Supporting Information

Designed pH-Responsive hollow mesoporous SiO_2 nanocarriers for ultrasensitive detection of Alpha-fetoprotein

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S1. Apparatus and materials

Ammonium Hydroxide ($NH_3 \cdot H_2O$) (25 %) was purchased from Aladdin. Hyaluronic acid(HA), Hexadecyl trimethyl ammonium bromid (CTAB), Lead (II) chloride ($PbCl_2$), tetraethylorthosilicate (TEOS), N-Hydroxysuccinimide (NHS), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 3-Aminopropyltriethoxysilane (APTES) are were obtained by Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Sodium carbonate anhydrous (Na_2CO_3) was provided by Tianjin Sheng Ao chemical reagent Co., Ltd. (Tianjin, China). AFP antigens and AFP antibodies were obtained from Zhengzhou Biocell Co. Ltd. (Zhengzhou, China). Bovine serum albumins (BSA) supplied by Alfa Aesar (Tianjin, China). The real sample of human serum was obtained from the First Affiliated Hospital of Shihezi University School of Medicine (incubated at 37 °C for 1 h and centrifuged at 3500 rpm to obtain the supernatant) and stored at 4 °C. The reagents are all analytical reagents. The experimental water was ultrapure water (18.25 MΩ).

An electrochemical workstation with a three-electrode system (CHI-760E, Shanghai, China), Scanning electron microscopy (SEM) (JEOLJSM-6700F, Hitachi, Japan), Transmission microscope (H600TEM, Tokyo, Japan), RG-160AT centrifuge (Hunan, China), X-ray diffractometer (D8 Advance, Bruker AG), pH measuring instrument (pHS-3B precision digital pH meter Shanghai Hongyi Instrument Co., Ltd.), the XPS is measured on a Thermo Scientific K-Alpha$^+$ photoelectron spectrometer (VG Co.) in which Mono Al Kα X-ray radiation as the X-ray source for excitation,

Ultrasonic3cleaning instrument (KQ-250B Kunshan Ultrasonic instrument Co. Ltd). Three station full function multi-purpose gas adsorption instrument (microTriStar II3flex, America), Zeta potential analyzer (Zetasizer Nano ZS, Britain).

S2. XPS characterization of HMSS before and after Pb^{2+} loading

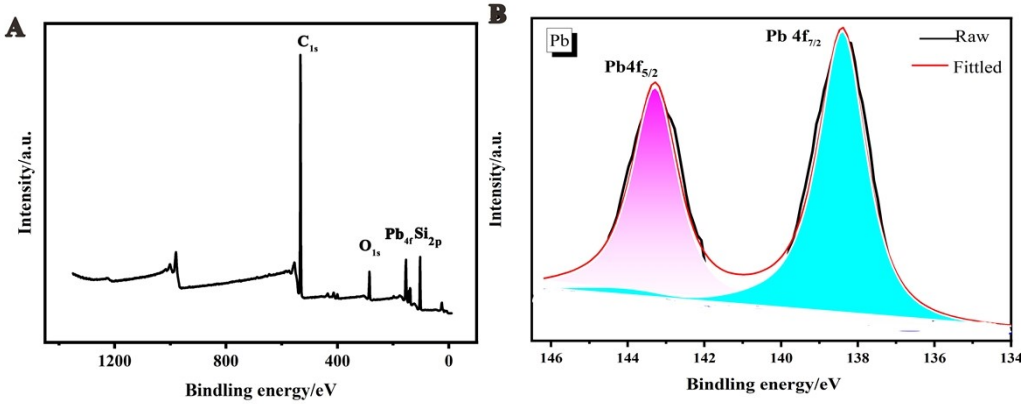

Figure S1. (A) XPS spectra of HMSS-Pb^{2+}; (B) High-resolution Pb 4f of XPS spectra of HMSS-Pb^{2+}.

