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# Hedgehog-like $Bi_2S_3$ nanostructures: novel composite soft template route to synthesis and sensitive electrochemical immunoassay of liver cancer biomarker

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## Experimental

### Materials and Reagents

Alpha-fetoprotein (AFP) ELISA kit was purchased from CanAg Diagnostics. The AFP ELISA kit contains a series of AFP standard solutions at concentration of 0-500 ng/mL, HRP-labeled AFP monoclonal antibodies and biotinylated AFP antibodies (1 μg/mL). Streptavidin was purchased from Sigma, and thionine was purchased from Acros Organics. Bismuth nitrate (Bi $(NO_3)_3$), CTAB ($C_{16}H_{33}$ $(CH_3)$ $_3NBr$), trimellitic acid ($C_9H_6O_6$), thiourea ($CH_4N_2S$), anhydrous ethanol ($C_2H_6O$), acetone ($C_2H_6O$), hydrogen peroxide ($H_2O_2$), potassium chloride (KCl), disodium hydrogen phosphate ($Na_2HPO_4$), sodium dihydrogen phosphate ($NaH_2PO_4$), potassium ferricyanide ($K_3Fe(CN)_6$), and potassium ferrocyanide ($K_4[Fe(CN)_6]_3 \cdot H_2O$) were purchased from Sinopharm Chemical Reagent Co., Ltd. Different pH values of 0.1 mol/L phosphate buffer solution (PBS) were prepared from $NaH_2PO_4$ and $Na_2HPO_4$ stock solutions, and the pH was adjusted with 0.1 mol/L NaOH and $H_3PO_4$. The serum samples were

provided by Jiangsu Cancer Hospital. The water used in the experiment was distilled water, and the other reagents were of analytical grade.

**Apparatus.** Electrochemical measurements were performed on a CHI852C workstation (Shanghai CH Instruments Co., China). A three-electrode system were employed using glassy carbon electrode as working electrode, the auxiliary electrode as a platinum electrode, and saturated calomel electrode as reference electrode. The pH of the buffer solution was adjusted using PHS-3C digital pH-meter (Shanghai Leici). The electrochemical impedance measurement was performed on an Autolab/PGSTAT30 electrochemical workstation (The Netherlands) in 0.1 M KCl solution containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. The amplitude of the sine wave potential was 5 mV, and the frequency range was from 0.1 to 10 kHz at a bias potential of 190 mV. Scanning electron microscope (SEM) images were obtained from Hitachi S-4800 scanning electron microscope (Japan) with an acceleration voltage of 15 kV. Transmission electron microscope images were measured on a Tecnai 12 transmission electron microscope (TEM, Philips, the Netherlands), and the operating voltage was 120 kV. X-ray diffraction pattern (XRD) was measured on a D8 Advance polycrystalline X-ray diffractometer (Bruker AXS, Germany). The static water contact angle was measured with a contact angle meter (Rame-Hart-100).

**Preparation of hedgehog-like bismuth sulfide nanostructure.** 0.0210 g of trimellitic acid, 0.0365 g of CTAB, 0.3806 g of thiourea, 0.50 mL (0.20 M) of bismuth nitrate solution, and 9.50 mL of distilled water were added to a 50 mL beaker, and ultrasonically mixed to obtain a homogeneous light yellow solution. The solution was then transferred to a Teflon-lined stainless-autoclave. After the autoclave was tightened, it was placed in an oven and heated at 120 ℃ for 12 h. After the reaction finished, the autoclave was taken out and cooled to room temperature. The resultant black $Bi_2S_3$ precipitates were washed with distilled water and anhydrous ethanol three times in sequence. Finally, $Bi_2S_3$ product was dried under vacuum at 60 ℃ for 12 h to obtain a black powder.

**Preparation of AFP electrochemical immunosensor.** Glassy carbon electrode (GCE) was polished on a homogeneous sandpaper, and then with 0.3, and 0.05 μm α-

$Al_2O_3$ slurry (Buehler), followed by sonication in a mixed solution of $HNO_3$, acetone and distilled water for 5 minutes to obtain a smooth and fresh electrode surface. 2 mg of $Bi_2S_3$ was dispersed in distilled water and then mixed with streptavidin (200 μg/mL) at 1:1 volume ratio. 5 μL of the mixed solution was dropped on the GCE, and dried in a refrigerator at 4 ℃. The streptavidin/$Bi_2S_3$/GCE was then coated with 0.5% Nafion, and dried at room temperature. Next, 6 μL of biotin-anti-AFP was dropped on the surface of the Nafion/streptavidin/$Bi_2S_3$/GCE for 40 min at room temperature, and washed with PBS to remove the unfixed biotin-anti-AFP. The biotin-anti-AFP/Nafion/streptavidin/$Bi_2S_3$/GCE was immersed in 1.0% BSA for 1.0 h at room temperature, and rinsed with PBS three times. The prepared immunosensor was placed in pH 7.0 PBS at 4 ℃.

