

Supplementary Information

Incorporation of Polymerizable Linkers into Aptamers for High-Affinity Nanoscale Molecularly Imprinted Polymer Hybrids: Analysis of Positional Selectivity

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Table S1: Synthesis Yields of polymeric nanoparticles

MIP	mass ($\mu\text{g mL}^{-1}$)
nanoMIP	90 (± 3)
AptaMIP-1	143 (± 7)
AptaMIP-2	136 (± 6)
AptaMIP-3	87 (± 5)
AptaMIP-4	213 (± 11)
AptaMIP-5	217 (± 16)
AptaMIP-6	237 (± 15)
AptaMIP-7	317 (± 17)
AptaMIP-8	287 (± 19)

Table S2: DLS particle sizing for synthesised MIPs. N = 5.

MIP	Size (nm)
nanoMIP	98.94 (± 7.4)
AptaMIP-1	101.48 (± 0.8)
AptaMIP-2	106.13 (± 6.3)
AptaMIP-3	105.47 (± 5.6)
AptaMIP-4	102.80 (± 2.8)
AptaMIP-5	101.91 (± 1.9)
AptaMIP-6	114.98 (± 1.1)
AptaMIP-7	113.76 (± 1.0)
AptaMIP-8	119.55 (± 9.5)

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Table S3: K_a and K_d values obtained for the rebinding studies from which K_D are calculated.

	Lysozyme	
Polymer	Ka	Kd
NanoMIP	$8.75 \times 10^5 (\pm 0.87 \times 10^4)$	$8.26 \times 10^{-2} (\pm 0.58 \times 10^{-3})$
AptaMIP-1	$1.60 \times 10^7 (\pm 0.23 \times 10^6)$	$2.05 \times 10^{-2} (\pm 0.33 \times 10^{-3})$
AptaMIP-2	$1.35 \times 10^7 (\pm 0.34 \times 10^6)$	$5.35 \times 10^{-2} (\pm 0.43 \times 10^{-3})$
AptaMIP-3	$1.36 \times 10^6 (\pm 0.64 \times 10^5)$	$1.63 \times 10^{-3} (\pm 0.90 \times 10^{-4})$
AptaMIP-4	$4.97 \times 10^5 (\pm 0.57 \times 10^4)$	$2.12 \times 10^{-3} (\pm 0.40 \times 10^{-4})$
AptaMIP-5	$5.01 \times 10^5 (\pm 0.36 \times 10^4)$	$4.18 \times 10^{-1} (\pm 0.89 \times 10^{-2})$
AptaMIP-6	$2.55 \times 10^5 (\pm 0.41 \times 10^4)$	$2.62 \times 10^{-1} (\pm 0.45 \times 10^{-2})$
AptaMIP-7	$2.35 \times 10^5 (\pm 0.72 \times 10^4)$	$3.60 \times 10^{-1} (\pm 0.33 \times 10^{-2})$
AptaMIP-8	$2.07 \times 10^5 (\pm 0.34 \times 10^4)$	$2.71 \times 10^{-1} (\pm 0.79 \times 10^{-2})$
	Trypsin	
Polymer	Ka	Kd
NanoMIP	$4.47 \times 10^4 (\pm 0.75 \times 10^3)$	$2.87 \times 10^{-1} (\pm 0.32 \times 10^{-2})$
AptaMIP-1	$5.88 \times 10^4 (\pm 0.62 \times 10^3)$	$2.75 \times 10^{-1} (\pm 0.10 \times 10^{-2})$
AptaMIP-2	$8.81 \times 10^4 (\pm 0.27 \times 10^3)$	$2.05 \times 10^{-1} (\pm 0.94 \times 10^{-2})$
AptaMIP-3	$8.44 \times 10^4 (\pm 0.60 \times 10^3)$	$8.23 \times 10^{-1} (\pm 0.96 \times 10^{-2})$
AptaMIP-4	$8.54 \times 10^4 (\pm 0.39 \times 10^3)$	$8.43 \times 10^{-1} (\pm 0.67 \times 10^{-2})$
AptaMIP-5	$9.06 \times 10^4 (\pm 0.98 \times 10^3)$	$8.11 \times 10^{-1} (\pm 0.70 \times 10^{-2})$
AptaMIP-6	$7.22 \times 10^4 (\pm 0.92 \times 10^3)$	$3.38 \times 10^{-1} (\pm 0.66 \times 10^{-2})$
AptaMIP-7	$7.36 \times 10^4 (\pm 0.81 \times 10^3)$	$3.60 \times 10^{-1} (\pm 0.23 \times 10^{-2})$
AptaMIP-8	$7.81 \times 10^4 (\pm 0.40 \times 10^3)$	$3.26 \times 10^{-1} (\pm 0.87 \times 10^{-2})$
	BSA	
Polymer	Ka	Kd
NanoMIP	$2.68 \times 10^4 (\pm 0.58 \times 10^3)$	$4.86 \times 10^{-1} (\pm 0.52 \times 10^{-2})$
AptaMIP-1	$9.68 \times 10^4 (\pm 0.33 \times 10^3)$	$8.71 \times 10^{-1} (\pm 0.76 \times 10^{-2})$
AptaMIP-2	$1.68 \times 10^4 (\pm 0.10 \times 10^3)$	$4.86 \times 10^{-1} (\pm 0.47 \times 10^{-2})$
AptaMIP-3	$2.51 \times 10^4 (\pm 0.24 \times 10^3)$	$5.17 \times 10^{-1} (\pm 0.88 \times 10^{-2})$
AptaMIP-4	$3.58 \times 10^4 (\pm 0.94 \times 10^3)$	$4.77 \times 10^{-1} (\pm 0.20 \times 10^{-2})$
AptaMIP-5	$4.29 \times 10^4 (\pm 0.13 \times 10^3)$	$5.03 \times 10^{-1} (\pm 0.12 \times 10^{-2})$
AptaMIP-6	$7.47 \times 10^4 (\pm 0.45 \times 10^3)$	$1.75 \times 10^{-1} (\pm 0.67 \times 10^{-2})$
AptaMIP-7	$6.97 \times 10^4 (\pm 0.51 \times 10^3)$	$2.09 \times 10^{-1} (\pm 0.84 \times 10^{-2})$
AptaMIP-8	$5.34 \times 10^4 (\pm 0.38 \times 10^3)$	$2.26 \times 10^{-1} (\pm 0.21 \times 10^{-2})$

