

Supplementary Information

Opto-Magnetic Interaction between Electrochemiluminescent CdS:Mn Film and Fe₃O₄ Nanoparticles and Its application to Immunosensing

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MATERIALS AND METHODS

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). Other chemicals were of analytical reagent grade. 0.1 M phosphate buffer solution (PB, pH 8.3) containing 0.05 M K₂S₂O₈ was used for ECL detection, and 0.01 M PB (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 ECL-time curves were recorded 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 PB containing 0.05 M K₂S₂O₈ 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 was 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.). The doped content of Mn in CdS NCs was determined by X-ray photoelectron spectroscopy (XPS, ESCALAB 250). Magnetic measurements were performed using a Superconducting Quantum Interference Device (SQUID) magnetometer (Quantum

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Design Co., Ltd., USA).

Synthesis of CdS:Mn and CdS NCs. 1.34 atom% manganese doped CdS NCs were prepared in the way we have reported previously.^{S1} Briefly, 0.1683 g of Cd(NO₃)₂•4H₂O and 0.0134 g of Mn(CH₃COO)₂•4H₂O were successively dissolved in 30 ml ultra-pure water. The reactant mixture was heated to 70°C under stirring, to which a freshly prepared solution of Na₂S in 30 ml ultra-pure water was added and instantly orange-yellow precipitates were obtained. The reaction was held at 70°C 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 Mn²⁺ and other ions remaining outside the clusters. The average size of the CdS:Mn NCs was about 5 nm, as indicated by transmission electron microscopy. (Fig. S1a) The 5 nm undoped CdS NCs were also synthesized by the same method. (Fig. S1b) For control experiments, 2.76 atom% manganese doped CdS nanocrystals were synthesized in the same way as that of 1.34 atom% manganese doped NCs except that 0.1592g of Cd(NO₃)₂•4H₂O and 0.0206 g of Mn(CH₃COO)₂•4H₂O were used. The doping content of manganese was determined by XPS. (Fig. S2)

Preparation of Fe₃O₄ nanoparticles and CdTe NCs. Fe₃O₄ nanoparticles were synthesized using a modified protocol.^{S2} Volumes of 0.425 mL of 12.1 M HCl and 12.5 mL of purified, deoxygenated water were mixed and bubbled by nitrogen gas for at least 30 min. Subsequently, 4.34 g of FeCl₃•6H₂O and 1.57 g of FeCl₂•4H₂O were successively dissolved in the solution with stirring and gas bubbling. The resulting solution was added dropwise into 125 mL of 1.5 M NaOH solution under vigorous stirring for 30 min at room temperature in a nitrogen gas atmosphere. The formed black Fe₃O₄ colloidal particles were separated by high speed centrifugation and further washed three times with ultra-pure water. The as-prepared Fe₃O₄ nanoparticles were dried in vacuum at room temperature for further use and characterization. CdTe NCs was synthesized following a modified method reported by Yang et al.^{S3} Briefly, 0.069g of CdCl₂•2.5H₂O was dissolved in 25 mL of water, and 55 μL of MPA was added followed by deaeration with N₂ for 30min. Next, oxygen-free NaHTe solution, which was freshly prepared from tellurium powder and NaBH₄ in water at 60°C, was injected into the above solution under vigorous stirring. The solution was then heated at 100°C for 3h. Finally, MPA capped CdTe NCs were obtained in aqueous media.

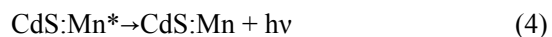
Preparation of colloidal Ab/Fe₃O₄ and Ab/CdTe Conjugates. 300 μL of 1 mg•mL⁻¹ 10 nm Fe₃O₄ nanoparticles in 0.01 M PB (pH 7.4) was mixed with 300 μL of 50 μg•mL⁻¹ Ab, followed by incubation at room temperature for 2 h. For the blocking of non-specific binding sites of Fe₃O₄ nanoparticles, 100 μL of

2 wt% BSA was added into the Fe₃O₄-Ab conjugates solution and incubated at room temperature for another 1 h. The resulting conjugates are denoted Ab/Fe₃O₄. Ab/CdTe conjugates were prepared by the same method as that of Ab/Fe₃O₄ for control experiment except that activated CdTe NCs were used. For the activation of carboxylic acid group on the surface of CdTe NCs, 1 mg MPA capped CdTe NCs was dispersed in 1 mL of 0.1 M 1-methylimidazol aqueous solution (pH 7.4) containing 20 mg EDC and 10 mg NHS and activated for 1.5 h at room temperature. Subsequently, the activated CdTe NCs were washed twice with 0.01M PB solution (pH 7.4) and redispersed in this PB solution.

