a result, an even NCs film was obtained. The CdS:Mn NCs film on GCE was immersed in 1.0 mL of 0.1 M NaCl + 0.1 M tris-HCl buffer (pH 7.4) containing 3 mM MPA for 5 h at 4ºC. After rinsed thoroughly with water and tris-HCl buffer, the terminal carboxylic acid groups of the MPA-CdS:Mn/GCE were activated by immersion in 1.0 mL 0.1 M 1-methylimidazol aqueous solution (pH 7.4) containing 20 mg EDC and 10 mg NHS for 1 h at room temperature. Then the electrode was rinsed with 0.1 M tris-HCl buffer (pH 7.4) to wash off the excess EDC and NHS. Subsequently, the resulting electrode was soaked in 40 μL of 50 μg•mL^{-1} Ab at 4 °C for at least 12 h. Finally, 40 μL of 2 wt% BSA was dropped on the electrode at 4°C for 2 h to block non-specific binding sites of CdS:Mn NCs film. The resulting Ab-BSA-MPA-CdS:Mn/GCE is denoted Ab/CdS:Mn. The CdS NCs film on GCE were prepared with the same method just using 5 nm CdS NCs instead of 5 nm CdS:Mn NCs. To study the influence of free NHS on ECL emission from CdS:Mn NCs film, the MPA-modified NCs film was also immersed in 1.0 mL 0.1 M 1-methylimidazol aqueous solution (pH 7.4) containing 10 mg NHS and activated for 1 h at room temperature followed by through washing with water and then underwent ECL detection.

ECL detection. The Ab/CdS:Mn was incubated in 40 μL mouse IgG for 1 h at room temperature for the formation of immuno-complex, followed by thoroughly washing with 0.01 M PBS (pH 7.4) to remove unbound Ag. The Ag-bound Ab/CdS:Mn is denoted Ag-Ab/CdS:Mn. For the further binding of Ab/CdTe, the Ag-Ab/CdS:Mn were incubated in 40 μL of Ab/CdTe solution at room temperature for 1 h. For direct adsorption of A-CdTe QDs, the CdS:Mn/GCE or CdS/GCE were incubated in 40 μL of A-CdTe NCs solution (with the same concentration of Ab/CdTe conjugates solution) for 1 h at room temperature, followed by thorough washing with water and 0.01 M PBS (pH 7.4). The electrodes in each step were in contact with 0.1 M PBS (pH 8.3) containing 0.05 M $K_2S_2O_8$ and scanned from 0 to -1.35 V. ECL signals related to the mouse IgG concentrations could be measured.

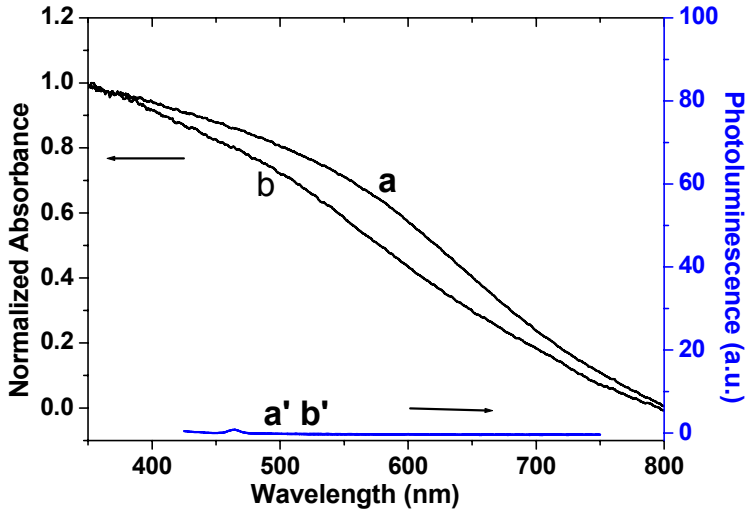

Figure S1. Normalized UV-Vis absorption and photoluminescence spectra of NHS-activated CdTe QDs(a,a') and EDC-activated CdTe QDs(b, b'). Photoluminescence spectra were recorded with an excitation at 400 nm.

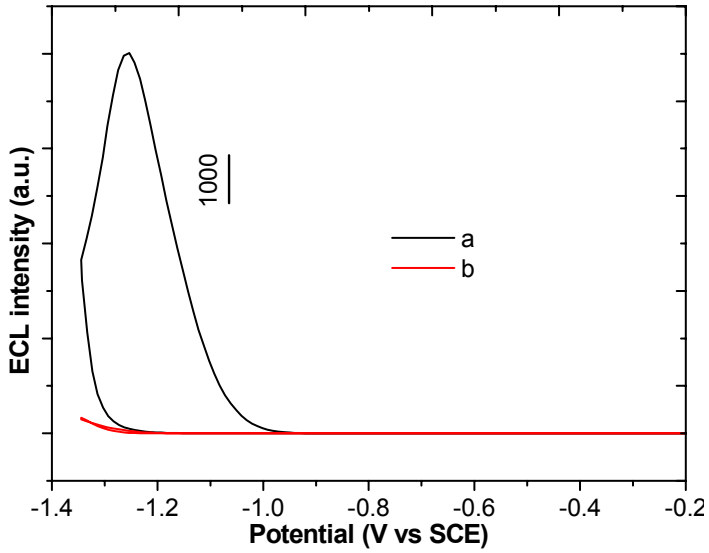

Figure S2. ECL quenching of CdS NCs film by A-CdTe QDs. (a) CdS film; (b) CdS film + A-CdTe QDs. ECL detection buffer: pH 8.3 0.1M PBS+0.05M $K_2S_2O_8$.

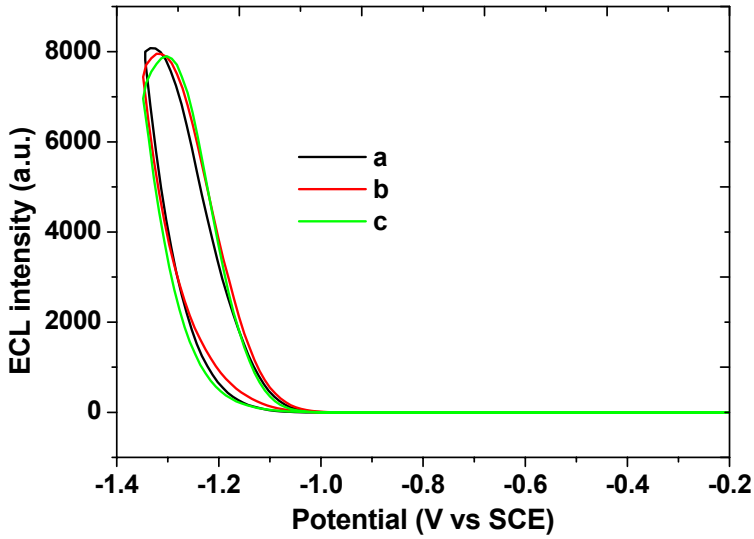

Figure S3. Cyclic ECL curves of CdS:Mn film (a), MPA modified CdS:Mn film after immersion in 1.0 mL 0.1 M 1-methylibmidazol aqueous solution (pH 7.4) containing 10 mg NHS for 1 h at room temperature (b) and MPA modified CdS:Mn film after activation in 1.0 mL 0.1 M 1-methylimidazol aqueous solution (pH 7.4) containing 20 mg EDC and 10 mg NHS for 1 h at room temperature (c). ECL detection buffer: pH 8.3 0.1M PBS+0.05M $K_2S_2O_8$.