Supplementary Information

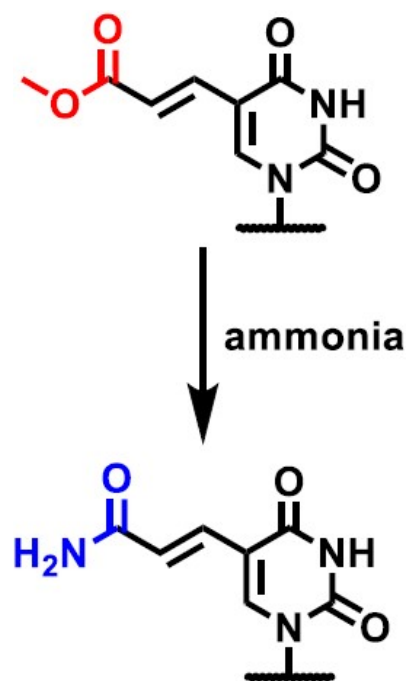
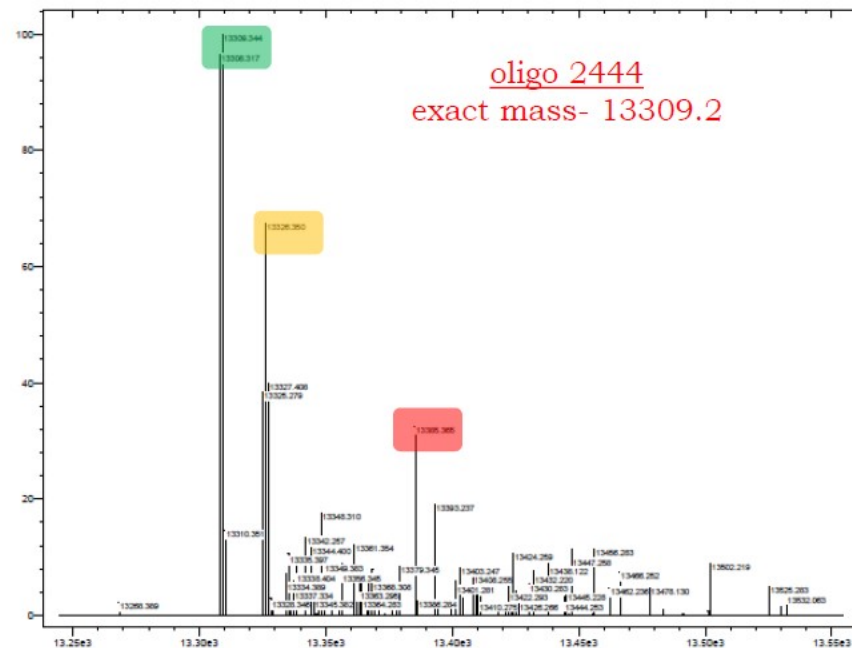
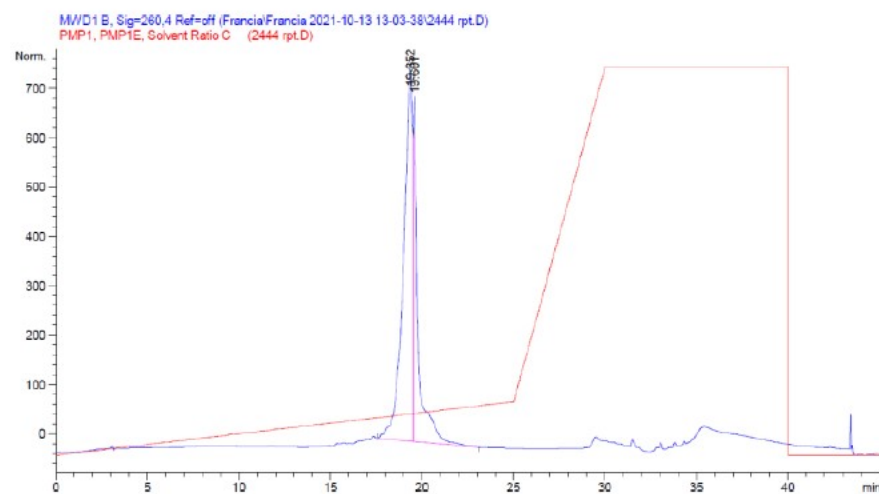


