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Supporting Information

An electro-responsive imprinted biosensor for switchable affinity to protein

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Contents

1. Experimental Part

1-1. Materials

N,N′-dimethylaminoethylmethacrylate (DMAEMA), N-isopropylacrylamide (NiPAAm), methylene N,N-bis(acrylamide) (MBAAm), acrylamide (AAm), N,N,N′,N′-tetramethylethylenediamine (TEMED), and ammonium persulfate (APS) were purchased from Aladdin Reagents. Bovine serum albumin (BSA), human serum albumin (HSA), human immunodeficiency virus p24 (HIV-p24), human chorionic gonadotropin (HCG), α-fetoprotein (AFP) and carcino-embryonic antigen (CEA) were purchased from Linc-Bio Science Co. Ltd (Shanghai, China). All chemicals were analytical grade and used without further purification. All aqueous solutions were prepared using ultra-pure water (purified by 18.2 MU, Milli-Q, Millipore).

1-2. Apparatus and procedures

The CHI660C electrochemical workstation (Chenhua, Shanghai, China) was used to measure the cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). A bare or modified glassy carbon electrode (GCE), a platinum wire, and a saturated calomel were used as the working electrode, auxiliary electrode, and reference electrode (SCE). The UV-3900H (Hitachi, Japan) spectrophotometer was used to measure HSA concentrations in real samples by a standard calibration curve method. The chemical character of EMIPs film was conducted on Fourier Transform infrared spectroscopy (FT-IR) (VERTEX 70 Bruker, Germany). The pore structure of the EMIPs film before application of voltage and after removal of the voltage was conducted on AutoPore IV 9500 (Micromeritics Instrument Corp., USA). The surface morphologies of EMIPs film and MIPs film without adding DMAEMA before and after applied -1.2 V for 150 s were monitor by field emission scanning electron micrographs (FE-SEM; SU-8220). The preparation method as follows: after treated with the required voltage, the EMIPs film was whipped into a bottle of liquid nitrogen for 3 min to “freeze” the structures. Subsequently, a freeze drier was used to dehydrate the obtained freeze film for 48 h (LGJ-18S; Beijing Songyuan Huaxing Technology).

1-3. Electrochemical property measurements

CVs were recorded in the potential range from - 0.2 V to + 0.6 V in a pH 5.5 PBS buffer containing 0.1 mol L^{-1} KCl and 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ at a scan rate of 100 mV s^{-1}. DPV was conducted for quantitative analysis basing on the variation of the oxidation peak current of 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ after and before being treated with HSA in a pH 5.5 PBS buffer containing 0.1 mol L^{-1} KCl from - 0.2 V to + 0.6 V. The pulse amplitude, pulse period, and pulse width of DPV were 50 mV, 0.2 s, and 50 ms, respectively.

1-4. Synthesis of EMIPs/GCE, ENIPs/GCE, and MIPs/GCE without DMAEMA

The EMIPs biosensor was fabricated through loading the 5.0 μL of pre-imprinted hydrogel solution onto the glassy carbon electrode under N_2. First, 100.0 mg of NiPAAm, 10.0 mg of DMAEMA, 5.0 mg of AAm, 5.0 mg of HSA, 5.0 mg MBAAm, 5.0 mg mL^{-1} APS and 3.0 μL TEMED were dissolved in 1.0 mL pH 5.5 PBS buffer to form clearly suspension. Then, 5.0 μL of pre-imprinted hydrogel was drop-casted onto the pre-polished glassy carbon electrode (3 mm) under N_2. The HSA imprinted hydrogel film was formed on the surface of bare electrode (HSA imprinted EMIPs/GCE) under room temperature for 30 min. For comparison, non-imprinted polymer film modified electrode (ENIPs/GCE) was prepared following the same procedure except adding any template. The MIPs without DMAEMA hydrogel film electrode (MIPs/GCE without DMAEMA) was prepared following the same procedure except adding DMAEMA.

In this work, the removal of HSA from HSA imprinted EMIPs/GCE was conditioned in a pH 5.5 PBS buffer using a voltage of -1.2 V for 150 s at 25 °C and the re-adsorption of HSA was conducted at 25 °C, which have been shown in Scheme S1 in details.