Modification of CdS:Mn NCs film. For the 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 °C. 10 μL of CdS:Mn NCs solution was drop-cast on the cleaned GCE and dried in air at room temperature, as a result, 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 6 h at 4°C for the assembly of MPA. 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 of 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 16 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 (and 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, and the resulting Ab (and BSA)-MPA-CdS/GCE is denoted Ab-CdS. For the preparation of MPA and BSA modified CdS:Mn NCs film, 50 μL of 2 wt% BSA was dropped on the MPA-modified CdS:Mn NCs film at room temperature for 1 h, followed by thorough washing with 0.01 M PB (pH 7.4) to remove un-adsorbed BSA.

In the presence of coreactant S₂O₈²⁻ ions, CdS:Mn NCs generated strong and stable ECL emission via the following ECL process:





Assembly of Fe₃O₄ nanoparticles to CdS:Mn NCs film. The Ab-CdS:Mn or Ab-CdS was incubated in 40 μL mouse IgG for 1 h at room temperature for the formation of immuno-complex, followed by thorough washing with 0.01 M PB (pH 7.4) to remove unbound Ag. For the further binding of Ab/Fe₃O₄, the electrodes were incubated in 40 μL of Ab/Fe₃O₄ solution at room temperature for 1 h. For assembly of Fe₃O₄ nanoparticles by adsorption, 40 μL of 1 $\text{mg}\cdot\text{mL}^{-1}$ Fe₃O₄ nanoparticles in 0.01 M PB (pH 7.4) was dropped on the naked CdS:Mn NCs film, MPA-modified and MPA-BSA-modified film at room temperature for 1h, respectively. ECL detection was performed for the electrode in each step of assembly by immersing in 0.1 M PB (pH 8.3) containing 0.05 M K₂S₂O₈ and scanning from 0 to -1.35 V. ECL signals related to the mouse IgG concentrations could be measured.

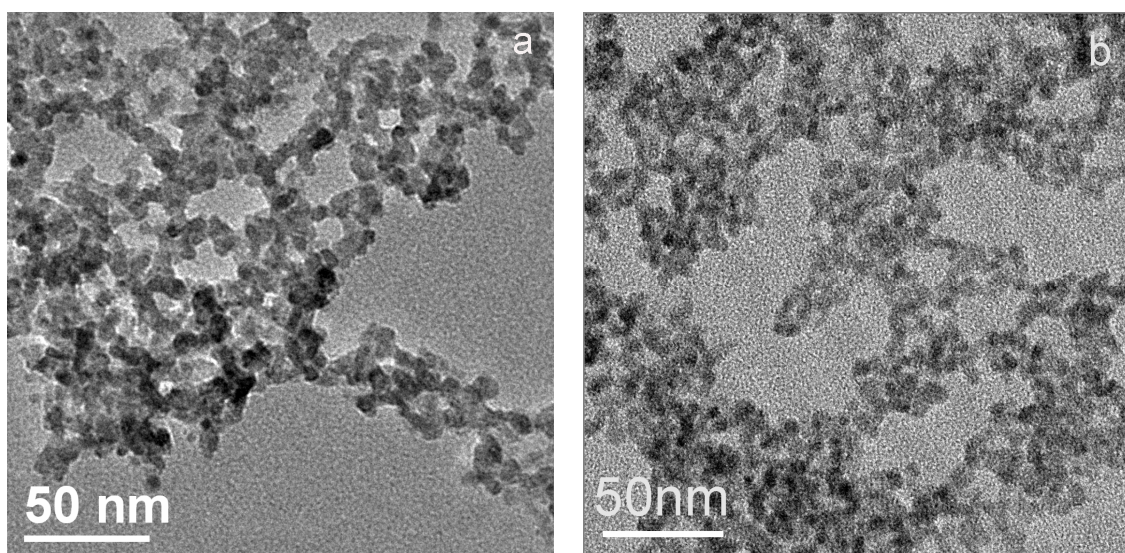


Figure S1. TEM images of (a) 1.34 atom% Mn²⁺ doped CdS:Mn NCs, (b) Undoped CdS NCs.