Figure S1: Amination of ester during synthesis

Supplementary Information

(a) AptaMIP-1 oligo

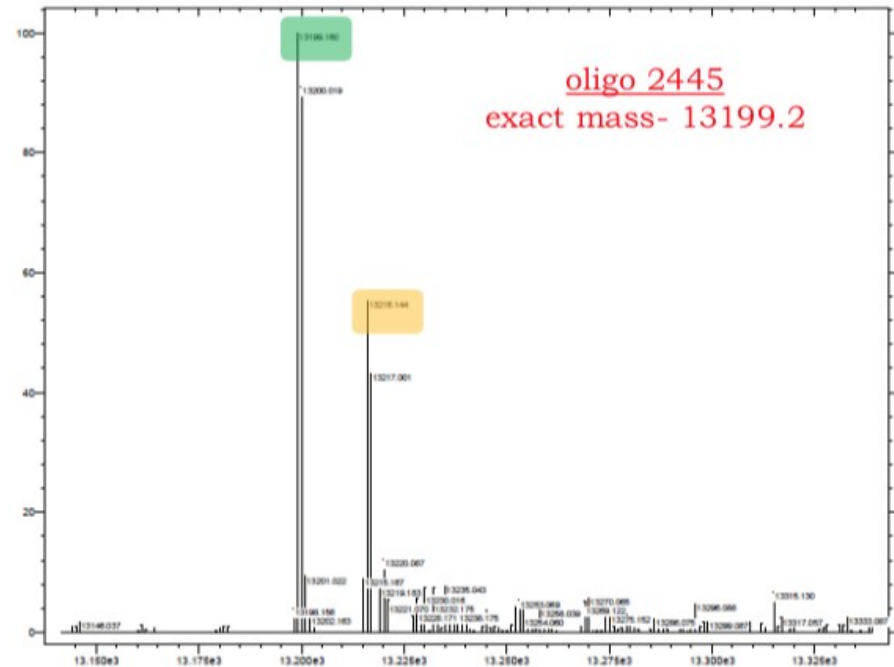
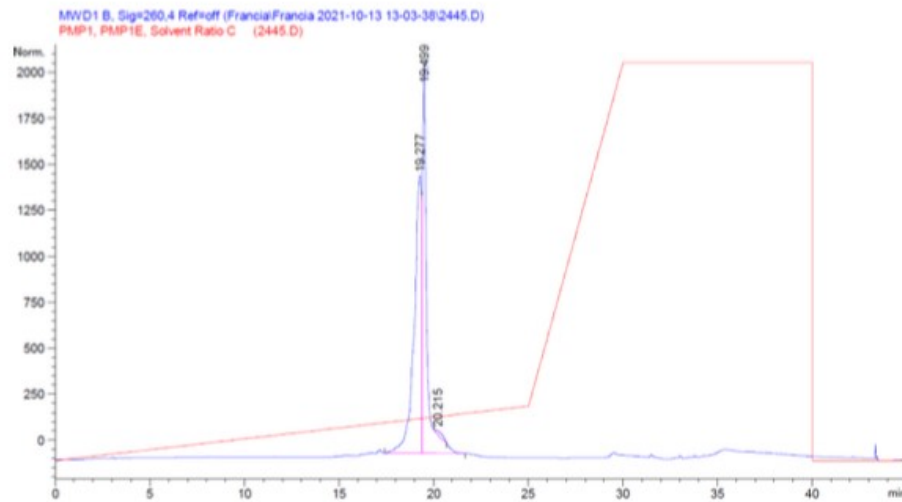
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(6 mods even spaced throughout)



