Appendix A-Supporting information

Protein adsorption behavior in batch and competitive conditions with nanoparticle surface imprinting

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A.1. Swelling experiments

The imprinted polymeric particles dispersed in water were recovered by centrifugation at 9000 rpm for 40 min. The swollen weight (W_W) of the particles was measured. Subsequently, the particles were freeze-dried for 24 h and weighed again to obtain the dry weight (W_D). The swelling ratio (S.R) of the polymer was then calculated as follows:

$$S.R = (W_W - W_D) / W_D \quad \text{Eq. (A.1)}$$

The S.R (Table A.1) is usually an indicator of the extent of cross-linking and hence the flexibility of the imprinted cavities. The S.R values obtained in this work were comparable to our previous work in which the values were in between 3-5.

A.2. Imprinting Efficiency

The parameter, imprinting efficiency (I.E), define the degree of template-monomer complexation[1]. Higher number of template-monomer interactions results in stronger template-monomer complexation and hence higher I.E values, assuming negligible template-template complexes. The I.E values of the protein imprinted particles were calculated based on the amount of protein adsorbed, as follows:

$$I.E = Q_{iMIP} / Q_{iNIP} \quad \text{Eq. (A.2)}$$

where Q_{iMIP} and Q_{iNIP} are the static equilibrium adsorption capacity of iMIPs and iNIPs respectively ($\mu mol\ g^{-1}$).

A.3. Selectivity parameters of the polymers

Molecular recognition selectivity was evaluated by the following parameters calculated according to the adsorbed protein concentrations of the protein imprinted and control particles obtained in the competitive adsorption tests under equilibrium conditions[2]:

Separation factor, $\alpha = K_{D1}/ K_{D2}$ Eq. (A.3)

where K_{D1} and K_{D2} are the static distribution coefficients of the template and the control molecules. K_D is the ratio of the amount of ligand adsorbed and free ligand concentration.

Relative separation factor, $\beta = \alpha_1/\alpha_2$ Eq. (A.4)

where α_1 and α_2 are the separation factors of the imprinted and control nanoparticles respectively.

A.4. Zeta potential of imprinted nanoparticles

A Zetasizer Nano-ZS (Malvern Instruments, UK) was used to measure the zeta potential of all the polymeric nanoparticles prepared.

A.5. Experimental and theoretical binding site density relation

We can theoretically calculate the maximum binding site density (N_{max}) taking RNase A as the protein of interest as follows. Based on the protein concentration used during the immobilization step, the maximum amount of RNase A immobilized on the core beads would be not more than 25 mg/g. The mass percentage of the external shell layer over the core beads in the final core–shell imprinted particles can be calculated from the magnetite encapsulation efficiency assuming negligible mass change of the core particles due to the surface modification reactions. TGA results showed that the magnetite encapsulation efficiency for the unmodified core particles and the resulting core–shell imprinted particles were 13.28 wt% and 4.44 wt%, respectively. Hence, the weight percentage of the

shell is calculated to be 66.6 % ((0.1328 – 0.0444) / 0.1328). Therefore, the maximum template amount embedded in the imprinted particles before the template removal is estimated to be 8.4 mg/g (25 / [1 + 0.666 / (1 – 0.666)]). Assuming that all the nitrogen originates from the template protein molecules, there are 169 nitrogen atoms in 1 RNase A molecule and each binding site is occupied/created by one template protein molecule, the total number of RNase A molecules immobilized ($N_{RNase\ A}$) can be calculated. Thus, N_{max} is the ratio of $N_{RNase\ A}$ to N_P, where is the total number of nanoparticles.

$$N_{\max} = \frac{N_{RNaseA}}{N_P} = \frac{2.14 \times 10^{18}}{N_P}$$

From the single protein adsorption kinetics, the measured specific RNase A rebinding on the imprinted particles achieved 89.3 mg/g. The experimental binding site density in this case is

$$N_{Exp} = \frac{N_{RNaseA}}{N_P} = \frac{2.27 \times 10^{19}}{N_P}$$

$$N_{Exp} = 10.6 N_{\max}$$

References

1. S. Srebnik, *Chem. Mater.*, 2004, **16**, 883-888.
2. S. Lu, G. Cheng and X. Pang, *J. Appl. Polym. Sci.*, 2006, **99**, 2401-2407.

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Table A.1 Sizes and Swelling measurements

Polymer particles	Mean diameter[a] (nm)	Polydispersity	Swelling ratio
Core	368 ± 3.7	0.205	-
LiMIP	582 ± 5.3	0.012	3.54 ± 0.70
iNIP	513 ± 4.8	0.065	2.28 ± 0.38
RiMIP	553 ± 4.6	0.109	3.94 ± 0.59

91 [a]Obtained from Dynamic light scattering (DLS) measurements.