1-5. HSA elution from the HSA-EMIPs/GCE

After polymerization, different applied voltages (i.e., -0.6 V, -0.8 V, -1.0 V, -1.2

V, and -1.4 V for 150 s) and time (50 s, 100 s, 150 s, and 200 s at -1.2 V) at 25 °C was applied to HSA imprinted EMIPs/GCE in a pH 5.5 PBS buffer. Subsequently, the sensor was rinsed with pH 5.5 PBS buffer and distilled deionized water, and then transferred into a fresh pH 5.5 PBS buffer for 55 min to restore the original structure. The following electrochemical measurements were all performed in a fresh pH 5.5 PBS buffer containing 0.1 mol L^{-1} KCl and 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$..

1-6. EMIPs film structure properties controlled by the voltage

After removal of HSA, different applied voltages (i.e., -0.4 V,-0.6 V, -0.8 V, -1.0 V, -1.2 V, -1.4 V, and -1.6 V for 150 s) and time (50 s, 100 s, 150 s, and 200 s at -1.2 V) at 25 °C was applied to EMIPs/GCE in a pH 5.5 PBS buffer. The electrochemical measurements were performed. Subsequently, the sensor was kept for 55 min in a pH 5.5 PBS buffer to restore the original structure. The following electrochemical measurements were performed in a fresh pH 5.5 PBS buffer containing 0.1 mol L^{-1} KCl and 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$.

1-7. Binding isotherms of HSA

The binding isotherms of HSA were investigated by the EMIPs/GCE. The typical procedure was described as follows. Standard HSA solutions were prepared in a pH 5.5 PBS buffer, by which the HSA concentrations varied within the range of 0.05 to 5.0 μmol L^{-1}. For equilibrium rebinding test, EMIPs/GCE was incubated with 0.5 mL 0.05 to 5.0 μmol L^{-1} of HSA solutions in a pH 5.5 PBS buffer for 12 min. Nonspecifically adsorbed samples were completely removed from the sensor by rinsing with pH 5.5 PBS buffer and double distilled deionized water. The mixture was discarded. Fresh pH 5.5 PBS buffer containing 0.1 mol L^{-1} KCl and 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ was then added for electrochemical measurements. For comparison, ENIPs/GCE was treated with the same procedure.

1-8. Selectivity test

HIV-p24, HCG, BSA, AFP, and CEA were selected as reference proteins. The

EMIPs/GCE was incubated with 0.5 mL 1.0 μmol L^{-1} of HIV-p24, HCG, BSA, AFP, or CEA solutions in a pH 5.5 PBS buffer for 12 min. Nonspecifically adsorbed samples were completely removed from the sensor by rinsing with pH 5.5 PBS buffer and double distilled deionized water. The mixture was discarded. Fresh pH 5.5 PBS buffer containing 0.1 mol L^{-1} KCl and 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ was then added for electrochemical measurements. For comparison, ENIPs/GCE was treated with the same procedure.