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(b) AptaMIP-2 oligo

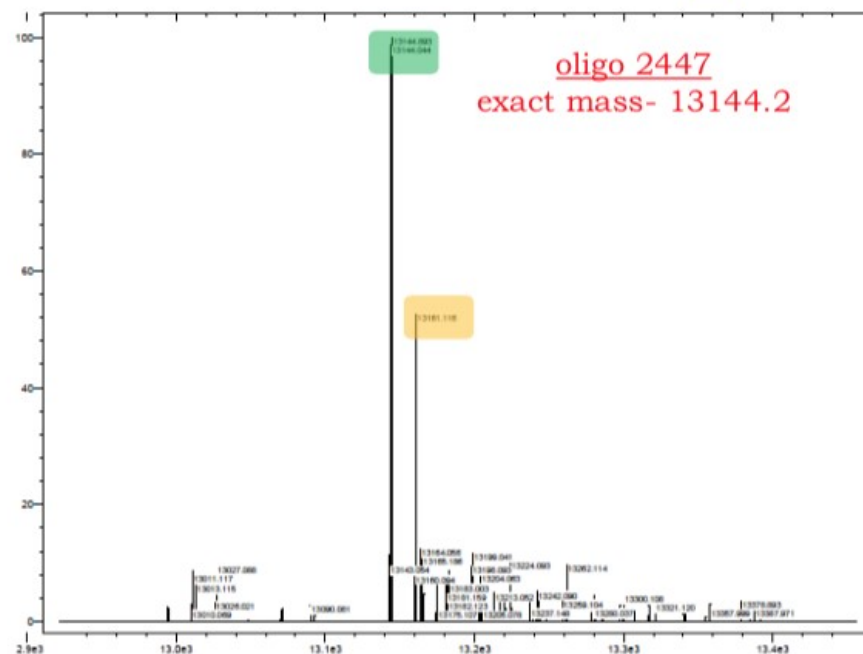
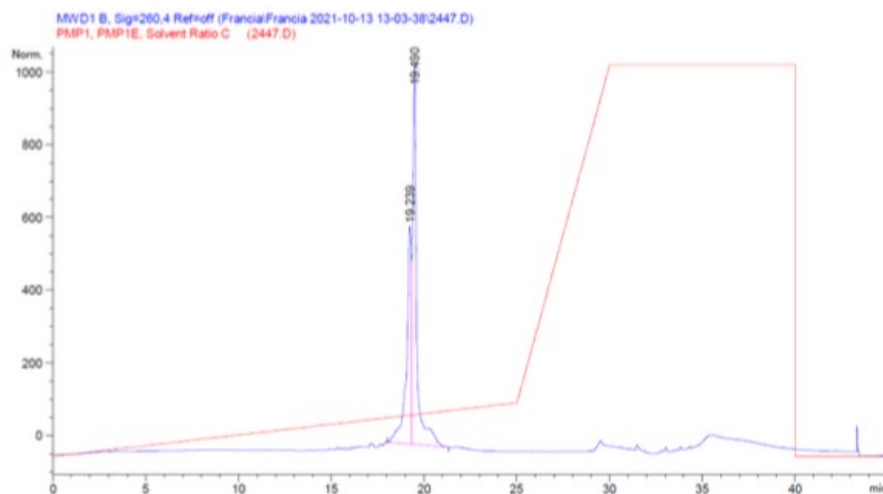
