Electrochemical protein recognition based on macromolecular self-assembly of molecularly imprinted polymer: a new strategy to mimic antibody for label-free biosensing

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Characterization and Measurement

The chemical structure of PDHS and UPDHS polymer was characterized by a Bruker (400 MHz) instrument using deuterated chloroform (CDCl$_3$) as solvent. Fourier-transform infrared spectroscopy (FT-IR) spectra was recorded on a Nicolet iS50 Fourier Infrared Spectrometer. The morphologies of BSA@UPDHS nanoparticles were observed using both a JEOL JEM-2100 transmission electron microscope (TEM) operating at 200 kV. Dynamic light scattering (DLS) experiment was conducted using an ALV-5000/E dynamic light scattering instrument at 90°, and all sample solutions were passed through 0.8 mm Millipore filters prior to load into the sample cell. Far UV circular dichroism (CD) was recorded on a MOS-450 spectropolarimeter (Biologic Co., Ltd., France). The irradiation light for photo-crosslinking was obtained from a UV–vis spot curing system (UV-100D, ANEST IWATA Co., Ltd., Japan) combined with a 365 nm filter, and was applied vertically above the electrode. The BSA@MIP coating morphologies on the electrode surface were observed using a Hitachi S-4800 field emission scanning electron microscope (FESEM).

Differential Pulse Stripping Voltammetry (DPSV), Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) were performed in a three-electrode system with an electrochemical workstation (CHI660E, Shanghai, China). CV was carried out between -0.20 V and 0.60 V in a 5 mmol L$^{-1}$ [Fe(CN)$_6$]$^{3-/-4-}$ and 0.2 mol L$^{-1}$ KCl solution at scan rate of 100 mV s$^{-1}$. EIS was also tested in a 5 mmol L$^{-1}$ [Fe(CN)$_6$]$^{3-/-4-}$ and 0.2 mol L$^{-1}$ KCl solution. DPSV curves were obtained in a 0.1 mol L$^{-1}$ PBS (pH=7.4) solution containing 5 mmol L$^{-1}$ [Fe(CN)$_6$]$^{3-/-4-}$ between -0.20 V and 0.40 V.
after incubating in various concentrations of templates. The bare gold electrode (4 mm diameter; Aida, Tianjin, China), and the BSA@MIP sensor were used as working electrode (WE). A platinum electrode and a saturated calomel electrode (SCE, Aida) were used as counter and reference electrode, respectively. All potentials applied to WE were referred to SCE.
Supplementary Results

Fig. S1. $^1$H NMR spectra (A) and FT-IR (B) of amphipathic copolymer PDHS and UV-crosslinkable macromonomer UPDHS.

Fig. S2. The diameters and TEM images of different ratio of monomers (DMAEMA:HEA:St): (A) and (D) 1:0.5:1; (B) and (E) 1:1:1; (C) and (F) 1:1.25:1. We controlled the HEA content to obtain different UPDHS with different double bond content, because the amount of UV-crosslinkable acrylate side groups (ICEA) was determined by HEA (Scheme 2). Double bond content is the key to command the
formation of BSA@UPDHS NPs and performance of MIP sensor originated from UV-crosslinkable macromonomer. The diameters and TEM images of different BSA@UPDHS NPs were shown in Fig.S2. With the increase of HEA, double bond content increased and the average diameter of BSA@UPDHS NPs increased as well. In a general way, the degree of UV-crosslinking would increase with more double bond in UPDHS, resulting in more robust and stable MIP layer. However, the BSA@UPDHS NPs with excess double bond would be irregular and varisized as shown in Fig.S2 C and F. Therefore, the copolymer UPDHS with the molar ratio of 1:1:1 (DMEEMA:HEA:St) was chosen to investigate the performance of MIP sensor.

Fig.S3. CV voltammograms of BSA@MIP sensor after different extraction time.
Fig. S4. DPSV peak currents of BSA@MIP sensor in PBS (pH 7.4) solution containing [Fe(CN)$_6$]$^{3-/4-}$ as probe after incubation in $10^{-11}$ mg mL$^{-1}$ BSA aqueous solution for different time.

Fig. S5. DPSV response currents of different BSA@MIP sensors after protein extraction. The sensors were constructed using the same procedures.

Table S1. Comparison of sensing performance data of proposed method with other materials applied for BSA detection.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Linear range (mg mL$^{-1}$)</th>
<th>References</th>
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<tbody>
<tr>
<td>BSA/WMMIP</td>
<td>$5 \times 10^{-10} \sim 5 \times 10^{-7}$</td>
<td>1</td>
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<tr>
<td>Epitope-based MIP/HRP amplification</td>
<td>$10^{-6} \sim 1.5 \times 10^{-4}$</td>
<td>2</td>
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</tbody>
</table>
MIP Microspheres  $10^5 \sim 5\times10^{-3}$  3
MIP IL/CNTs  $10^{-4} \sim 10^{-1}$  4
Chitosan/CNTs  $10^{-7} \sim 10^{-1}$  5
Graphite-based ink/MIP  $10^3 \sim 10^2$  6
TGA-CdTe/MIP  $4.6 \sim 1.5\times10^2$  7
BSA@UPDHS  $10^{-14} \sim 10^{-9}$  This work

References