S3. Changes of specific surface area and pore diameter of HMSS before and after

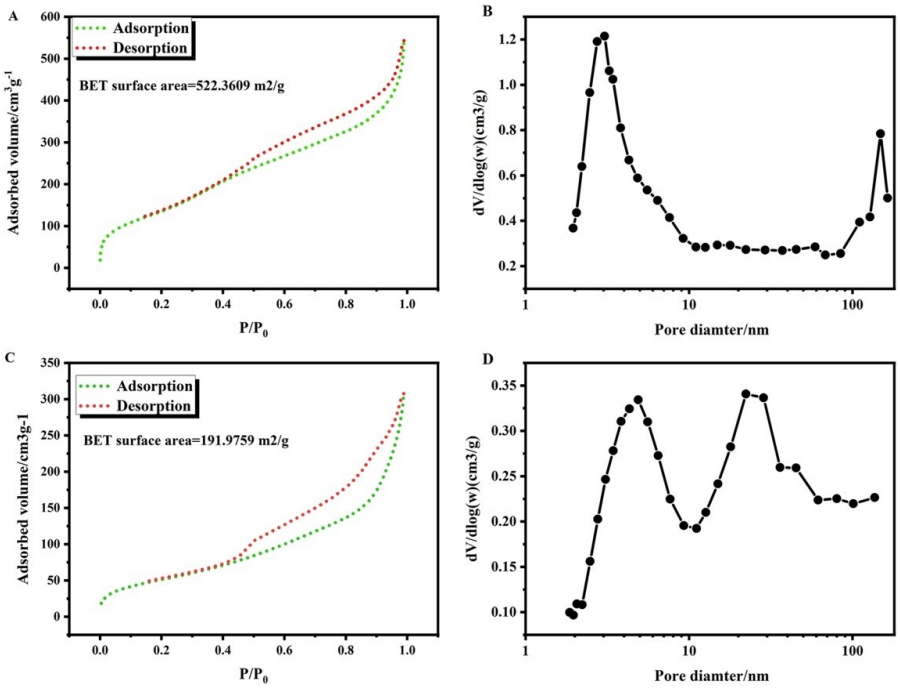

Figure S2. N_2 adsorption–desorption isotherm and the corresponding pore size distribution of (A-B) HMSS and (C-D) HMSS-NH_2-Pb^{2+}@HA.

HA coating

S4. FT-IR and Zeta characterization of nanomaterials

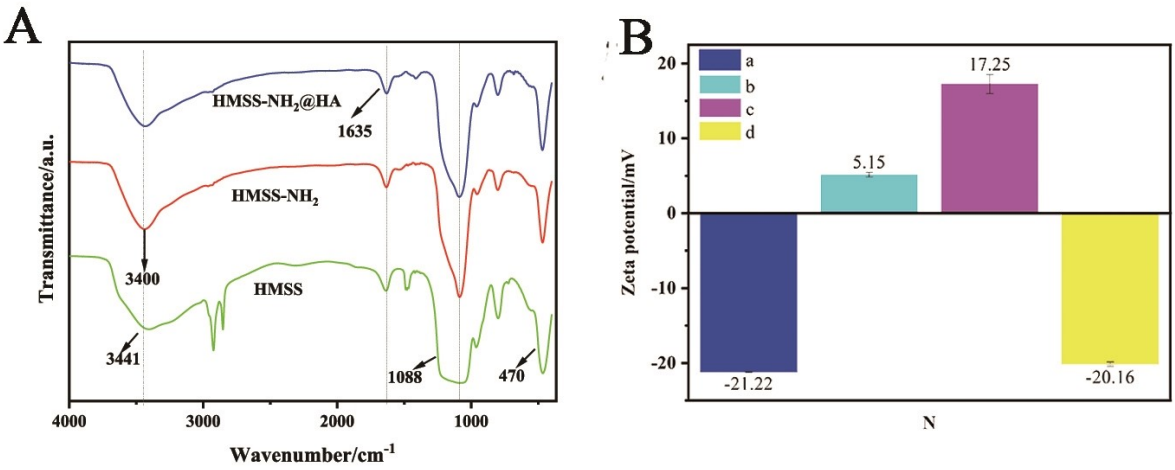

Figure S3. (A) FT-IR spectra of HMSS, HMSS-NH_2, HMSS-NH_2@ HA; (B) Zeta potential of (a) HMSS, (b) HMSS-NH_2, (c) NH_2-HMSS-Pb^{2+}, (d) NH_2-HMSS-Pb^{2+}@HA.

S5. Calculation of the active area of the electrode

To further characterize the effective area on the glassy carbon electrode, CV tests at various velocity scan rates (0.01 V/s~0.1 V/s) were performed on the bare electrode and after deposition of Au. The test substrate was 5 mM $Fe(CN)_6]^{3-/4-}$. As shown in Figure S4, the cathode and anode peak currents were linearly related to the square root of the scan rate, indicating that the process was diffusion controlled. The effective area of the electrode is obtained according to the Randles-Sevcik formula (S-1):

$$Ip = 2.69 \times 105A \times n^{3/2} \times D^{1/2}\, C \times V^{1/2} \qquad (S\text{-}1)$$

where Ip refers to the maximum current (A); D is the diffusion coefficient (cm^2/s); C is the concentration (mol/cm^3) of $[Fe(CN)_6]^{3-/4-}$; n is the transferred electron number; V is the scan rate (V/s); and A is the effective working area of the modified electrode (cm^2).

