Supporting Information

CdTe quantum dots functionalized silica nanospheres as labels for ultrasensitive detection of protein

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Experimental Details

**Chemicals and Reagents.** 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-Hydroxysuccinimide (NHS) and (3-aminopropyl)-triethoxysilane 99% (APTS) were obtained from Sigma-Aldrich. 25% glutaraldehyde in water was from Sinopharm Chemical Regent Co., Ltd. (Shanghai, China). α-Fetoprotein (AFP) antibody and antigen were obtained from Sunshine Biotechnology Co. Ltd. (Nanjing, China). Bismuth nitrate pentahydrate was purchased from Kermel Chemical Reagents Development Centre (Tianjin, China). CdTe QDs with a diameter of 3.2 nm and UV-Vis absorption at 547 nm was a gift from Dr. Chunwang Ge (Department of Chemistry, Nanjing University, Nanjing, China). The surface of the QDs was covered with carboxyl group by using mercaptoacetic acid as stabilizers.\textsuperscript{S1} 0.05 M phosphate buffer at pH 7.0 was prepared by mixing the stock standard solutions of Na\textsubscript{2}HPO\textsubscript{4} and NaH\textsubscript{2}PO\textsubscript{4}. All other chemicals were analytical grade and were used as received. All stock solutions were prepared by twice-distilled water.

**Synthesis of AFP antibody-Fe\textsubscript{3}O\textsubscript{4} magnetic nanoparticle conjugates (MB/Ab\textsubscript{1}).** The Fe\textsubscript{3}O\textsubscript{4} magnetic nanoparticles was prepared by a conventional coprecipitation method.\textsuperscript{S2} Briefly, 5.2 g of FeCl\textsubscript{3}, 2.0 g of FeCl\textsubscript{2}, and 0.85 mL of 12 M HCl were mixed in 25 mL water under N\textsubscript{2} protection. Then the mixture was added dropwisely into 250 mL 1.5 M NaOH solution under vigorous stirring. After washed thoroughly with water and separated under the magnetic field, the deposit was dispersed in ethanol to obtain the Fe\textsubscript{3}O\textsubscript{4} magnetic beads. The bioconjugation of Fe\textsubscript{3}O\textsubscript{4} magnetic
beads with AFP antibody was included the follow three steps as illustrated in this Supporting Information in Scheme S1.

(a) Preparation of amino group-functionalized Fe₃O₄ MBs. The Fe₃O₄ magnetic beads were functioned with amino group by addition of 0.4 mL APTS into 150 mL magnetic beads suspension in ethanol containing 1 mL water. After stirring at 37 °C for 7 hours, the particles were centrifuged and washed with ethanol for 5 times and redispersed in 50 mL ethanol to give the amino-terminated Fe₃O₄ MBs.

(b) Preparation of glutaraldehyde ester-functionalized Fe₃O₄ MBs. The amino groups of Fe₃O₄ MBs were reacted with glutaraldehyde by adding 100 μL glutaraldehyde solution (3%) into 50 mL APTS (a silane coupling agent) modified Fe₃O₄ magnetic beads suspension. Then the mixture was stirred at 37 °C for 3 hours. The excess glutaraldehyde was removed by external magnetic force and separation, and the particles were washed with twice-distilled water for three times. The Fe₃O₄ MBs were finally redispersed in water to 2 mL.

(c) Preparation of AFP antibody-Fe₃O₄ magnetic nanoparticle conjugates. The glutaraldehyde modified Fe₃O₄ magnetic beads were added into 2 mL of AFP antibody (model protein, the first antibody donated Ab₁) with a photometric concentration (280 nm) of 12 μg/mL and stirred overnight. Excess AFP antibody was removed by successive washing of the Fe₃O₄ MBs conjugates with an external magnetic force. The AFP antibody conjugated Fe₃O₄ MBs were further incubated in 1 wt.% BSA solution for 30 min to block nonspecific binding sites of the Fe₃O₄ MBs. Excess BSA were removed by successive washing of the Fe₃O₄ MBs with external
magnetic force. The deposit was dispersed in water to final volume of 4 mL to obtain AFP antibody- \(\text{Fe}_3\text{O}_4\) magnetic nanoparticle conjugates (MB/Ab\(_1\)) and kept at 4 °C for follow use.