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Table A.2 Surface atomic composition (%) of all particles from XPS wide scan spectra

Polymer particles	C	O	N
Core	71.63	28.37	0.00
RNase A immobilized core	68.50	27.01	4.49
Lys immobilized core	72.37	23.06	4.57
RiMIP (Before hydrolysis)	44.98	52.89	2.13
LiMIP (Before hydrolysis)	73.42	24.42	2.16
RiMIP (After hydrolysis)	63.98	35.62	0.40
LiMIP (After hydrolysis)	73.36	26.64	0.35

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Table A.3 Physico-chemical properties of the proteins

Property	Lys	RNase A	BSA
Molecular mass ($gmol^{-1}$)	14600	13600	66000
Size (nm^3)	4.5 x 3.0 x 3.0	3.8 x 2.8 x 2.2	4.0 x 4.0 x 14
Isoelectric point (IEP)	11.1	9.4	4.8
Adiabatic compressibility (k_S) ($x10^{-6}$ cm^3 g^{-1} bar^{-1})	3.6	0.8	6.5

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Table A.4 Imprinting efficiencies

Particles imprinted with	Single[a]	Binary[b]	Ternary[c]
Lys	7.70	6.75	1.93
RNase A	6.27	1.20	0.74

[a]Initial concentration of Lys: 1.8 mg mL^{-1} & RNase A: 2 mg mL^{-1}.
[b]An equimolar mixture of Lys & RNase A were used.
[c]A 1:1:2 mixture of Lys: RNase A: Albumin was used.

Table A.5 Selectivity parameters of the polymers

Polymer particles	K_D (mL g^{-1})		α	β
	Lys	RNase A		
LiMIP	59.94	7.26	8.26 (Lys-RNase A)	27.07
iNIP	5.71	18.73	0.31	
RiMIP	87.48	33.19	0.38 (RNase A- Lys)	0.12
iNIP	5.71	18.73	3.28	

Table A.6 Zeta potential measurements

Polymer particles	Zeta potential[a] (mV)
LiMIP	-0.0798 ± 0.09
iNIP	-0.0888 ± 0.02
RiMIP	-0.0169 ± 0.04

[a]All particles were measured in 0.01M phosphate buffered saline (PBS), pH 7.4 at 25°C