1-9. Electro-controlled uptake and release study

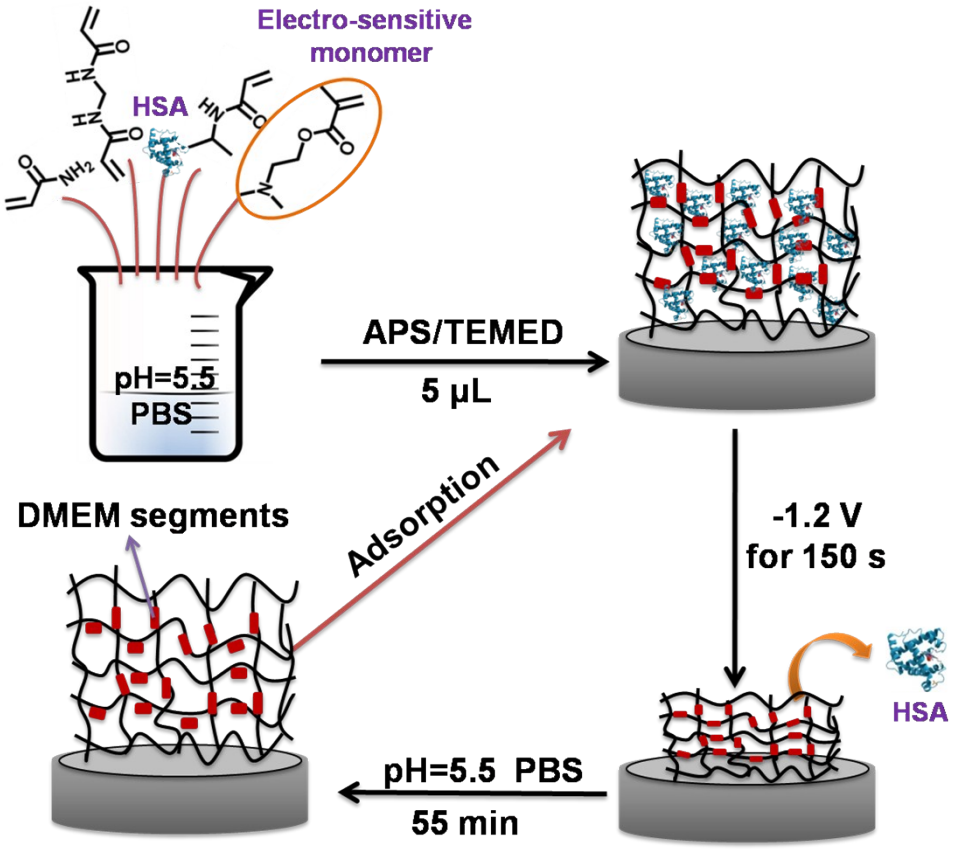
For the electro-controlled uptake and release of HSA study, the EMIPs/GCE was first immersed into 0.5 mL 1.0 μmol L^{-1} of HSA for 12 min. Nonspecifically adsorbed samples were completely removed from the sensor by rinsing with pH 5.5 PBS buffer and distilled deionized water, and then the resulting sensor was transferred into a fresh pH 5.5 PBS buffer containing 0.1 mol L^{-1} KCl and 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ for electrochemical measurements. For the electro-controlled release of HSA, a voltage of -1.2 V was applied for 150 s to remove HSA from the HSA imprinted EMIPs/GCE at 25 °C firstly, and then using pH 5.5 PBS buffer and distilled deionized water to rinse the sensor. Subsequent keeping for 55 min in a pH 5.5 PBS buffer to restore the original structure. The following electrochemical measurements were all performed in a fresh pH 5.5 PBS buffer containing 0.1 mol L^{-1} KCl and 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$. For comparison, ENIPs/GCE and MIPs/GCE without DMAEMA were treated with the same procedure.

1-10. Electro-controlled extraction of HSA from complex serum sample

Human blood serum was collected from a healthy volunteer. Human blood (5.0 mL) was coagulated in blood coagulation-promoting tubes for 2 h and subsequently centrifuged at 5000 rpm. Approximately 1.0 mL of serum was extracted with an equal volume of methanol, vortexed for 1 min, and centrifuged at 5000 rpm for 5 min. The supernatant was filtered through a 0.22-μm microporous membrane and dried using a N_2 stream, to which PBS (1.0 mL) was introduced. The pH of the solution was also

adjusted to 5.5. The EMIPs/GCE was incubated in the serum samples for 12 min to rebind all the HSA from the serum sample. Nonspecifically adsorbed samples were completely removed from the sensor by rinsing with pH 5.5 PBS buffer and double distilled deionized water. The mixture was discarded. Fresh pH 5.5 PBS was then added. For the electro-extracting of HSA shown in Scheme S2, -1.2 V for 150 s was applied to the HSA imprinted EMIPs/GCE. The released concentration was determined by the UV–vis spectroscopic method[1,2] and the results obtained are concluded in Table S2.

2. Preparation of EMIPs films on electrode surface



Scheme S1 Schematic illustration of the preparation of electro-responsive HSA imprinted EMIPs/GCE via free radical polymerization and the process of self-cleaned HSA imprinted EMIPs/GCE.