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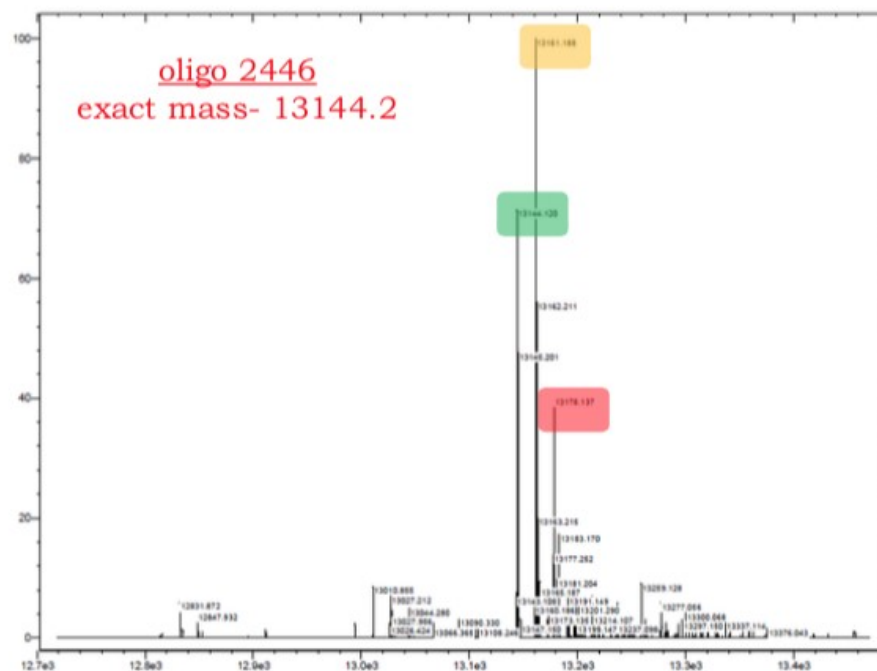
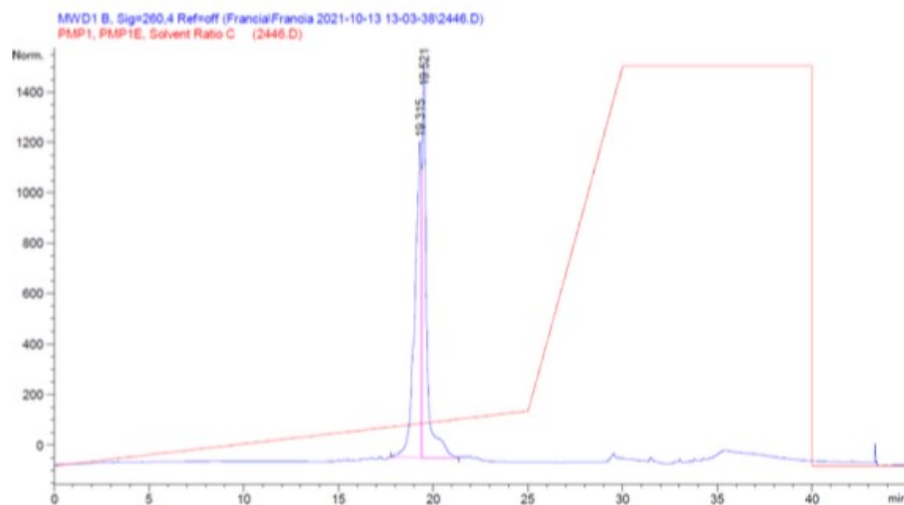
(c) AptaMIP-3 oligo

