Electronic supplementary information (ESI) for:

Preparation of surface–imprinted microspheres with effectively controlled by orientated template immobilization using highly cross–linked raspberry–like microspheres for selective recognition of immunostimulating peptide

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1. Adsorption isotherms of P(PEGDMA-VI) and different IL-functionalized microspheres

In order to investigate the optimal mass ratio of IHH and IL-functionalized microspheres, adsorption isotherms were performed at different initial IHH concentrations from 0.025 to 0.25 mg mL^{-1}. The microspheres (10 mg) were washed once with 0.01 M phosphate buffer saline (PBS) and then 10 mL IHH PBS solution was added. The mixture was magnetically stirred at 300 rpm for 24 h at 25 ^{o}C for the self–assembly to occur. The IHH–immobilized microspheres were then washed with DI water until no IHH was detected by UV–2550 spectrophotometer at 276.5 nm detection wavelength. As shown in Fig. S1, the amount of IHH bound to P(PEGDMA-VI) microspheres reaches saturation at 0.075 mg mL^{-1}. For P(PEGDMA-VI)@CB, P(PEGDMA-VI)@CO, P(PEGDMA-VI)@CD and P(PEGDMA-VI)@CAA, the maximum adsorption capacities could be observed when the IHH concentration was 0.15 mg mL^{-1}. The amount of IHH bound to P(PEGDMA-VI)@CA, P(PEGDMA-VI)@ECA and P(PEGDMA-VI)@ACA increases with the initial concentration of IHH and reaches saturation below 0.20 mg mL^{-1}. When the initial concentration of IHH was above 0.20 mg mL^{-1}, all microspheres could reach saturation. Note that all microspheres have been washed with DI water until no IHH

was detected, indicating that the adsorption capacities obtained were the suitable capacities for the following research. In order to conduct the research under identical experimental conditions, 0.20 mg mL^{-1} of IHH concentration was chose in the preparation of MIMs.

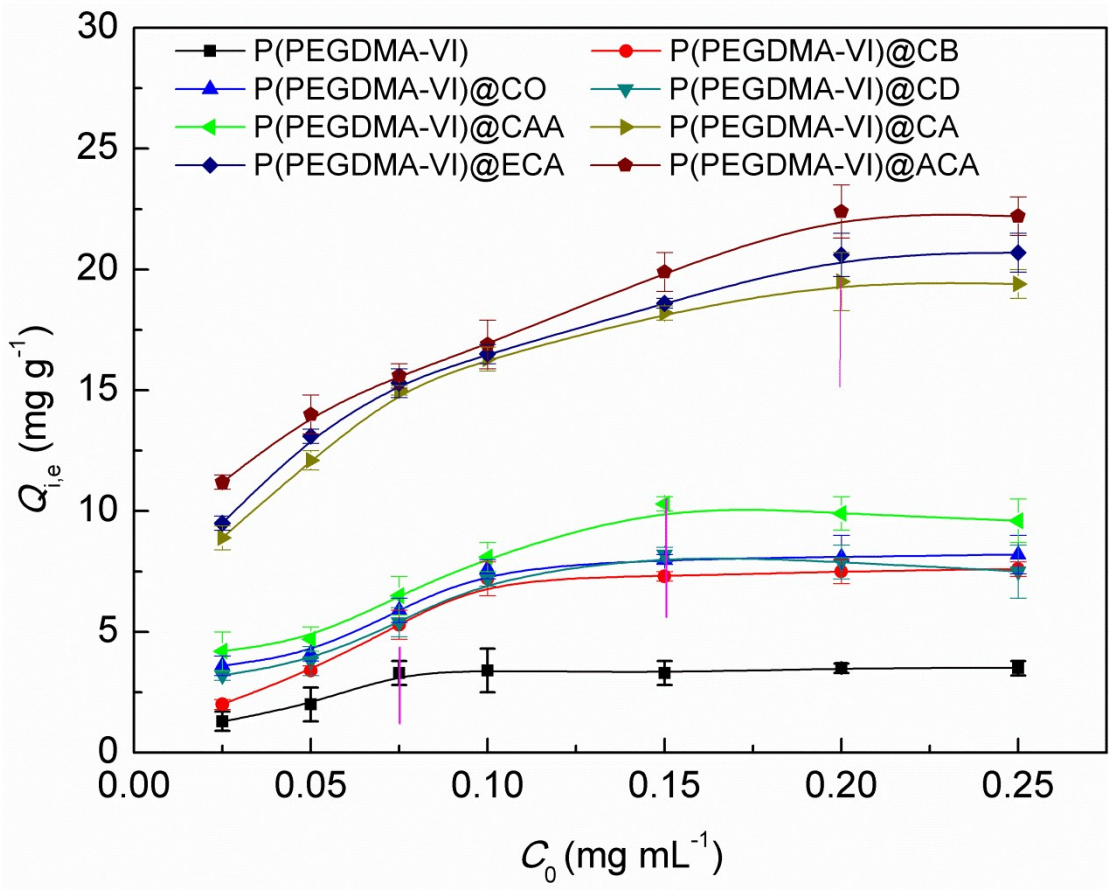

Fig. S1 Adsorption isotherms of P(PEGDMA-VI) and different IL-functionalized microspheres (10.0 mg microspheres were incubated in 10.0 mL of IHH PBS solution for 24 h at 25 °C).