3. IR spectra characterization of EMIPs

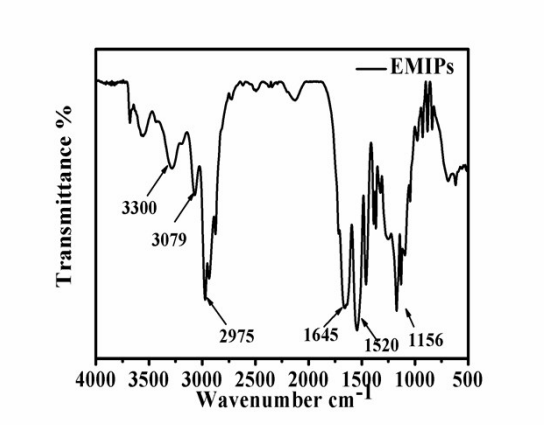


Fig. S1 IR spectra of EMIPs.

4. Electro-responsive property of the EMIPs/GCE

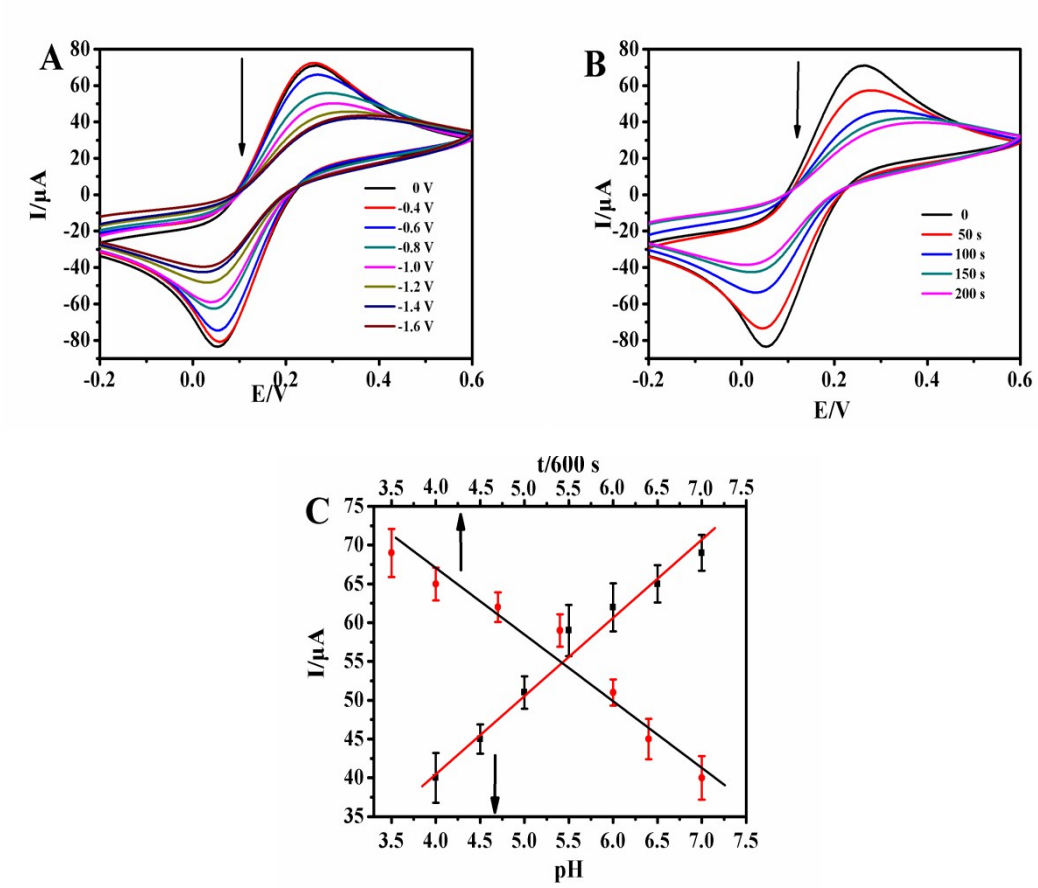


Fig. S2 (A) CV responses of the EMIPs/GCE in a pH 5.5 PBS buffer at voltage from 0 V to -1.6 V for 150 s at 25 °C; (B) CV responses of the EMIPs/GCE in a pH 5.5 PBS buffer applied -1.2 V for 50 s to 200 s at 25 °C; (C) the redox peak currents of $[Fe(CN)_6]^{3-/4-}$ and recovery time of the EMIPs/GCE in a PBS buffer applied -1.2 V for 150 s with pH from 4.0 to 7.0 at 25 °C.