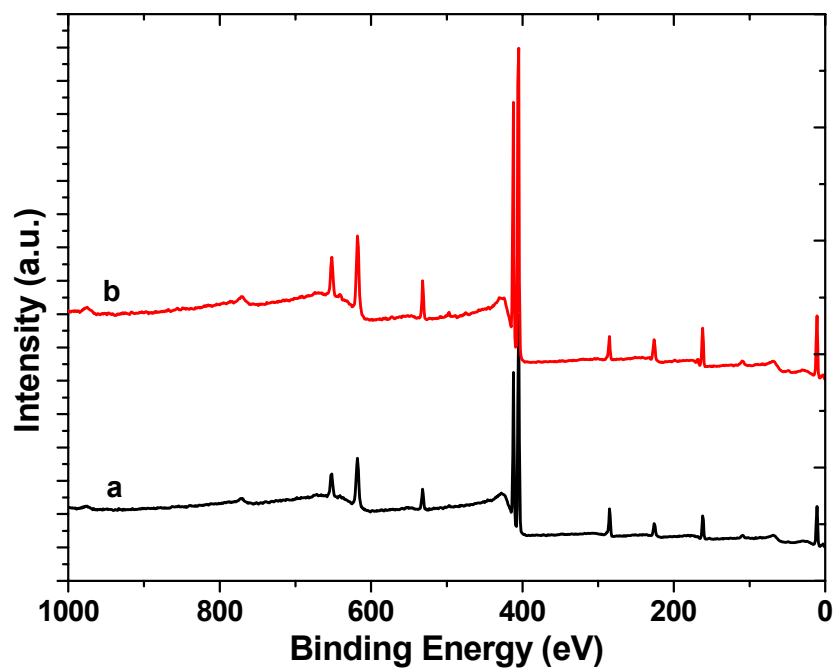
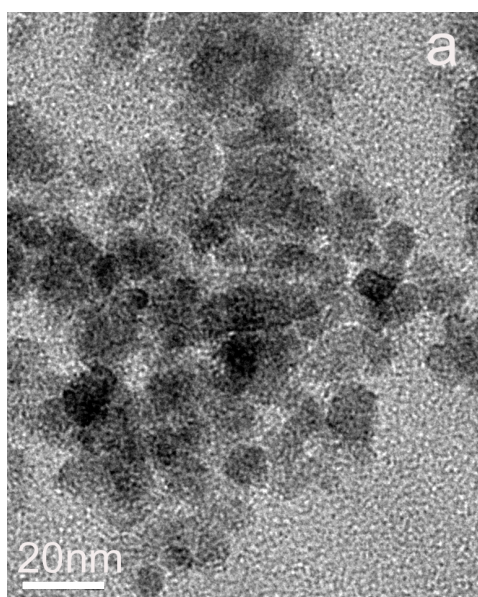


Figure S2. XPS survey scans of the 1.34 atom% (a) and 2.76 atom% (b) manganese doped CdS NCs.