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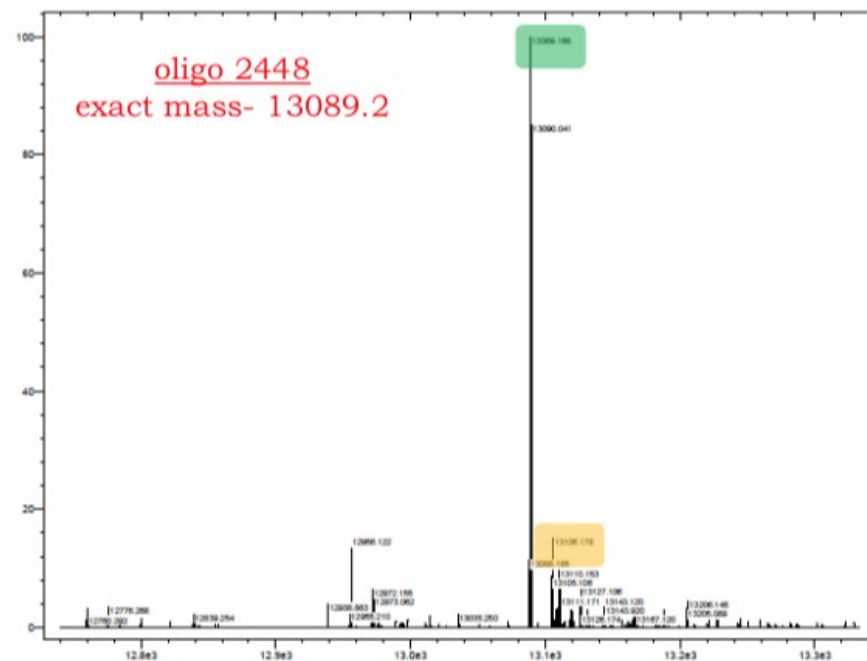
(d) AptaMIP-4 oligo