5. EIS responses of the EMIPs/GCE

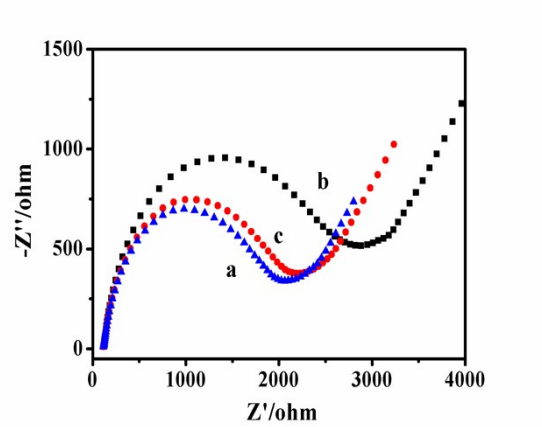


Fig. S3 EIS responses of the EMIPs/GCE before (curve a) and after applied -1.2 V for 150 s (curve b) and subsequent withdrew the voltage and keep for 55 min (curve c) in a pH 5.5 PBS buffer at 25 °C;

6. SEM of MIPs film without DMAEMA before and after applied voltage

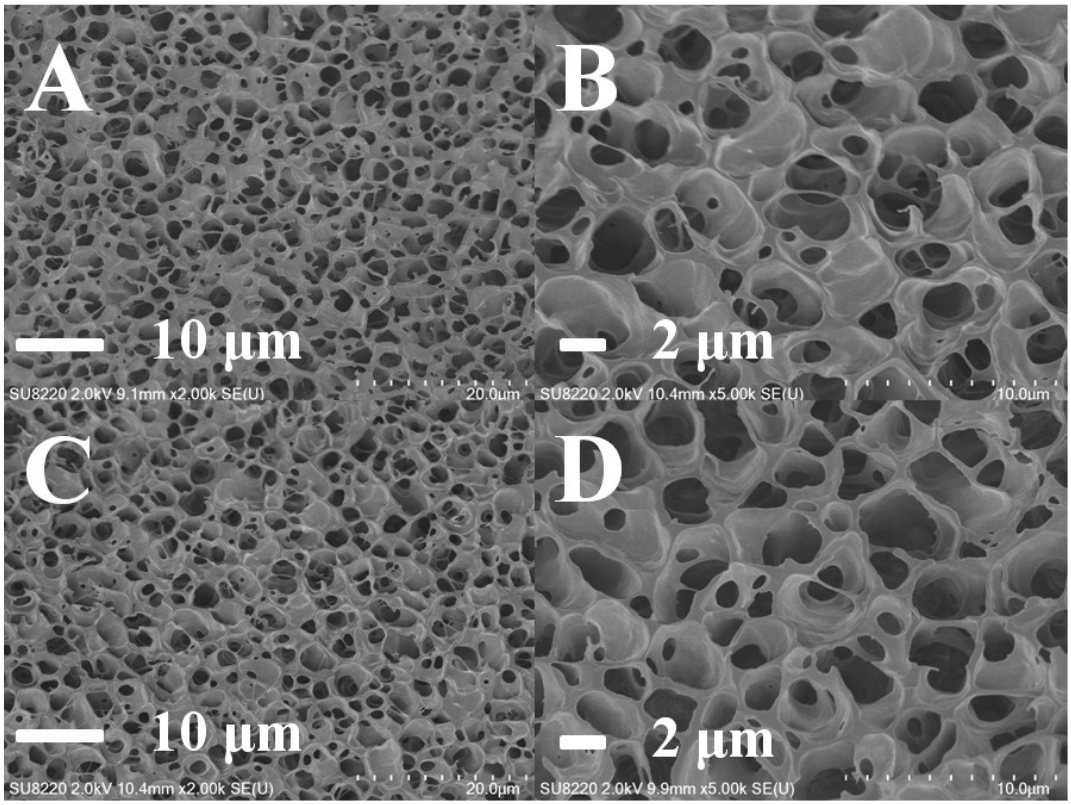


Fig. S4 SEM of MIPs film without DMAEMA prepared on the GCE surface before (A, B) and after applied -1.2 V for 150 s (C, D).