**Synthesis of antibody/QDs-coated SiO\(_2\) nanosphere labels (Si/QD/Ab\(_2\)).** The SiO\(_2\) nanosphere was fabricated by seed-growth methods. First of all, we obtained the preliminary seeds for the particle growth. 80 mL ethanol, 4.85 mL H\(_2\)O and 3.6 mL NH\(_3\).H\(_2\)O were respectively added to 250 mL flask and heated gradually to 50 °C under continuous fierce stirring. Then the mixed solution containing 3.1 mL tetraethoxysilane (TEOS) and 8 mL ethanol were added to the above solution quickly. During the 5 h reaction process, the mixed solution temperature was kept at 50 °C, then the colloids suspension was obtained. Secondly, 10 mL of the above colloids suspension were mixing with 70 mL ethanol, 13 mL H\(_2\)O and 7.5 mL NH\(_3\).H\(_2\)O in a 250 mL flask. And then a mixture solution of 1 mL TEOS and 10 mL ethanol were dropped into the flask slowly followed continous stiring for 5 h. Repeatitation this step for four times (total added 4 mL of TEOS), then it was centrifugated, washed with ethanol four times and dried under vacuum, the grown SiO\(_2\) nanoparticles with a diameter of 200 ± 3.0 nm determined by a transmission electron microscopy (TEM, JEM-2100, Jeol, Japan, at a accelerate voltage of 200 kV) was obtained for the follow use. The bioconjugation of antibody/QDs-coated SiO\(_2\) nanosphere labels was included the follow three steps as illustrated in Scheme 1.

**a) Preparation of amino group-functionalized SiO\(_2\) nanosphere.** 0.022 g SiO\(_2\) nanospheres were dispersed in 2 mL ethanol and treated with 0.4 mL APTS, which
reacted with the surface silanol groups to produce the silica surface functionalized with -NH₂ groups. Surging for 6 h and then centrifugation, washing with ethanol four times, the amino-functionalized nanoparticles were obtained.

(b) Preparation of QDs-coated SiO₂ nanosphere. The surface amino-functionalized silica nanoparticles were dispersed in the mixture of 1 mL 3-mercaptopropionic acid coated CdTe QDs and 1 mL 20 mg/mL EDC. The solution was stirred at 4 °C for 12 h. Unbound QDs (an orange color solution) was removed by successively centrifugation and washing the SiO₂ nanoparticles with PBS. After that the CdTe QDs coated silica nanosphere with the same orange color as CdTe QDs itself was obtained as show in Fig. S2A and dispersed in water to final volume of 1 mL for the follow use.

c) Preparation of anti-AFP/QDs-coated SiO₂ nanosphere conjugates. To generate QDs-coated silica nanosphere immunological labels, 1 mL above QDs-coated SiO₂ suspension was mixed with 1 mL 12 μg/mL AFP secondary antibody solution followed addition of 100 μL 20 mg/mL EDC and 100 μL 10 mg/mL NHS. After 2 hours incubation at room temperature, the free AFP antibody were removed by centrifugation and washing with water. The deposit was collected and diluted with water to 1 mL to obtain anti-AFP/QDs-coated SiO₂ labels (Si/QD/Ab₂) and stored at 4 °C for follow use.

Sandwich immunoassay with Si/QD/Ab₂ as label. The sandwich immunoassay process was showed in Scheme 2. 40 μL of MB/Ab₁ suspension was mixed with 40 μL antigen solution with different concentrations and incubated in the well at 37 °C for 30 min. The wells were washed with water for 3 times with external magnetic force holding the antigen-MBA conjugates (Ag-MB/Ab₁). After that, 40 μL
Si/QD/Ab₂ suspension was added into these wells, and incubated at 37 °C for 45 min to form MB/Ab₁-Ag-Si/QD/Ab₂ sandwich immunocomplex. And then the wells were washed with water for 3 times with external magnetic force holding the magnetic beads. 20 μL 0.05M H₂SO₄ solutions in water were added into above wells to release cadmium ions from the captured QDs for electrochemical analysis.