**Immunoassay process for AFP.** The schematic illustration of the fabrication of the electrochemical immunosensor and detection of AFP was shown in Scheme 1. The AFP immunosensor was immersed in 50 μL of incubation solution containing AFP sample and HRP-labeled AFP antibody for 40 min at room temperature. After incubation step, the AFP immunosensor was rinsed with PBS several times. DPV signals were recorded from -0.5 to 0.3 V with a pulse amplitude of 50 mV and a pulse width of 50 ms.

**The FT-IR and XPS spectra of different modified electrodes**

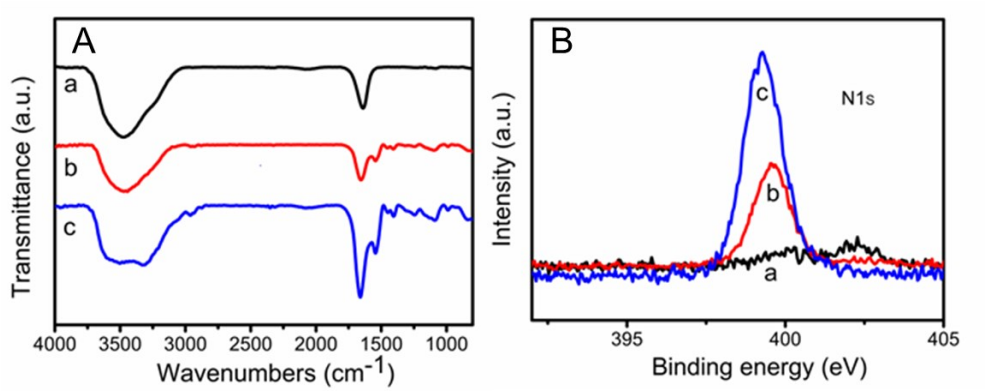


**Fig.S1** (A) FT-IR and (B) XPS spectra of $Bi_2S_3$ (a), streptavidin/$Bi_2S_3$ (b) and biotin-anti-AFP/streptavidin/$Bi_2S_3$ (c).

**The characterization of electrochemical impedance spectrum of different modified electrodes**

Electrochemical impedance is an important tool to study the change of electrode interface during modifications. Fig. S2 (ESI†) shows electrochemical impedance spectra of the modification process of electrode surface layers. The electron transfer resistance ($R_{ct}$) value of the bare glassy carbon electrode (GCE) is 305 Ω (Fig. S2a), which is smaller than that of the HL-$Bi_2S_3$ modified GCE (440 Ω, Fig. S2b), indicating that the HL-$Bi_2S_3$ film was coated to the electrode surface. When streptavidin/HL-$Bi_2S_3$was modified on the electrode, its $R_{ct}$ value (716 Ω, Fig. S2c) continued to increase. It was also proved that streptavidin was functionalized to $Bi_2S_3$ nanostructure. After biotinylated AFP antibody was captured by streptavidin/HL-$Bi_2S_3$ modified GCE, the $R_{ct}$ of AFP antibody-modified GEC increased to 1673 Ω (Fig. S2d). This is because that the biotinylated AFP antibody molecules hindered the electron transfer on the electrode surface, thus producing the greatly increased $R_{ct}$ value. After blocking the active site of the immunosensor with BSA (Fig. S2e), the $R_{ct}$ further slightly increased to 1869 Ω, suggesting the successful fabrication of the immunosensor.