7. CVs of MIPs/GCE without DMAEMA before and after applied voltage

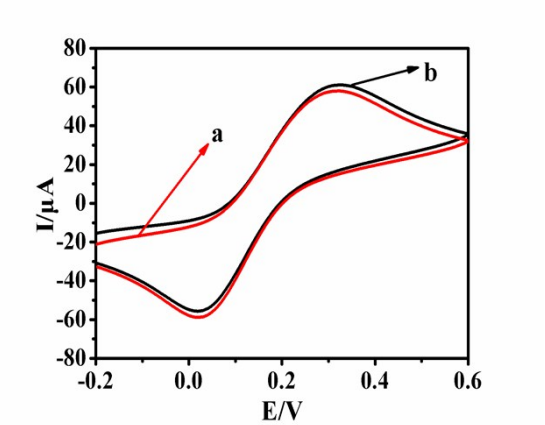


Fig. S5 CVs responses of the MIPs/GCE without DMAEMA before (curve a) and after (curve b) applied -1.2 V for 150 s.

8. The Cumulative intrusion curve and pore size distribution of EMIPs

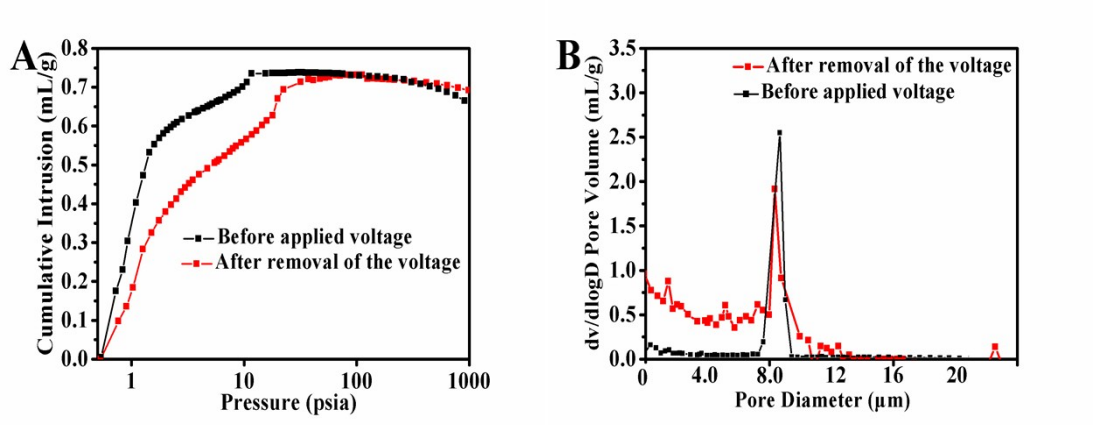


Fig. S6 Cumulative intrusion curve (A) and pore size distribution (B) from mercury intrusion porosimetry of each sample.

9. The efficiency evaluation of self-cleaned HSA imprinted EMIPs/GCE

Table S1. Controlled experiments.

