Solid-Phase Engineering of Molecularly Imprinted Nanoparticles (NanoMIPs): How Template and Support Drive Polymer Composition and Binding Performance

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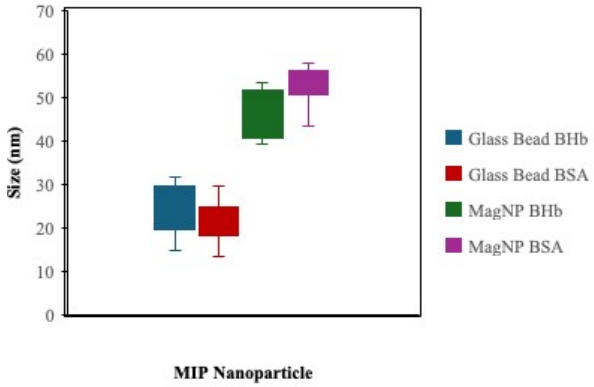

Supplementary Information

Figure S1. *Particle size distribution as estimated from SEM images (Figure 4).*

Table S1*. Calculated nanoMIP sizes (nm) as estimated from SEM images (Figure 4)*

Protein template	Magnetic Nanoparticle Yield (mg)	Glass Bead Size (nm)
BHb	52.7 ± 8.4	27.4 ± 6.0
BSA	58.6 ± 4.4	24.0 ± 4.7

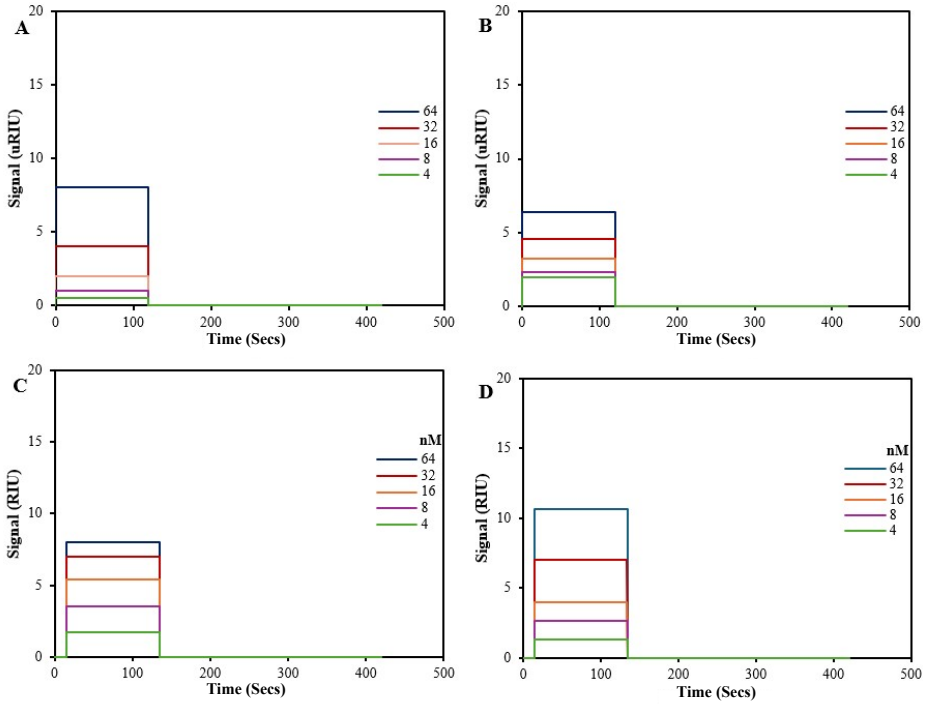

Figure S2. *Representative SPR curves showing the binding of non-target proteins (BSA, BHb) to the immobilized nanoMIPs. Five concentrations of the analyte in PBST. (**A**) BSA binding to a BHb-imprinted nanoMIP produced using glass bead solid phase. (**B**) BSA binding to a BHb-imprinted nanoMIP produced using magnetic nanoparticle solid phase. (**C**) BHb binding to a BSA-imprinted nanoMIP produced using glass bead solid phase. (**D**) BHb binding to a BSA-imprinted nanoMIP produced using magnetic nanoparticle solid phase.*

