Boronate affinity MIP-based resonance light scattering sensor for sensitive detection of glycoprotein

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Glycoproteins have extraordinary significance for the diagnosis of tumors or cancers. However, in untreated complex biological samples, highly selective and sensitive determination of glycoproteins is still a huge challenge. In this study, a boronate affinity surface molecular imprinted resonance light scattering (RLS) sensor was constructed to detect glycoproteins. In the RLS sensor, 4-vinylbenzeneboronic acid, which was used as functional monomer for molecular imprinted polymers (MIPs), are pH sensitive and can reversibly bind to glycoproteins. In addition, 2-(Dimethylamino) ethyl methacrylate was also applied as a pH-responsive component. Its property of swelling under lower pH is beneficial to rapidly remove template glycoproteins from MIPs. Combining the boronate affinity and MIPs, the RLS sensor can specifically recognize target glycoproteins from sample solutions and sensitively detect them. Under optimum conditions, the RLS sensor has a linear response to ovalbumin (OVA) in the range of 0.5–15 nM with a low detection limit of 0.13 nM. The RLS sensor has been successfully utilized for the detection of OVA in real human blood serum, with good recovery of 94.0%–104%. The approach will provide a promising way to detect glycoproteins in clinical diagnostics.

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[b]

Experimental

Measurement of swelling ratio

To evaluate the pH-responsive swelling/shrinking behavior of Fe_3O_4@MIPs, the swelling ratios at different pH were investigated and the experiments were performed as follows: a certain amount of Fe_3O_4@MIPs particles were dispersed in phosphate buffer (PB, 20 mM, pH=7.0) by ultrasonication, then transferred into the NMR tube. The same static liquid level was recorded as H_0. After that, Fe_3O_4@MIPs particles were isolated by magnetic separation, and then the supernatant was discarded. Subsequently, the Fe_3O_4@MIPs particles were incubated in PB solutions with different pH (20 mM, pH=2, 3, 4, 5, 6, 7, 8, respectively) for 3 h at 25 °C. After equilibrium, the resultant liquid level (H_W) was measured. The swelling ratio (SR) was calculated according to the following equation:

$$SR = \frac{H_W - H_0}{H_0}$$

Isothermal adsorption experiments

Isothermal adsorption experiments were performed as follows: 5 mg of Fe_3O_4@MIPs or Fe_3O_4@NIPs was dispersed in 5 mL of the OVA solutions with different initial concentrations (ranging from 0.2 to 1.0 mg/mL) prepared in phosphate buffer (20 mM, pH=7.0). Then the mixtures were shaking at 25 °C for 30 min. After adsorption equilibrium, the supernatants were determined. The amount of OVA (Q) adsorbed by Fe_3O_4@MIPs/ Fe_3O_4@NIPs was calculated as:

$$Q = \frac{(c_0 - c)V}{m}$$

where c_0 (mg/mL) and c (mg/mL) are the initial and final concentrations of the OVA, respectively; V (mL) is the volume of the OVA solution, and m (mg) is the dry mass of Fe_3O_4@MIPs/ Fe_3O_4@NIPs.

The experimental results were analyzed by Langmuir model, which was expressed as follow equation:

$$\frac{c_e}{q_e} = \frac{c_e}{q_{max}} + \frac{K}{q_{max}}$$

where q_e (mg/g) is the equilibrium adsorption amount of OVA adsorbed by MIPs or NIPs nanoparticles, c_e (mg/mL) represents the equilibrium concentration of OVA in the solution, q_{max} (mg/g) is the maximum adsorption capacity of OVA, K represents the Langmuir apparent equilibrium dissociation constant.

Supporting data **Effects of pH on RLS sensor**

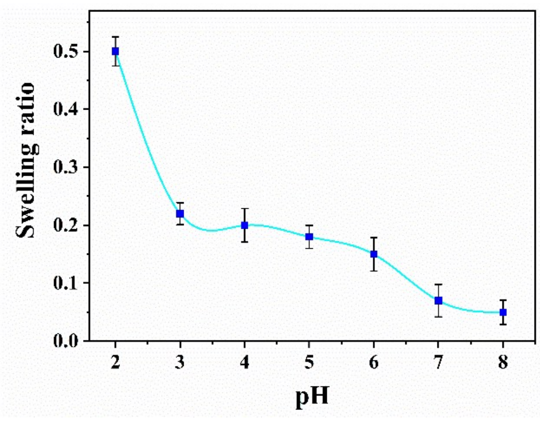

Fig. S1 Swelling ratio of Fe_3O_4@MIPs.

Selectivity and affinity evaluation

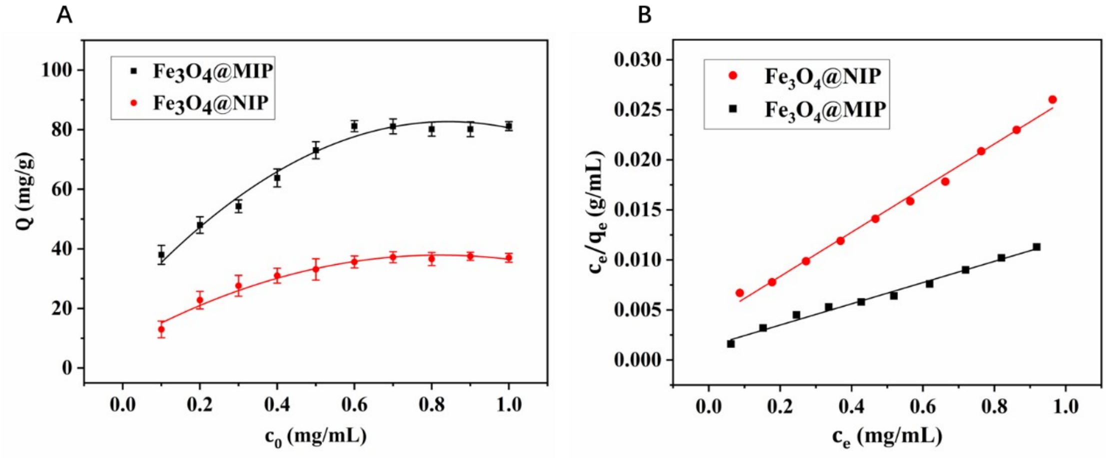

Fig. S2 (A) adsorption isotherms of Fe_3O_4@MIPs/ Fe_3O_4@NIPs and (B) linear fitting of adsorption isotherms using Langmuir model.

Table S1 Parameters K and q_{max} from Langmuir model.

Sample	R^2	K (mg/mL)	q_{max} (mg/g)
MIP	0.9878	0.13	94.3
NIP	0.9939	0.18	45.5