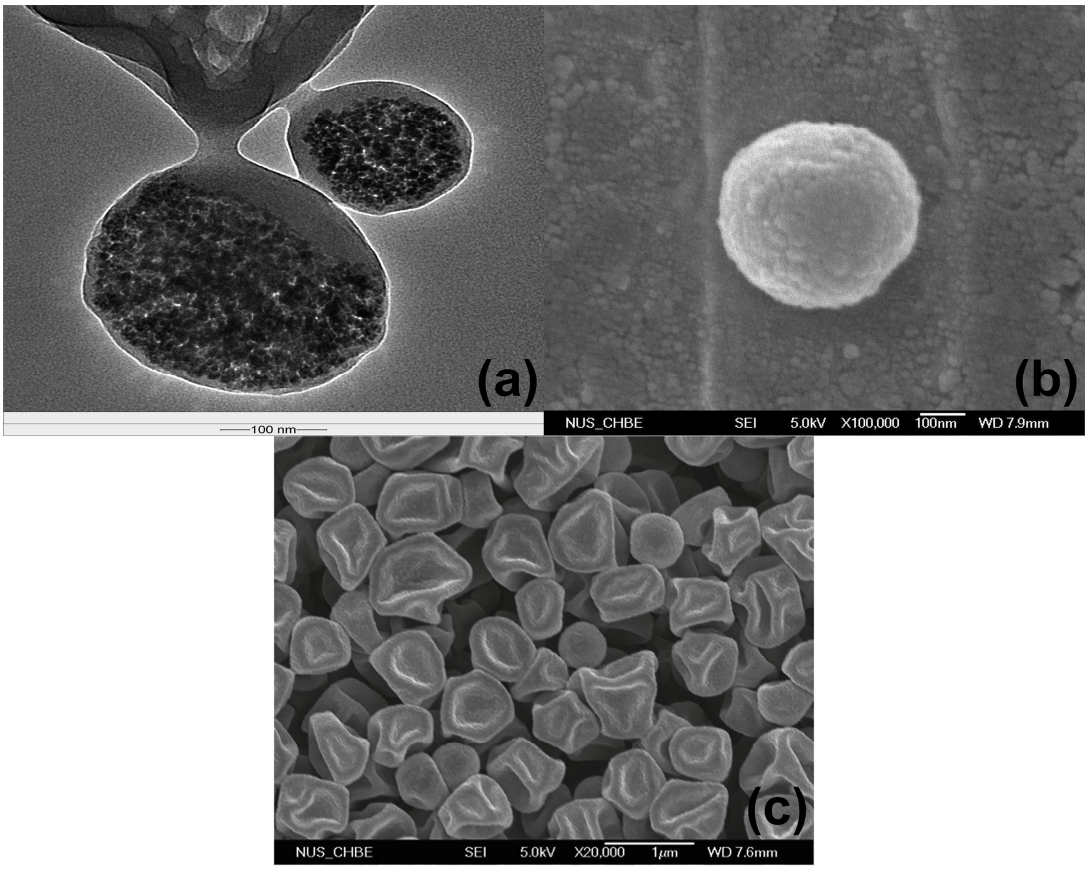


Fig. A.1 Electron micrographs of particles (a) TEM image of core particles (b) FESEM images of core and (c) core-shell nanoparticles. There was no significant morphological difference between the imprinted and control particles in the SEM images.

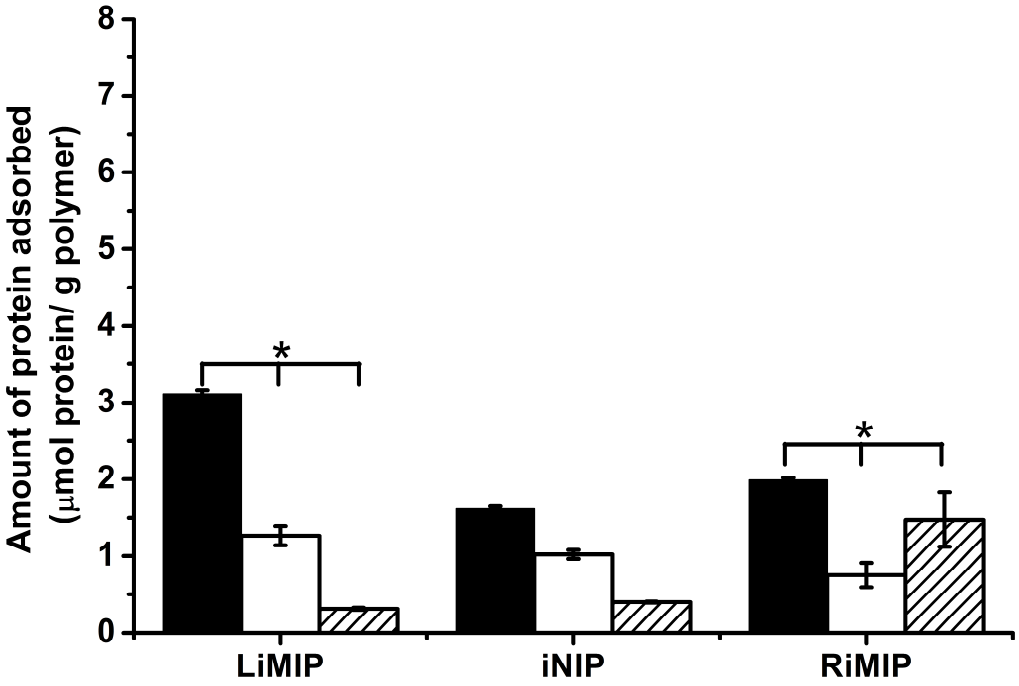
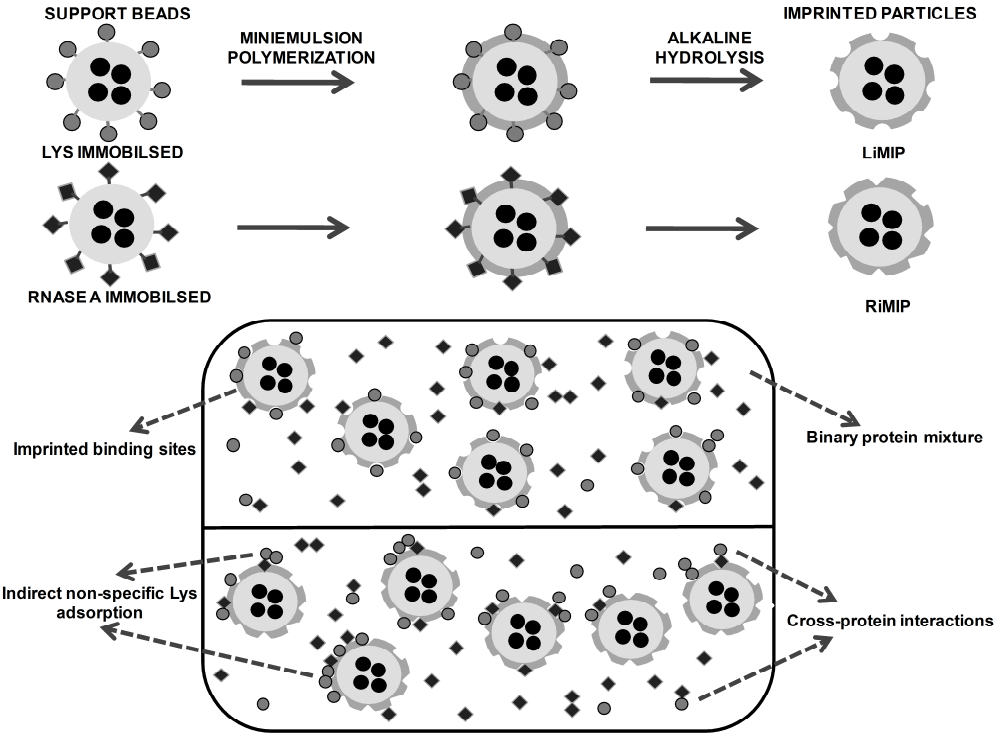