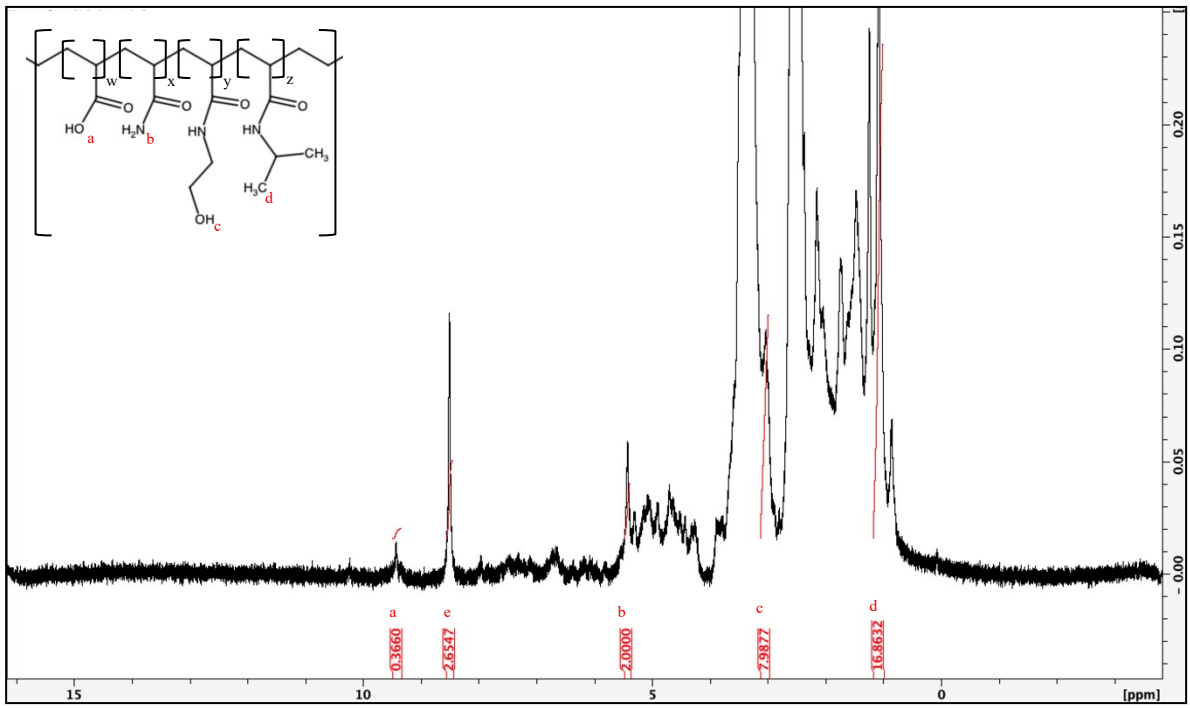

Figure S3. *[1]HNMR specrum of the BHb-imprinted nanoMIP produced using glass bead solid phase, where a = AAc, b = AAm , c = NHMAm and d = NIPAm and e = BIS.*