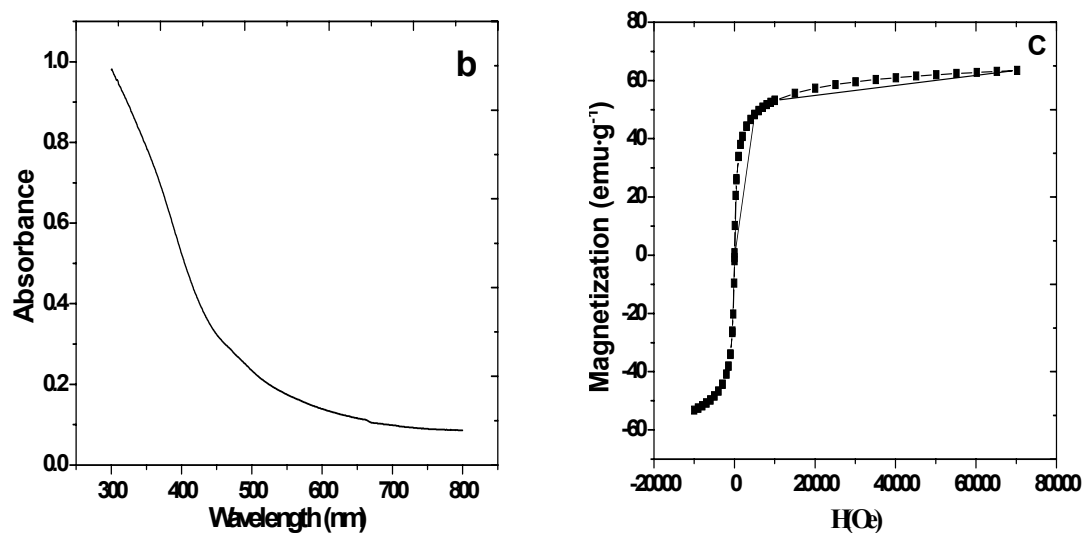


Figure S3. (a) TEM image of Fe₃O₄ nanoparticles. (b) UV-Vis absorption spectrum of Fe₃O₄ nanoparticles. (c) Magnetization curve of Fe₃O₄ nanoparticles at 300K.

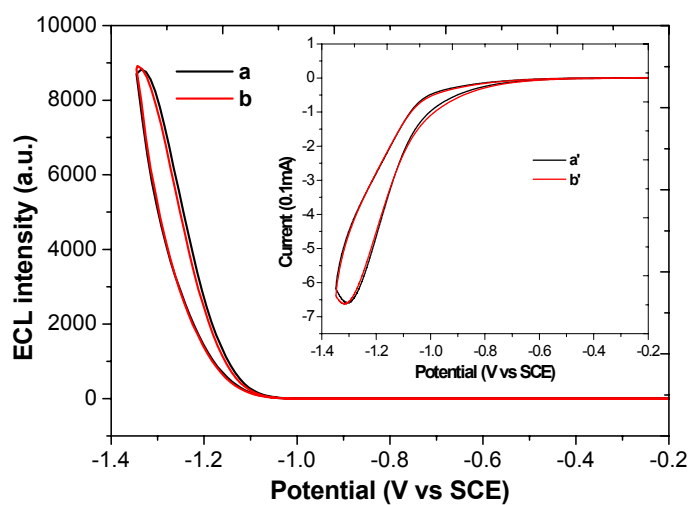


Figure S4. Cyclic ECL curves of CdS:Mn NCs film on GCE in ECL detection buffer before (a) and after (b) deoxygenization. Inset: the corresponding cyclic voltammograms. a' to a; b' to b. Scan rate: 0.1V s⁻¹. ECL detection buffer: pH 8.3 0.1M PB containing 0.05M K₂S₂O₈. The photomultiplier tube is biased at 500V.

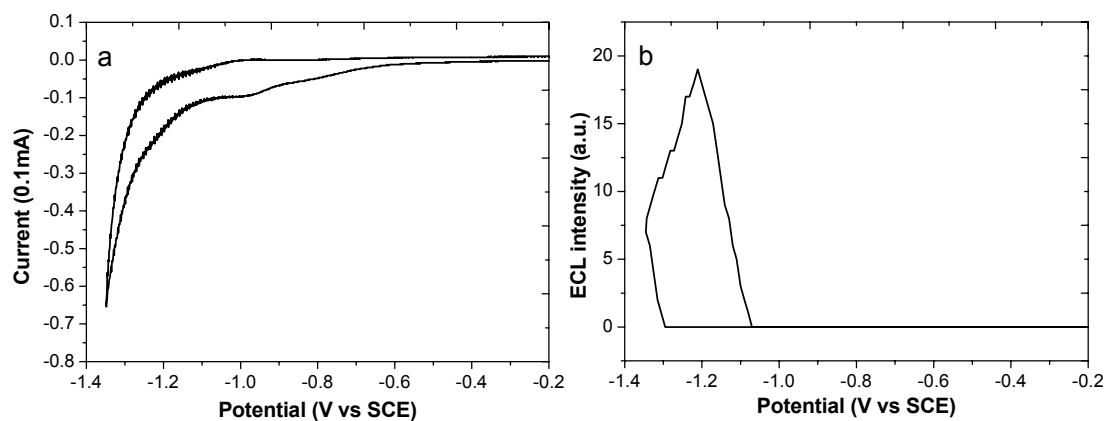


Figure S5. Cyclic voltammogram (a) and cyclic ECL curve (b) of CdS:Mn film on GCE in pH 8.3 0.1M PB. scan rate: 0.1V s^{-1} . The photomultiplier tube is biased at 500V.

