Electronic supplementary information (ESI) for:

Preparation of highly cross-linked raspberry-like nano-microspheres and surface tailoring for controlled an immunostimulating peptide adsorption

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1. Adsorption isotherms of ionic liquid–functionalized raspberry–like nano–microspheres

Adsorption isotherms were performed at different initial peptide concentrations from 0.025 to 0.25 mg mL^{-1} (PBS solution, pH=7.40). The microspheres (10 mg) were washed once with 0.01 M PBS solution (pH=7.40) and then 10 mL IHH PBS solution was added. The mixture was magnetically stirred at 300 rpm for 2 h at 25 ^{o}C for the adsorption to occur. The IL-functionalized raspberry–like nano–microspheres adsorbed IHH were then collected and rinsed with PBS solution (pH=7.40) to remove any excess peptide solution 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 reached saturation at 0.075 mg mL^{-1}. For 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 and P(PEGDMA-VI)@ECA increased with the initial concentration of IHH and reached 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 PBS (pH=7.40) until no IHH was detected, indicating that the adsorption capacities obtained were the saturated capacities.

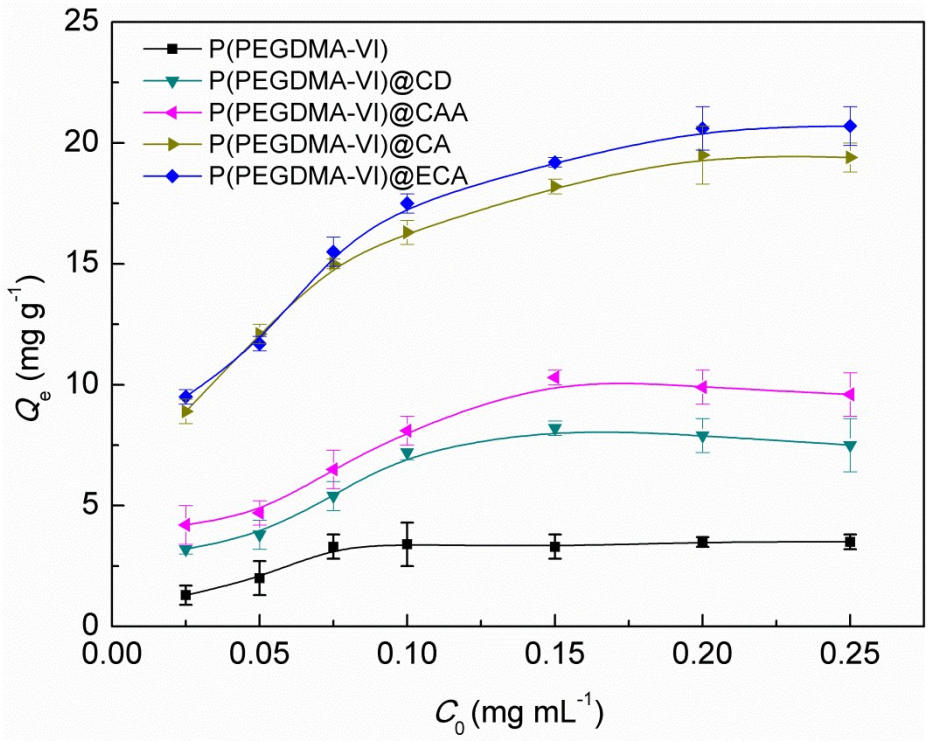

Fig. S1 Adsorption isotherms of P(PEGDMA-VI) and IL-functionalized raspberry–like nano–microspheres

Since P(PEGDMA-VI)@CA and P(PEGDMA-VI)@ECA nano-microspheres showed relatively more excellent binding properties for IHH than the other nano-microspheres, 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 nano-microspheres (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.

The calculated Langmuir and Freundlich adsorption equilibrium constants of P(PEGDMA-VI)@CA and P(PEGDMA-VI)@ECA nano-microspheres 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 sites on the surface of P(PEGDMA-VI)@CA and P(PEGDMA-VI)@ECA nano-microspheres, and the binding sites were homogeneous in respect to the affinity for IHH molecules. K_L of P(PEGDMA-VI)@CA and P(PEGDMA-VI)@ECA nano-microspheres were estimated to be 0.0226 and 0.0202 L mg^{-1}, respectively. The difference of K_L of P(PEGDMA-VI)@CA and P(PEGDMA-VI)@ECA indicated that the properties of alkyl chains played a different role in adsorbing IHH molecules. That is to say, the binding of nano-microspheres for biomolecules could be controlled by well-designed surface chemical properties.