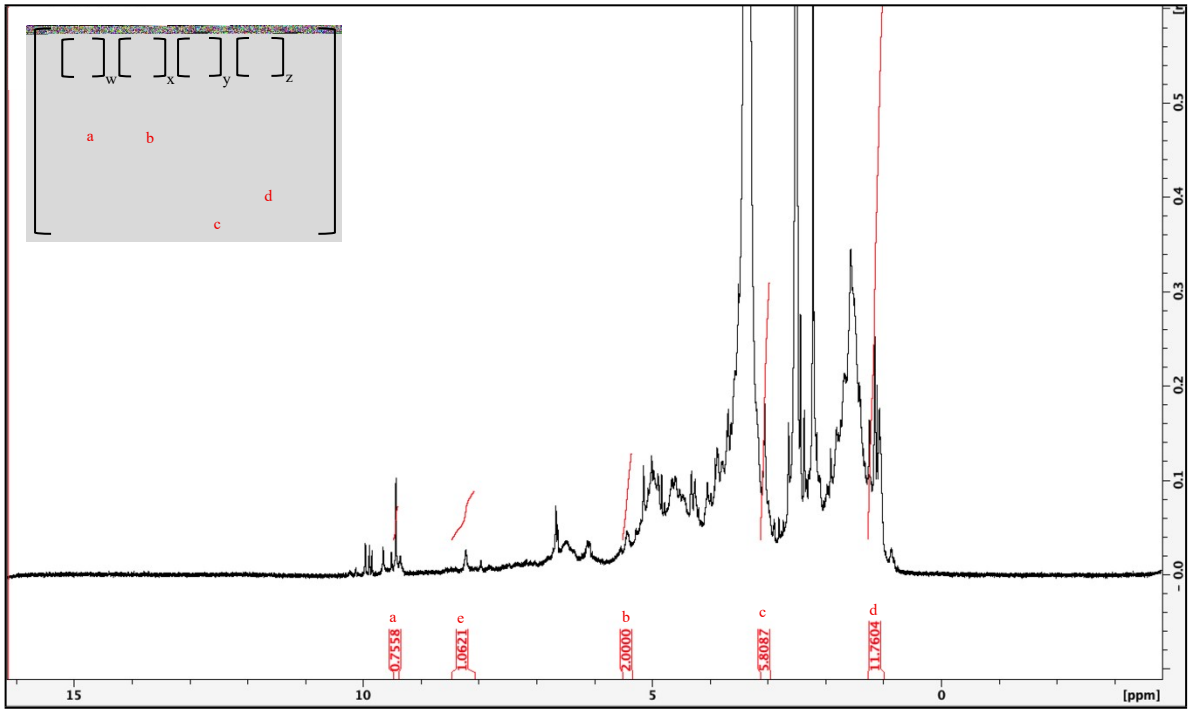

Figure S4. *[1]HNMR specrum of the BHb-imprinted nanoMIP produced using magnetic nanoparticle where a = AAc, b = AAm , c = NHMAm and d = NIPAm and e = BIS solid phase.*