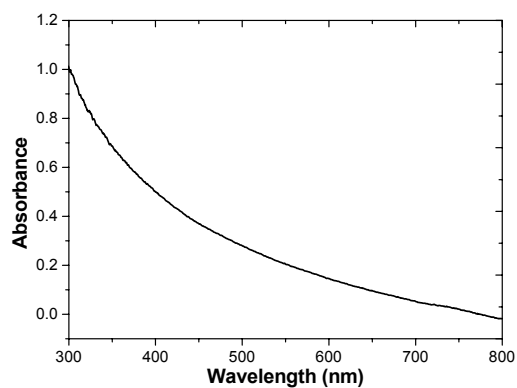
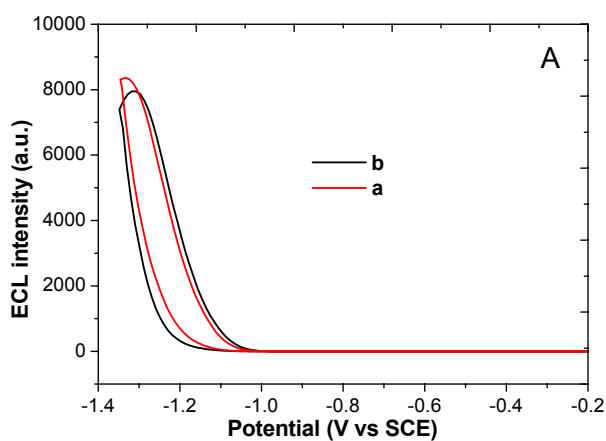


Figure S6. Normalized UV-Vis absorption spectrum of the blackish CdTe NCs



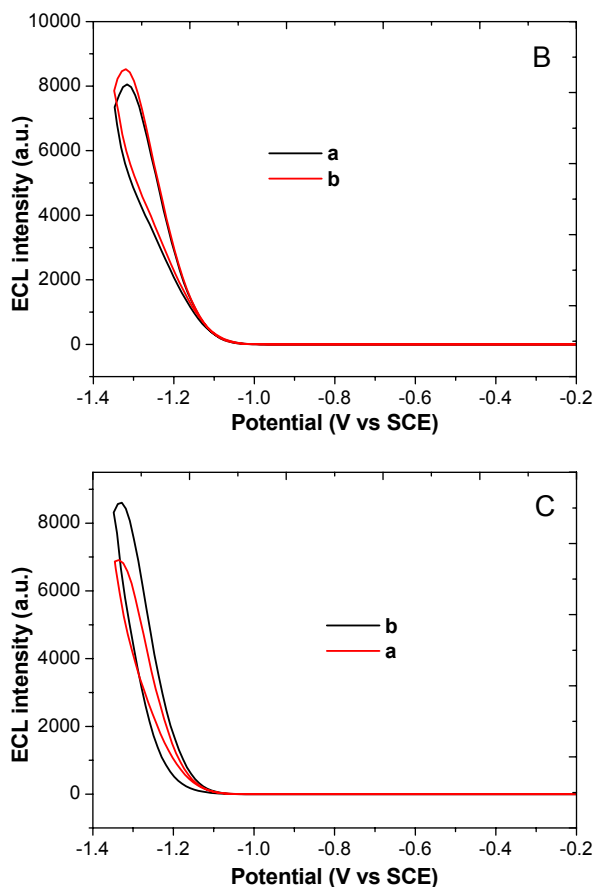


Figure S7. Effect of adsorbed Fe_3O_4 nanoparticles on the ECL emission from the naked film, MPA-modified CdS:Mn film, and the MPA and BSA modified CdS:Mn film. (A) (a) naked CdS:Mn film; (b) naked CdS:Mn film + Fe_3O_4 nanoparticles. (B) (a) MPA modified CdS:Mn film; (b) MPA modified CdS:Mn film + Fe_3O_4 nanoparticles. (C) (a) MPA and BSA modified CdS:Mn film; (b) MPA and BSA modified CdS:Mn film + Fe_3O_4 nanoparticles. ECL detection buffer: 0.1 M pH 8.3 PB containing 0.05 M $\text{K}_2\text{S}_2\text{O}_8$. The photomultiplier tube is biased at 500V. Compared ECL intensity from the MPA modified CdS:Mn film with that from the MPA and BSA modified film, an ECL intensity drop was observed due to the blocking of ECL emission reaction by BSA protein^{S4}, which also occurred in the assembly of capturing antibody to the CdS:Mn and undoped CdS NCs film.

References

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- S4. G. Jie, J. Zhang, D. Wang, C. Cheng, H.-Y. Chen, J. J. Zhu, *Anal. Chem.* 2008, **80**, 4033-4039