The equation Ip = 558.56 $V^{1/2}$ + 26.44, R^2 = 0.997 (Figure S4B), Ip = 795.21 $V^{1/2}$ + 8.08, R^2 = 0.998 (Figure S4D), calculates the effective area of the bare electrode to be 0.44 cm^2. After deposition of Au at the electrode, the effective area of the electrode increases to 0.60 cm^2. This is due to the excellent electrical conductivity of AuNPs, which results in faster electron transfer efficiency.

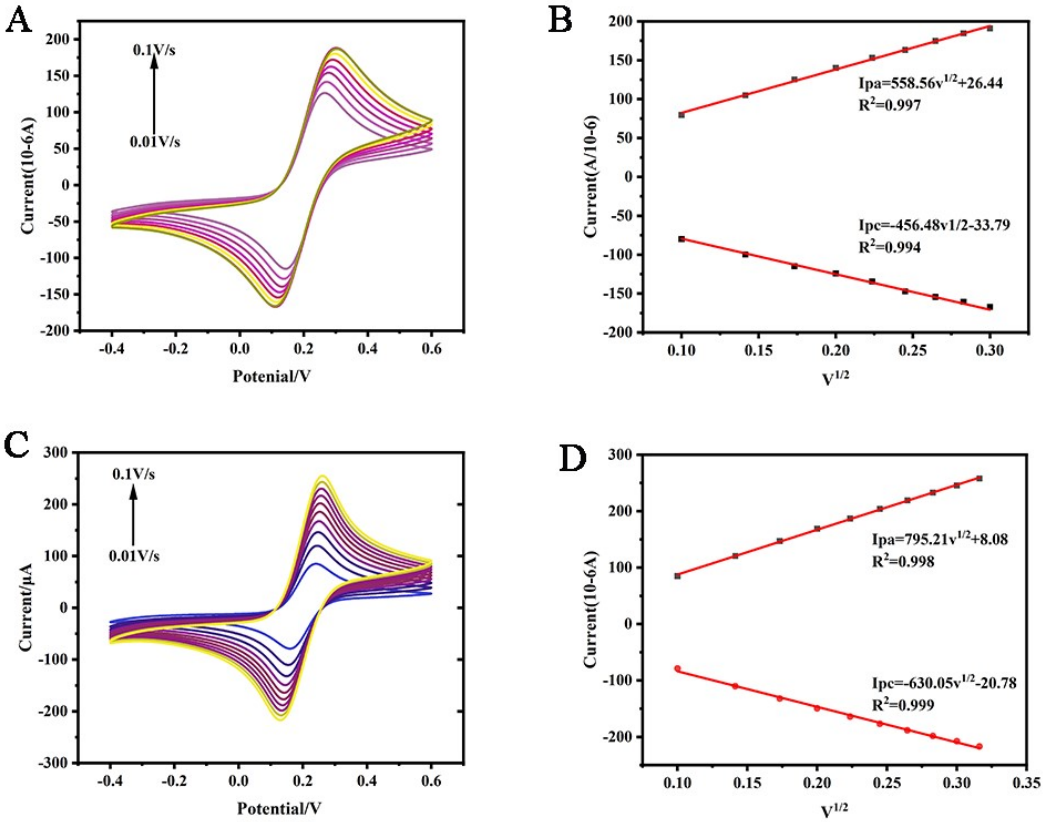

Figure S4. The CV curves of the bare (A) GCE and (C) Au-modified electrodes at different scan rates; linear relationship between the anodic and cathodic peak currents versus scan rate of bare (B) GCE and (D) Au-modified electrodes.

S6. Optimization of the best detection conditions

Pb^{2+} is easy to precipitate in an alkaline environment, so the pH of the test environment is very important for the SWV test of AFP. Therefore, we chose the buffer solution of HAc-NaAc. In addition, in the case of over-acid or over-alkali, it will also have a certain impact on antigen and antibody. As shown in Figure S5A, with the increase of pH, the current response gradually increases. When pH = 4.5, the current response no longer increases but gradually decreases. Therefore, the best detection condition is that the pH value is 4.5. Figure S5B showed the optimization about concentration of hydrochloric acid. The detection results showed that the electrochemical response signal of Pb^{2+} was the largest when the concentration of hydrochloric acid is 0.06 M. When the concentration of HCl is greater than 0.06 M, biological products will be destroyed, resulting in signal weakening. The Figure S5C showed the time required for the secondary antibody label was treated with acid to obtain the maximum electrochemical signal. As shown in Figure S5C, when the acid is added to the electrode for 15 min, the square wave voltammetry test current reached the obvious value. This may be because HA was not destroyed, and signal molecules were not completely released less than 15 min. When the destruction time is longer than 15 min, the SWV current signal gradually decreased. It is speculated that this may be due to the destruction of antigen and antibody, resulting in the loss of signal molecules, or it may have separated from the electrode surface. Finally, the concentration of label material was optimized (Figure S5D). After testing, the concentration of 2 mg/mL is more beneficial in increasing the

current response of the electroactive substance. This is because when the concentration of the secondary antibody label is too high, the overall resistance of the interface will increase, thus hindering the transmission of electrons and affecting the electrochemical response signal.