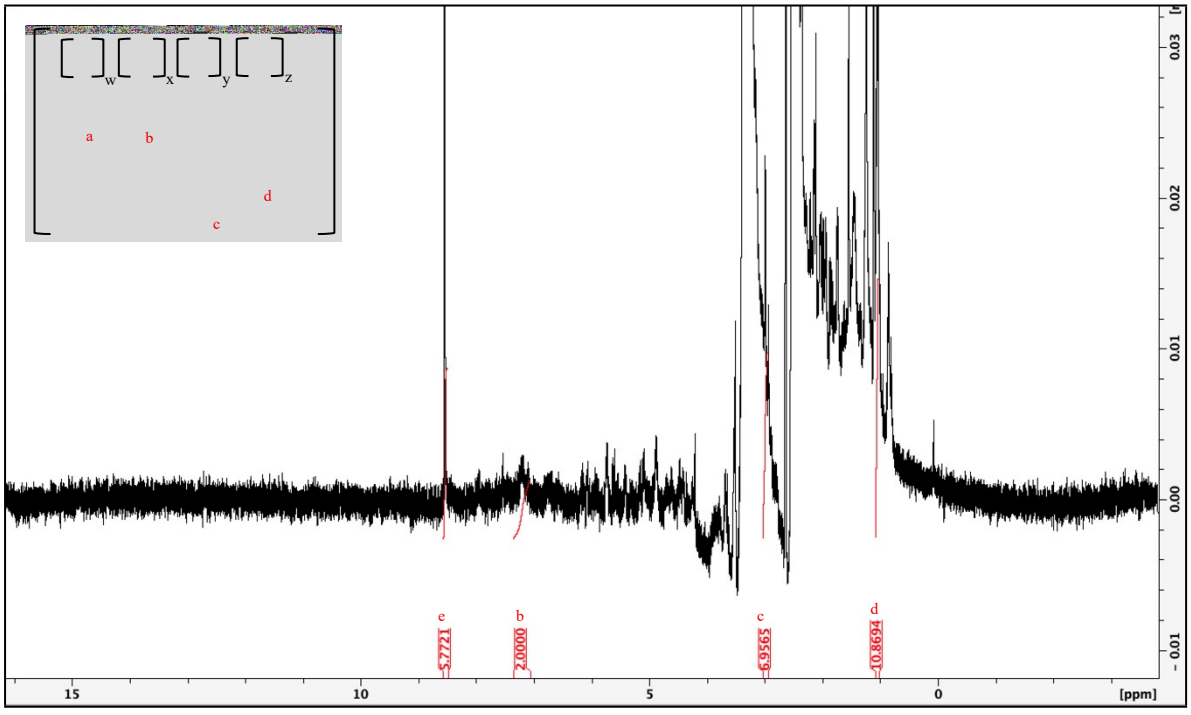

Figure S5. *[1]HNMR specrum of the BSA-imprinted nanoMIP produced using glass bead solid phase where a = AAc, b = AAm , c = NHMAm and d = NIPAm and e = BIS.*

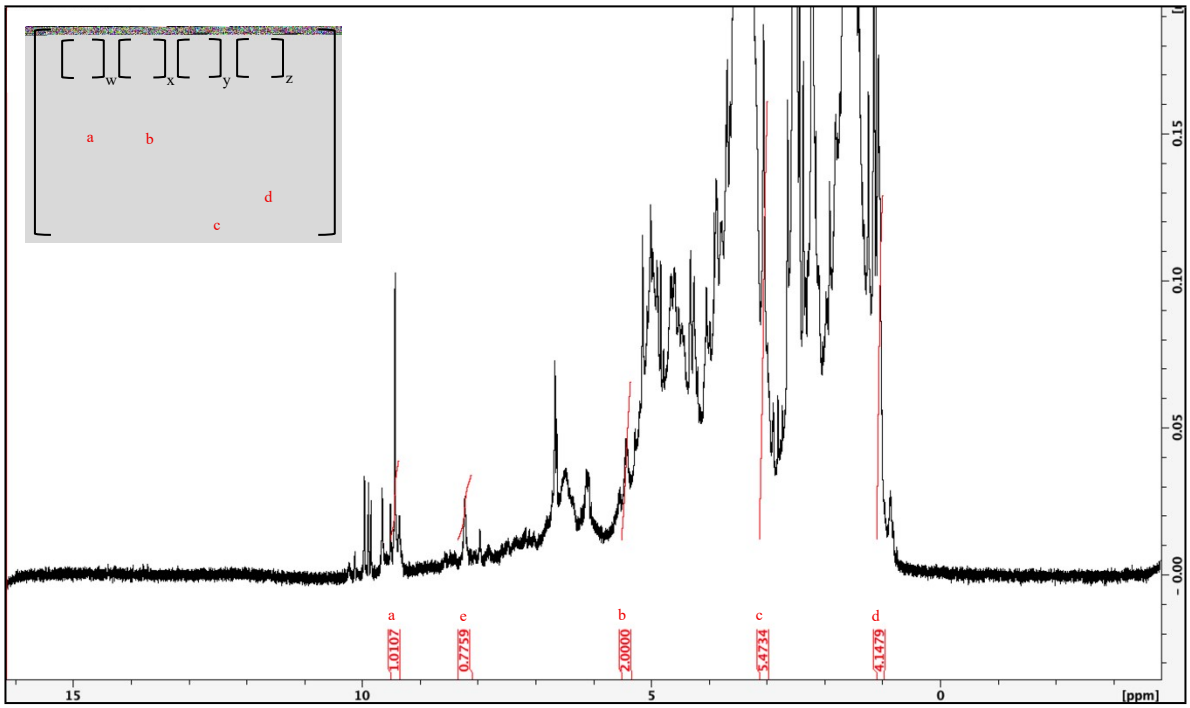

Figure S6. *[1]HNMR specrum of the BSA-imprinted nanoMIP produced using magnetic nanoparticle solid phase where a = AAc, b = AAm , c = NHMAm and d = NIPAm and e = BIS.*