Fig. A.2 Ternary protein equilibrium adsorption analyses of LiMIPs and RiMIPs. A mixture containing 25 mol% each of Lys & RNase A and 50 mol% BSA was mixed with the nanoparticles. Error bars represent standard error. Statistical significance was denoted by *. One-way ANOVA: $p<0.05$. iNIPs were used as control samples (■, Lys; □, RNase A; ▨, BSA)



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122 **Fig. A.3** An illustration of competitive protein adsorption behaviour of Lys and RNase A imprinted
123 nanoparticles. The figure depicts the specific adsorption of Lys (template) by the LiMIPs due to the
124 imparted molecular affinity and the high non-specific adsorption of Lys (non-template in this case) by
125 the RiMIPs owing to strong cross-protein interactions during binary protein adsorption process.

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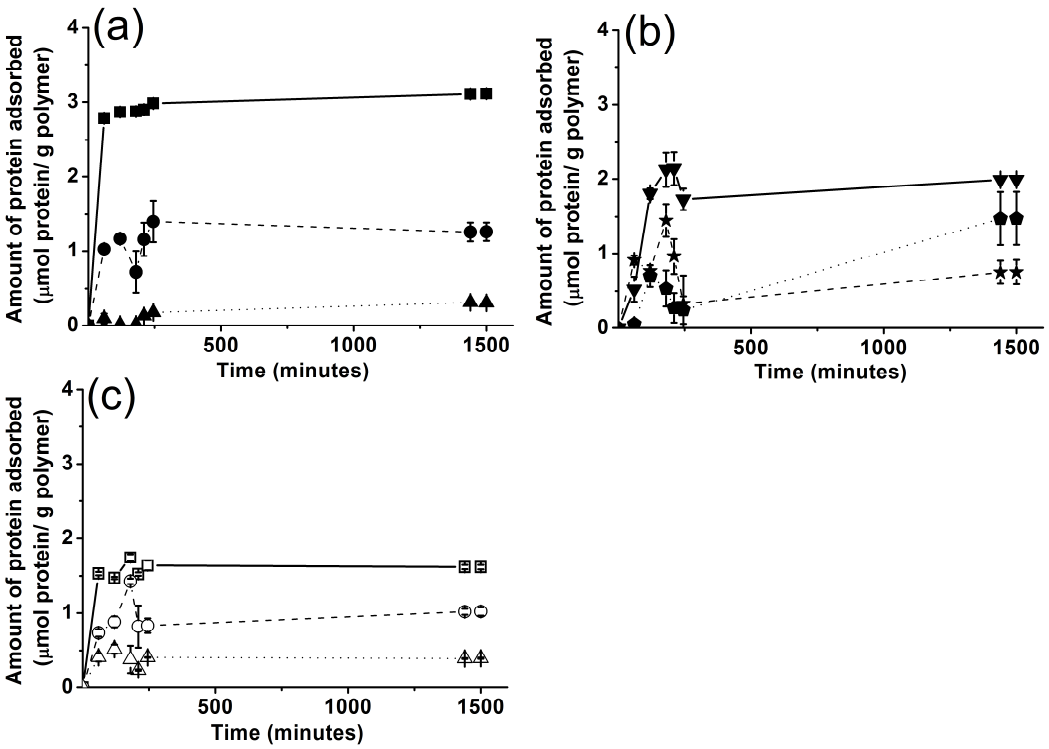

Fig. A.4 Ternary protein adsorption kinetics of (a) LiMIP (b) RiMIP (c) iNIP. iNIPs were used as control samples (■, LiMIP-Lys; ●, LiMIP-RNase A; ▲, LiMIP-BSA; ▼, RiMIP-Lys; ★, RiMIP-RNase A; ⬟, RiMIP-BSA; □, iNIP-Lys; ○, iNIP-RNase A; △, iNIP-BSA).