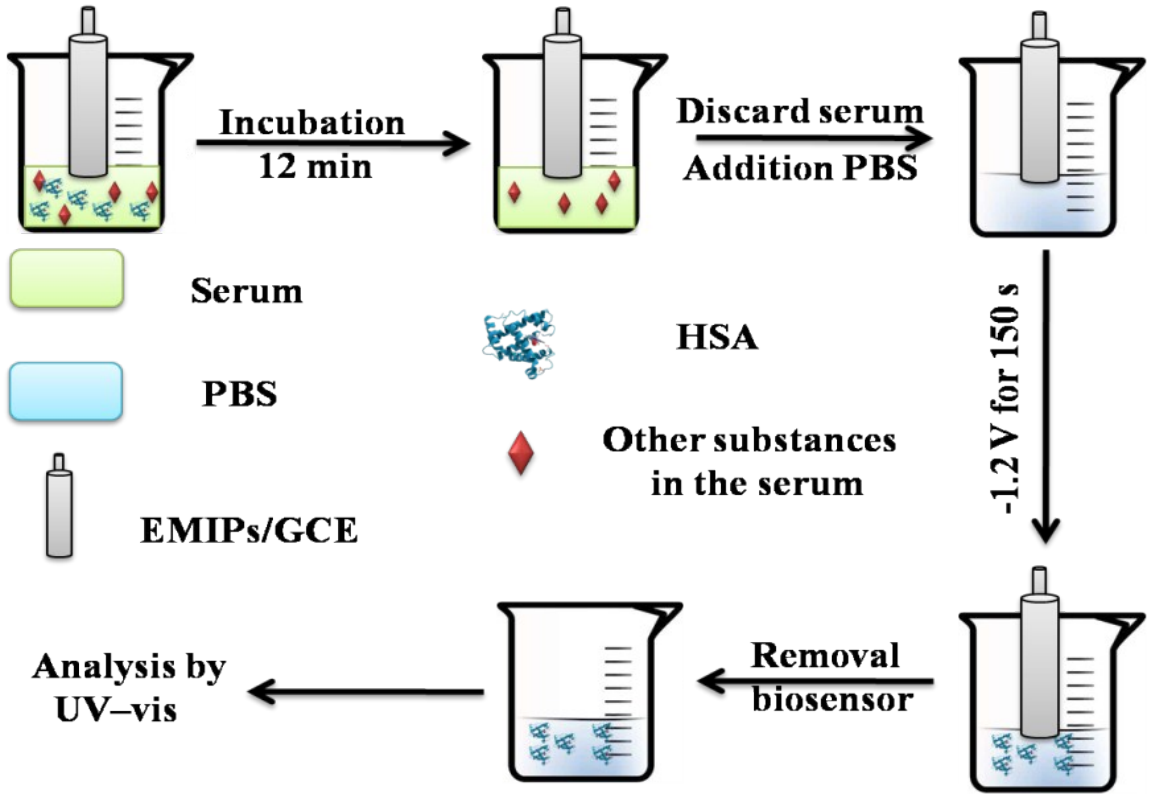
Entry	Voltage (V)	Time (s)	Current (μA)	Fig. 1B
1	0	150	16±3	a
2	-0.6	150	17±2	b
3	-0.8	150	34±1	c
4	-1.0	150	47±3	d
5	-1.2	150	67±2	e
6	-1.4	150	68±1	f
7	-1.2	50	37±2	-
8	-1.2	100	52±1	-
9	-1.2	200	68±2	-
10	0	150	14±2	-
11	-1.2	150	15±2	-

Conditions: The HSA imprinted EMIPs/GCE and HSA imprinted MIPs/GCE without DMAEMA were conditioned in a pH 5.5 PBS buffer containing 0.1 mol L^{-1} KCl and 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$; Scan rate:100 mV s^{-1}.

10. Application of the EMIPs/GCE

Table S2 Electro-controlled extraction of HSA from serum.

Sample	Content (μmol L^{-1})	Added (μmol L^{-1})	Detected content by UV (μmol L^{-1})	Recovery (%)	RSD (%) (n=3)
	0.15	0.2	0.33	94.3	3.4
1	0.15	1.5	1.66	101.2	2.6
	0.15	3.0	3.11	98.7	2.5
	1.65	0.2	1.71	92.4	1.3
2	1.65	1.5	3.10	98.4	3.5
	1.65	3.0	4.64	99.8	2.7



Scheme S2 Electro-controlled extraction of HSA from serum using EMIPs/GCE.

1. Li, X., Zhang, B., Li, W., Lei, X., Fan, X., Tian, L., Zhang, H., Zhang, Q., 2014b. Biosens. Bioelectron. 51, 261-267.
2. Liu, M., Pi, J., Wang, X., Huang, R., Du, Y., Yu, X., Tan, W., Liu, F., Shea, K.J., 2016. Anal. Chim. Acta 932, 29-40.