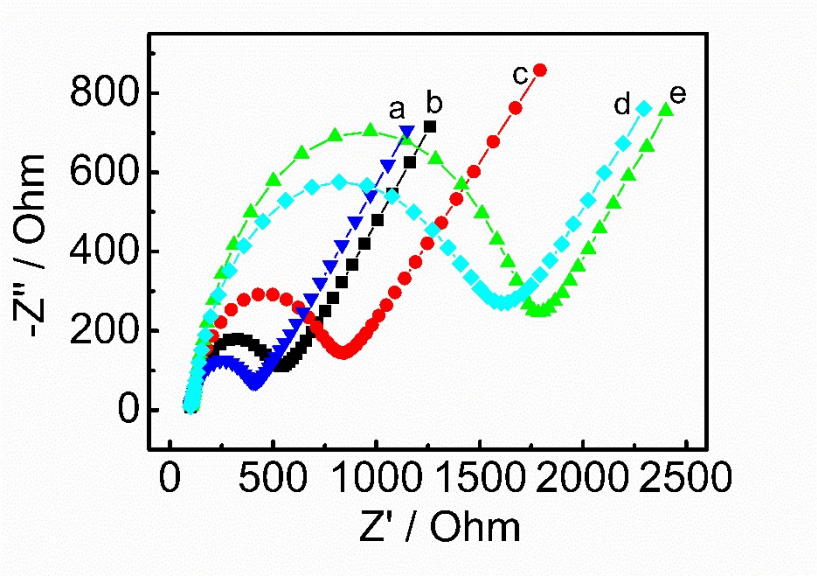


**Fig.S2** Nyquist plot of GCE (a), $Bi_2S_3$/GCE (b), streptavidin/$Bi_2S_3$/GCE(c), biotin-anti-AFP/streptavidin/$Bi_2S_3$/GCE (d) and BSA /biotin-anti-AFP/streptavidin/ $Bi_2S_3$ /GCE(e). Nyquist plots were recorded in 0.1 M pH 7.0 PBS containing 5.0 mM $Fe(CN)_6^{3-/4-}$ and 0.1 M KCl.

**The characterization of contact angles of different modified electrodes**

The hydrophilicity of a sensing interface is very important in development of an immunosensor and can be evaluated by measuring the static water contact angle of the substrate. Fig. S3 (ESI†) shows the contact angles of bare electrode (a), HL-$Bi_2S_3$ modified GCE (b), streptavidin-functionalized HL-$Bi_2S_3$/GCE (c), and biotinylated antibody immobilized electrode (d). Their measurement results are 67.7°, 55.1°, 45.7° and 39.1°, respectively. Compared with the bare electrode, the contact angle of the HL-$Bi_2S_3$ modified electrode became smaller, indicating that the hydrophilicity of electrode surface increased. After HL-$Bi_2S_3$ was functionalized with streptavidin, the contact angle of streptavidin-$Bi_2S_3$/GCE further reduced, that is, the hydrophilicity became better. This indicates that streptavidin functionalized HL-$Bi_2S_3$ sensing platform provides a biocompatible environment for the immobilization of antibody molecules. The biotinylated antibody immobilized electrode shows the smallest contact angle, suggesting the successful modification of AFP antibody on the HL-$Bi_2S_3$ sensing platform.

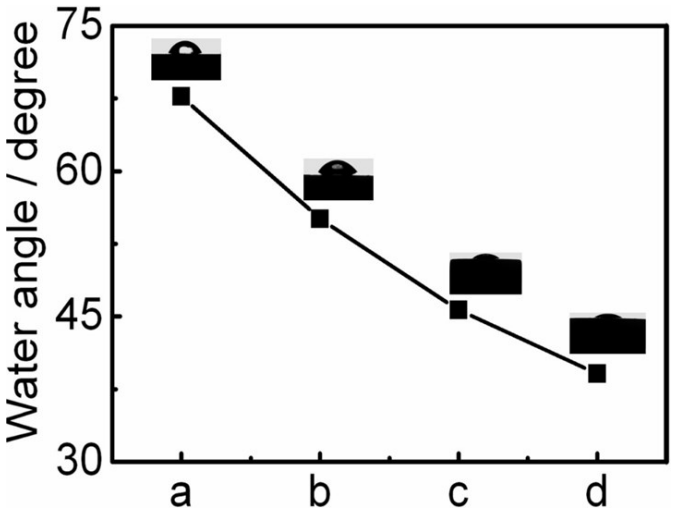


**Fig. S3** Contact angles of GCE (a), $Bi_2S_3$/GCE(b), streptavidin/$Bi_2S_3$/GCE(c), and biotin-anti-AFP/ streptavidin/$Bi_2S_3$/GCE (d).

**Optimization of the concentration of thionine and $H_2O_2$ and solution pH**

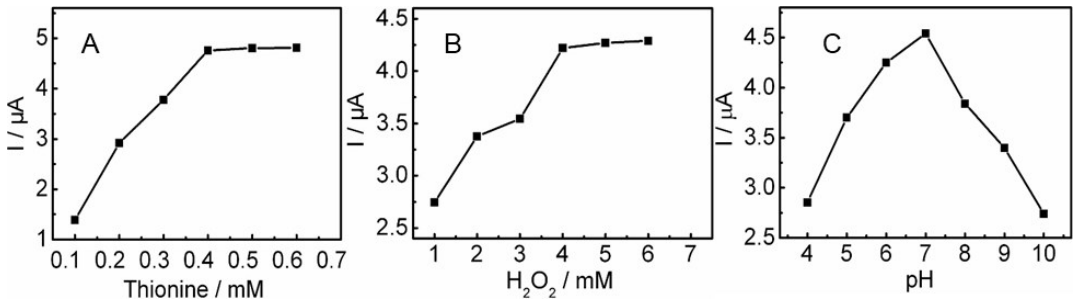


**Fig. S4** Dependence of amperometric response of immunosensor on thionine concentration (A), $H_2O_2$ concentration (B) and pH (C) of detection solution at 100 mV/s scan rate.