2. Adsorption isotherms of MIMs

In order to evaluate the adsorption behaviors of MIMs ($MIMs^{CA}$, $MIMs^{ECA}$ and $MIMs^{ACA}$), adsorption isotherm experiments were performed at different initial IHH concentrations from 0.025 to 0.2 mg mL^{-1}. Here, the nonlinear form of the Langmuir[1] and Freundlich[2] isotherm models were used to analyze experimental data as follows:

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_{\max}} + \frac{1}{K_L Q_{\max}} \quad (1)$$

$$Q_e = K_F C_e^{1/n} \quad (2)$$

where Q_e and Q_{max} were the experimental adsorption capacity to IHH and theoretical

maximum adsorption capacity of polymer (mg g^{-1}), respectively; C_e was the concentration of IHH in equilibrium solution (mg mL^{-1}); K_L (L mg^{-1}) was the Langmuir adsorption constant; K_F (mg g^{-1}) and n were the Freundlich adsorption equilibrium constants.

As illustrated in Fig. S2, the adsorption capacities of $MIMs^{CA}$, $MIMs^{ECA}$ and $MIMs^{ACA}$ for IHH increased with the increasing initial concentration of IHH. Compared with adsorption capacities of $MIMs^{CA}$ and $MIMs^{ECA}$, the adsorption capacity of was $MIMs^{ACA}$ higher. The calculated Langmuir and Freundlich adsorption equilibrium constants were summarized in Table S1. According to correlation coefficients (R^2), it was noted that Langmuir isotherm model gave a better fit than Freundlich in the range of concentrations, suggesting that there was only one kind of binding site in $MIMs^{CA}$, $MIMs^{ECA}$ and $MIMs^{ACA}$, and the binding sites were homogeneous in respect to the affinity for IHH which were formed due to the template effect in the imprinting process. K_L of $MIMs^{CA}$, $MIMs^{ECA}$ and $MIMs^{ACA}$ are estimated to be 0.0190, 0.0242 and 0.0251 L mg^{-1}, respectively. The difference of K_L of $MIMs^{CA}$, $MIMs^{ECA}$ and $MIMs^{ACA}$ indicated that the properties of alkyl chains and imprinting cavities played an important role in adsorbing the template. Particularly, $MIMs^{ACA}$ showed relatively better affinity and higher maximum adsorption capacity for IHH than the other.

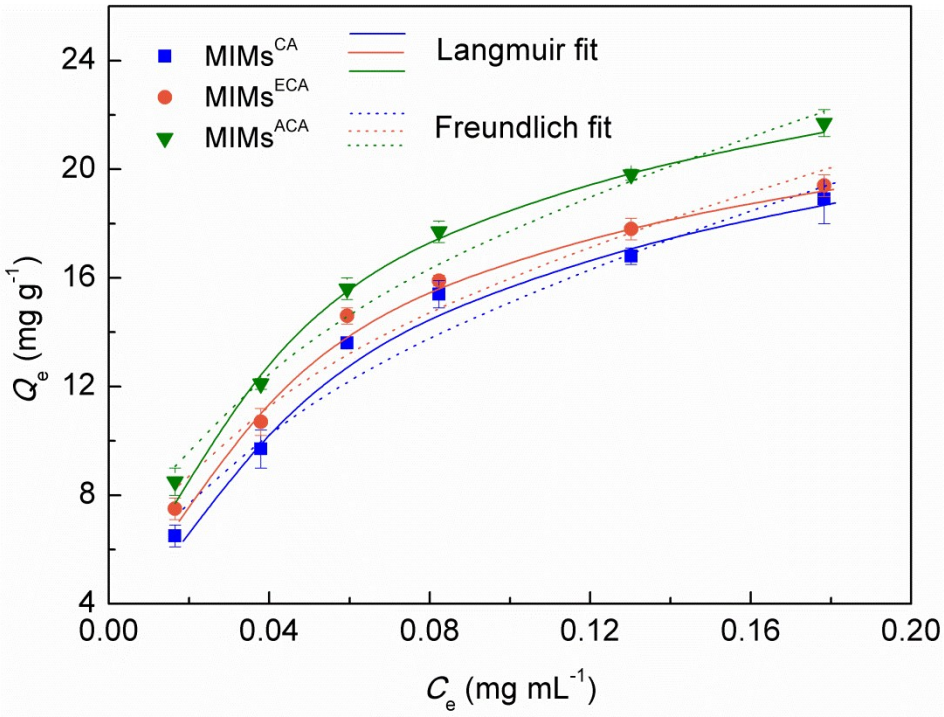

Fig. S2 Adsorption isotherms of MIMs^{CA}, MIMs^{ECA} and MIMs^{ACA} (10.0 mg microspheres were incubated in 10.0 mL IHH PBS solution for 24 h at 25 °C).