**Fabrication of bismuth film electrodes.** The glassy carbon electrodes (GCE) with a geometric area of 7.06 mm² were polished before each experiment with 1.0 and 0.3 μm α-alumina slurry, respectively, rinsed thoroughly with doubly distilled water between each polishing step, then sonicated in 1:1 ethanol/water and doubly distilled water successively, and allowed to dry at room temperature. Bismuth film was electrodeposited on the surface of glassy carbon electrode for 120 s by using a deposition potential of -1.0 V (vs. SCE) under stirring. The electrolyte was a bismuth nitrate solution in acetate with a final pH of 2.0 and Bi(III) ion concentration of 1.25 mg/mL.

**Spectroscopic analysis and protein activity measurements.** For Fourier Transform Infrared spectra (FT-IR) analysis, MB/Ab₁ and sandwich immune conjugates of MB/Ab₁-Ag-Si/QD/Ab₂ suspension were cast on a glass slice and then stripped off after dried in air, respectively. The FT-IR spectra were recorded with a Nicolet 380 FT-IR spectrometer (ThermoFisher, USA) in reflection mode. 32 scans were collected and averaged for each spectrum. UV-Vis spectrum was performed with a UV-2450 spectrophotometer (Shimadzu, Japan).

Fluorescence microscopy images were performed with an Olympus IX71 Inverted Optical Microscope (Olympus, Japan). Each sample was dropped on the glass slide
and evaporated at the room temperature overnight.

**Stripping voltammetric analysis.** The MB/Ab1-Ag-Si/QD/Ab2 were dissolved by 20 $\mu$L H$_2$SO$_4$ (0.05 M) solution. The solution was transferred to 3 mL 0.05 M phosphate buffer at pH 7.0, the amount of dissolved Cd ion was detected by electrochemical stripping techniques. The square wave voltammetric analysis (SWV) was performed with CHI 830 electrochemical workstation (CH Instrument Co. Shanghai). The conventional three-electrode system was applied for running the SWV consisted of a BFE, a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference against which all potentials were measured.
Scheme S1. Preparation of Mb/Ab₁
Fig. S1 the energy dispersive x-ray spectrometric analysis (JEOL JSM 5900LV equipped with Oxford Links ISIS) of QDs coated silica nanoparticles. The Cd peak suggested the presence of CdTe QDs on the silica spheres’ surface.
The coating of CdTe QDs loaded on silica nanospheres can be confirmed by (A) the color change (from white to bisque) of the silica nanosphere before and after the CdTe QDs loaded on silica nanospheres. (B) a sharp and strong photoluminescence emission peak at 524 nm when using $\lambda_{\text{ex}}=300$ nm.

Fig. S2(A) the color change of silica nanoparticles. (B) photoluminescence emission peak at 524 nm when using $\lambda_{\text{ex}}=300$ nm.
Fig. S3 Characterization of Si/QD/Ab$_2$. UV-vis spectra of Si/QD/Ab$_2$. The strong absorbance at 288 nm was attributed to the specific absorbance of protein, while the weak absorbance band at 523 nm caused by the absorption of CdTe QDs itself.
Fig. S4 The TEM images of silica spheres (a,b), CdTe coated silica spheres (c,d).
**Fig.S5** FT-IR reflective spectra of MBs (1a) and MB/Ab1 (1b).

**The formation of MB/Ab1 can be confirmed by FT-IR measurement.** It is well known that the shapes of the infrared absorption bands of amylose aether bond in AFP molecule can provide detailed information on the secondary structure of the glycoprotein. The addition absorption bands at 1070 cm\(^{-1}\) comparing with the MBs without protein coating was attributed to the amylose aether bond in the MBAb\(_1\), which confirm the coating of AFP antibody on Fe\(_3\)O\(_4\) nanoparticles.
Reference