Table S1 Isotherm constants for IHH adsorption onto P(PEGDMA-VI)@CA and P(PEGDMA-VI)@ECA

Isotherm model	Langmuir			Freundlich		
Nano-microsphere	Q_m (mg g^{-1})	K_L (L mg^{-1})	R^2	K_F (mg g^{-1})	$1/n$	R^2
P(PEGDMA-VI)@CA	23.6	0.0226	0.9986	35.6	0.3544	0.9930
P(PEGDMA-VI)@ECA	25.6	0.0202	0.9924	38.8	0.3734	0.9894

2. Rebinding kinetics study of ionic liquid–functionalized raspberry–like nano–microspheres

The adsorption dynamic of IL-functionalized raspberry–like nano–microspheres were investigated. As shown in Fig. S2, all IL-functionalized raspberry–like nano–microspheres showed higher adsorption capacities than P(PEGDMA-VI) microspheres in the whole adsorption process. Moreover, all IL-functionalized raspberry–like nano–microspheres showed a rapid increase in the initial stage and then the adsorption rate became slow along with the increase of adsorption time. After about 20 min, the adsorption process reached equilibrium which indicated that the IHH molecules could reach the binding sites of IL-functionalized raspberry–like nano–microspheres in a short time. Particularly, for P(PEGDMA-VI)@CD and

P(PEGDMA-VI)@CAA, favourable hydrophilicity and a large amount of electrostatic binding sites of imidazolium groups determined the excellent binding capacities and induced the rapid matching of IHH molecules. In addition, the binding sites distributed on the surface of P(PEGDMA-VI)@CD and P(PEGDMA-VI)@CAA also could reduce the mass-transfer resistance of IHH molecules [3], which endow them with rapid binding properties.

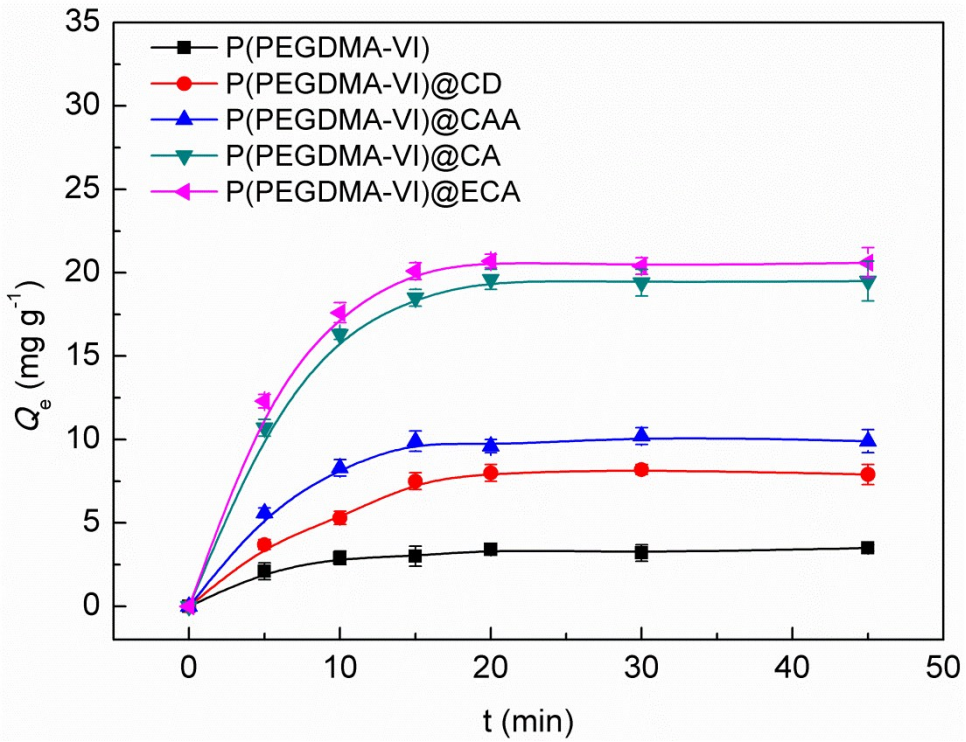

Fig. S2 Adsorption kinetic curves of IHH on P(PEGDMA-VI) and IL-functionalized raspberry–like nano–microspheres

References

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