Table S1 Isotherm constants for IHH adsorption onto MIMs^{CA}, MIMs^{ECA} and MIMs^{ACA}

Isotherm model	Langmuir			Freundlich		
Sorbents	Q_m (mg g^{-1})	K_L (L mg^{-1})	R^2	K_F (mg g^{-1})	$1/n$	R^2
MIMs^{CA}	24.2	0.0190	0.9855	39.96	0.4196	0.9549
MIMs^{ECA}	23.7	0.0242	0.9927	38.19	0.3754	0.9611
MIMs^{ACA}	25.9	0.0251	0.9902	42.38	0.3718	0.9789

3. Rebinding kinetics study of MIMs

The adsorption dynamic of MIMs^{CA}, MIMs^{ECA} and MIMs^{ACA} were investigated. In Fig. S3, it can be seen that MIMs^{CA}, MIMs^{ECA} and MIMs^{ACA} showed a rapid increase in the first 15 min and then the adsorption rate became slow from 15 min to 30 min. After about 30 min, the adsorption process reached equilibrium which indicated that the template molecules could reach the imprinted cavities of MIMs^{CA}, MIMs^{ECA} and MIMs^{ACA} easily and quickly. A large amount of electrostatic binding sites provided by imidazolium groups of MIMs^{CA}, MIMs^{ECA} and MIMs^{ACA} could

attract oppositely charged sites of carboxyl groups in IHH, which induced the rapid matching of IHH. Moreover, the combination of the surface imprinting technology also could endow the surface–imprinted microspheres with lower mass–transfer resistance.[3] This confirmed that the imprinted materials obtained in this work exhibited a rapid adsorption rate owing to the electrostatic driving force and existence of surface–imprinted cavities.

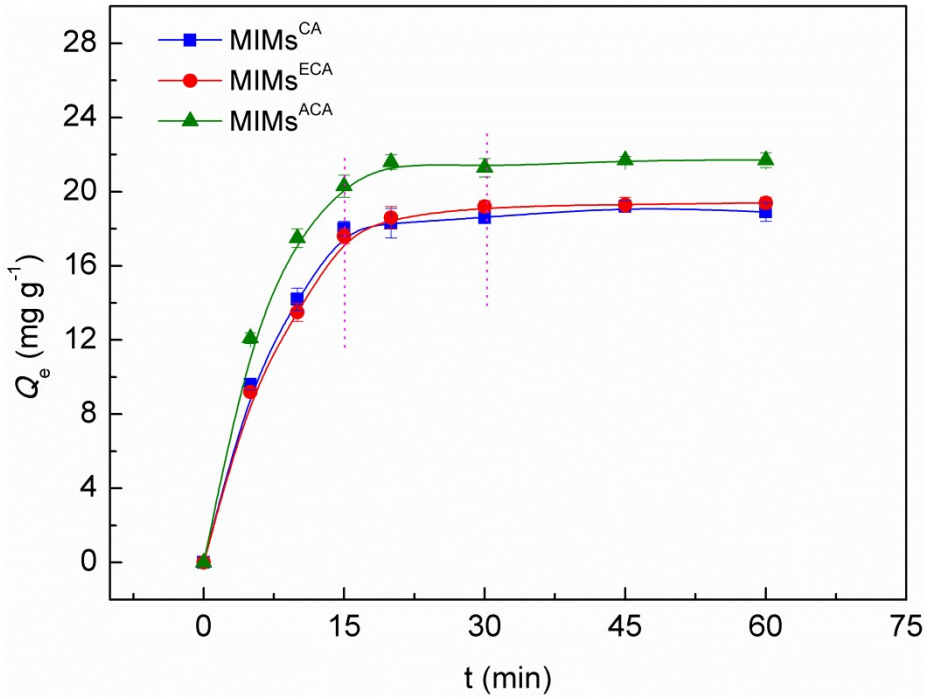

Fig. S3 Adsorption kinetic curves of IHH on $MIMs^{CA}$, $MIMs^{ECA}$ and $MIMs^{ACA}$ (10.0 mg microspheres were incubated in 10.0 mL 0.2mg mL^{-1} IHH PBS solution for 24 h at 25 ^{o}C).

References

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