Calculations

Calculations for copolymer composition were made using the following equations, Firstly, the integration value per proton is calculated via the sum of integrations per repeat unit via peaks, where a = AAc, b = AAm , c = NHMAm and d = NIPAm and e = BIS and v, w, x, and z = the number of hydrogen atoms in the peak for AAc, AAm, NHAm, NIPAm and BIS respectively (Equation S1).

$$\int Per\ H = \frac{\int a}{v} = \frac{\int b}{w} = \frac{\int c}{x} = \frac{\int d}{y} = \frac{\int e}{z}$$

(S1)

For *BHb-imprinted nanoMIP produced using glass bead solid phase*

Secondly, the ratio of a, b, c, d and e is formed through the values calculated in Equation S1 and used to calculate the number of repeat units in the polymer (Equation S2), with any decimal points disregarded (as units need to be whole numbers within the polymer).

$$\frac{0.366}{1}:\frac{2}{2}:\frac{7.9877}{2}:\frac{16.8632}{6}:\frac{2.6547}{2} * 100\% = 37:100:399:281:133 = 949\ RU$$

(S2)

The sum of the ratio of repeat units is then used to calculate the overall polymer % composition (Equation S3).

$$\%\ Composition = \frac{37}{949}:\frac{100}{949}:\frac{399}{949}:\frac{281}{949}:\frac{133}{949} * 100 = 4:11:42:30:14 \quad \text{(S3)}$$

This was repeated for the rest of the nanoMIPs.

For *BHb-imprinted nanoMIP produced using magnetic nanoparticle solid phase*

The ratio of a, b, c, d and e is formed through the values calculated in Equation S1 and used to calculate the number of repeat units in the polymer (Equation S4), with any decimal points disregarded (as units need to be whole numbers within the polymer)

$$\frac{5.8087}{1}:\frac{2}{2}:\frac{0.7558}{2}:\frac{11.7604}{6}:\frac{1.0621}{2} * 100\% = 581:100:38:196:53 = 968\ RU \quad \text{(S4)}$$

The sum of the ratio of repeat units is then used to calculate the overall polymer % composition (Equation S5).

$$\%\ Composition = \frac{581}{968}:\frac{100}{968}:\frac{38}{968}:\frac{196}{968}:\frac{53}{968} * 100 = 17:10:4:20:6 \quad \text{(S5)}$$

For *BSA-imprinted nanoMIP produced using glass bead solid phase*

The ratio of a, b, c, d and e is formed through the values calculated in Equation S1 and used to calculate the number of repeat units in the polymer (Equation S6), with any decimal points disregarded (as units need to be whole numbers within the polymer).

$$\frac{0}{1}:\frac{2}{2}:\frac{6.9565}{2}:\frac{10.8694}{6}:\frac{5.27721}{2} * 100\% = 0:100:348:181:264 = 893\ RU \quad \text{(S6)}$$

The sum of the ratio of repeat units is then used to calculate the overall polymer % composition (Equation S7).

$$\%\ Composition = \frac{0}{893}:\frac{100}{893}:\frac{348}{893}:\frac{181}{893}:\frac{264}{893} * 100 = 0{:}11{:}39{:}20{:}30 \quad \text{(S7)}$$

For *BSA-imprinted nanoMIP produced using magnetic nanoparticle solid phase*

The ratio of a, b, c, d and e is formed through the values calculated in Equation S1 and used to calculate the number of repeat units in the polymer (Equation S8), with any decimal points disregarded (as units need to be whole numbers within the polymer)

$$\frac{1.0107}{1}:\frac{2}{2}:\frac{5.4734}{2}:\frac{4.1479}{6}:\frac{1.0621}{2} * 100\% = 101{:}100{:}274{:}69{:}39 = 583\ RU \quad \text{(S8)}$$

The sum of the ratio of repeat units is then used to calculate the overall polymer % composition (Equation S9).

$$\%\ Composition = \frac{101}{583}:\frac{100}{583}:\frac{274}{583}:\frac{69}{583}:\frac{39}{583} * 100 = 17{:}17{:}47{:}12{:}7 \quad \text{(S9)}$$

This gives the overall composition of the formed nanoMIP polymer, with a best estimation of the incorporated acrylic acid. The compositions of the polymers are found in Table 6 alongside the monomer:crosslinker ratio.