**Optimization of the HRP-anti-AFP concentration and incubation time**

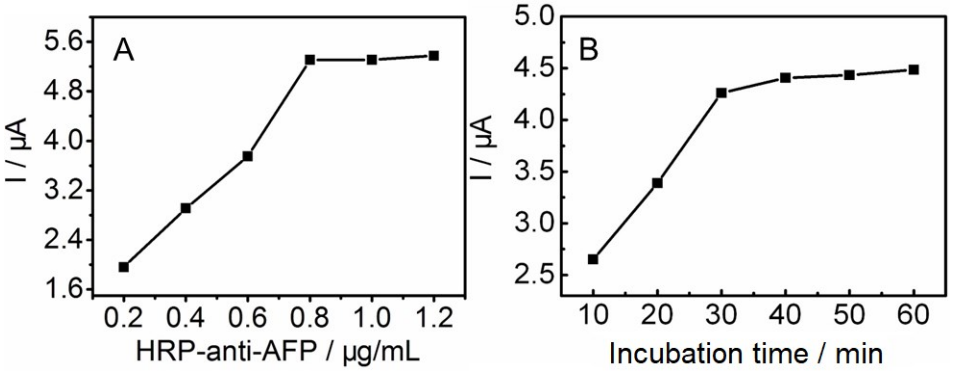


**Fig. S5** Dependence of amperometric response of immunosensor on HRP-anti-AFP concentration (A) and incubation time (B) at 100 mV/s scan rate.

**The specificity test of the electrochemical immunosensor**

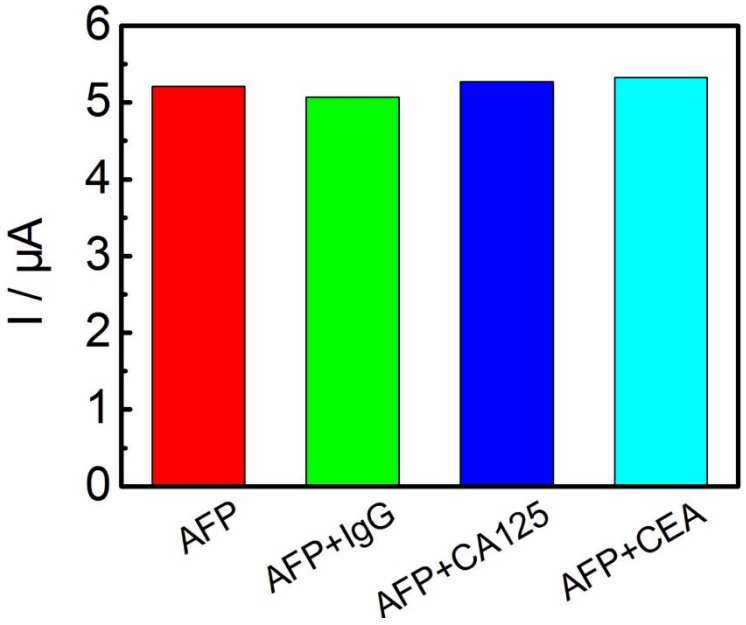


**Fig. S6** Specificity of immunosensor in the presence of 5 ng/mL AFP, 5 ng/mL AFP+50 ng/mL IgG, 5 ng/mL AFP+50 ng/mL CA125, and 5 ng/mL AFP+50 ng/mL CEA.

**Table S1 Comparison of the proposed immunosensor and other methods for AFP detection**

| Immunoassay methods | Linear range (ng $mL^{-1}$) | Detection limit (ng $mL^{-1}$) | References |
|---|---|---|---|
| Electrochemistry | 0.01-20 | 0.005 | This work |
| Surface plasmon resonance | 1.0-200 | 0.65 | S1 |
| Inductively coupled plasma mass spectrometry | 1.5-1000 | 0.34 | S2 |
| Chemiluminescence | 0.05-50 | 0.04 | S3 |
| electrochemiluminescence | 0.05-50 | 0.03 | S4 |
| Electrochemistry | 0.1-50 | 0.04 | S5 |
| Electrochemistry | 0.2 - 100 | 0.04 | S6 |
| Electrochemistry | 0.03-100 | 0.015 | S7 |
| Electrochemistry | 0.05-20 | 0.02 | S8 |

**Table S2 Detection results of clinical serum samples using proposed and reference methods (n = 5).**

| Sample | Proposed method (ng/mL) | Reference method (ng/mL) | Relative error (%) |
|---|---|---|---|
| 1 | 15.22 | 16.38 | 7.62 |
| 2 | 3.03 | 3.31 | 9.24 |
| 3 | 4.85 | 4.55 | -6.19 |
| 4 | 3.99 | 3.89 | -2.51 |
| 5 | 2.16 | 2.34 | 8.33 |

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