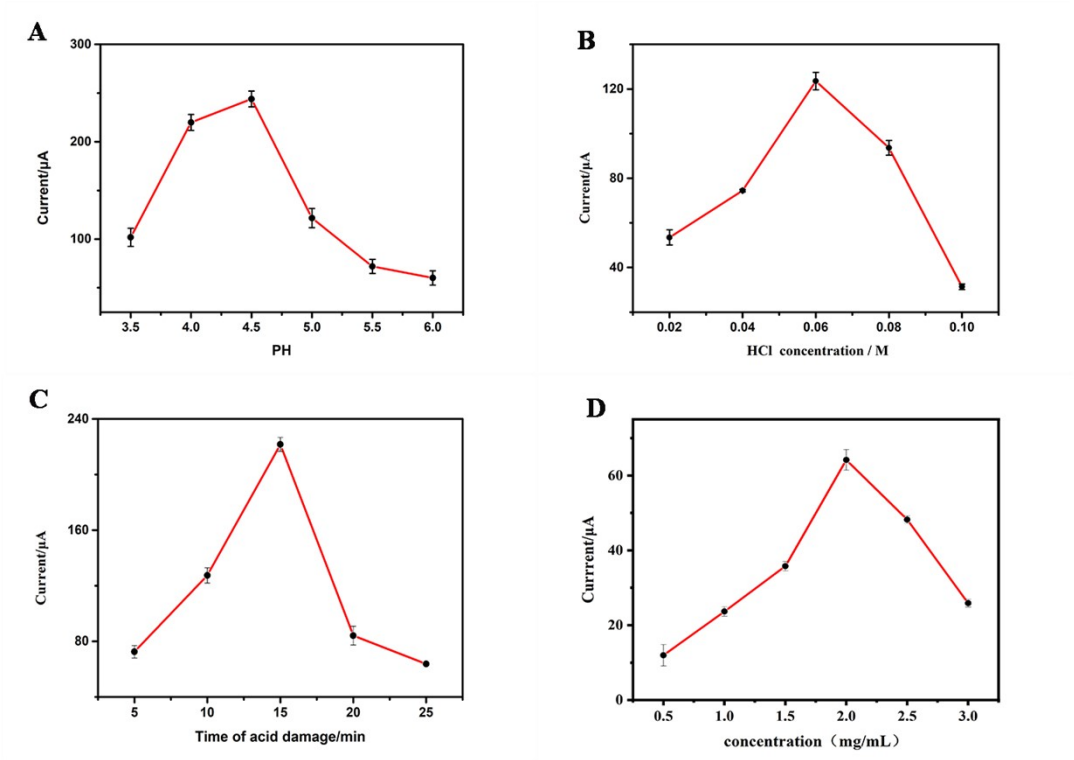

Figure S5. The optimization of experimental conditions with (A) pH of the test solution, (B) the concentration of HCl, (C) time of acid damage on the electrode, and (D) concentration of the Second antibody labeling material. Error bar = SD (n = 3).

S7. Calculation of detection limit

The definition of the LOD was calculated according to the definition of LOD of the International Union of Pure and Applied Chemistry (IUPAC). LOD refers to the lowest

concentration (C_L) corresponding to the smallest analytical signal (xL) detected. When calculating the LOD of the ratiometric sensor, first perform 10 parallel determinations on the blank sample to obtain the corresponding average (xb) and standard deviation (σ). The minimum value of I (Pb^{2+}) is calculated according to the formula (S-2):

$$xL = xb + k * \sigma \quad \text{(S-2)}$$

In the formula, xb was the average value of the blank sample; σ was the standard deviation of the blank sample; k was a numerical factor selected according to the desired confidence level. IUPAC recommended k = 3 as the detection limit calculation standard, and the corresponding confidence was about 90 %. After taking ten blank measurements, the xb = 12.31 and σ = 1.10 was obtained in this work. Therefore, xL = xb + k* σ = 12.31+ 3 * 1.10= 15.61 The calibration plot of this proposed immunosensor is I (μA) = 3.557 * log C_{CEA} +33.41. Therefore, the C_L = 10 * [(15.61-33.41) / (3.557)] = 9.9 * 10^{-6} ng/mL = 9.9 fg/mL. In conclusion, the LOD of this proposed immunosensor is 9.9 fg/mL.