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(e) AptaMIP-5 oligo

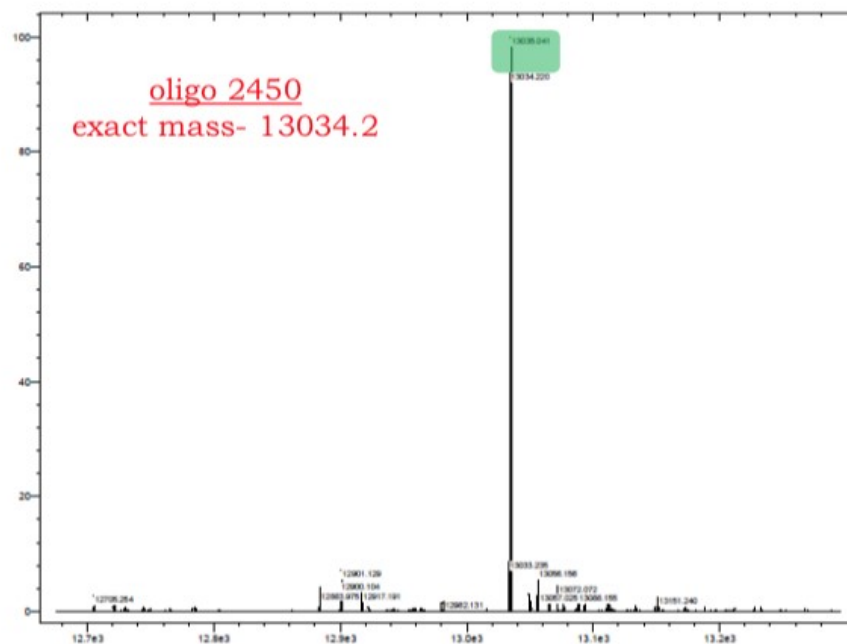
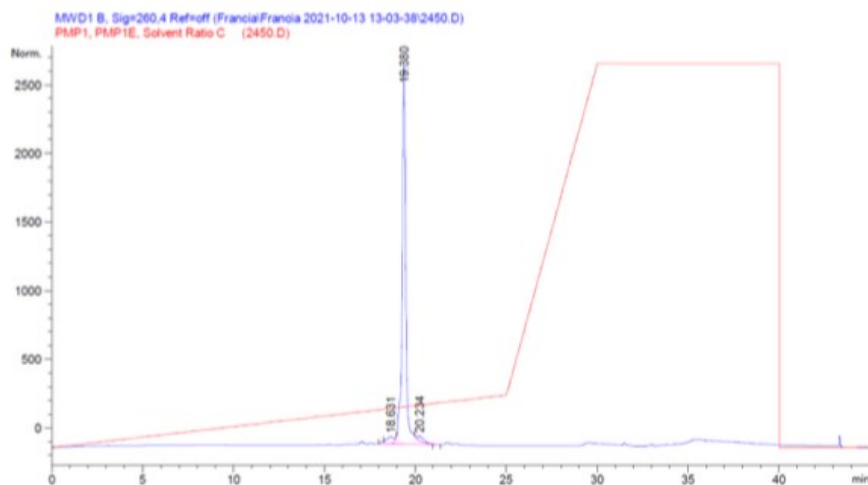
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PMP1, PMP1E, Solvent Ratio C (2448.D)



Supplementary Information

(f) AptaMIP-6 oligo

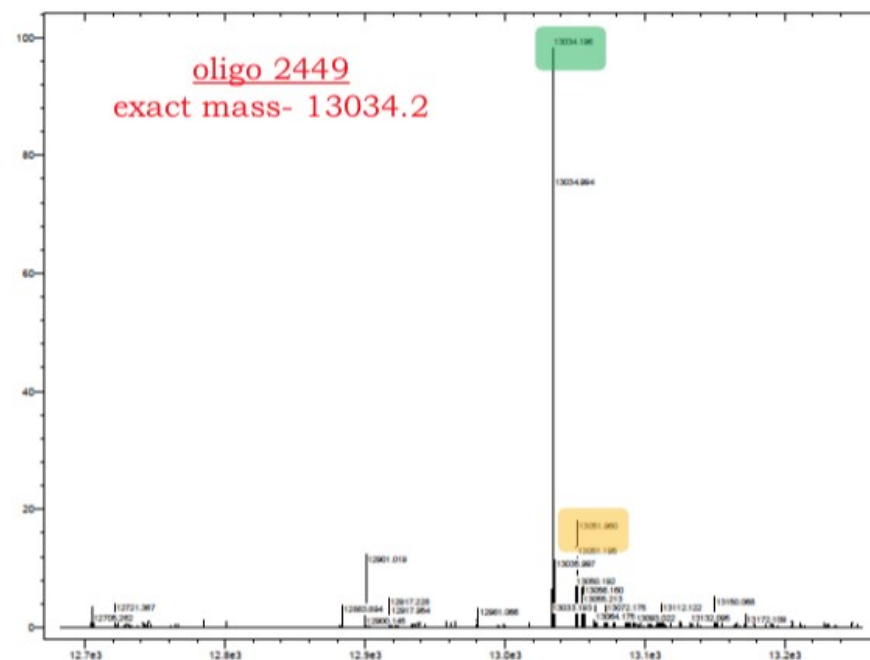
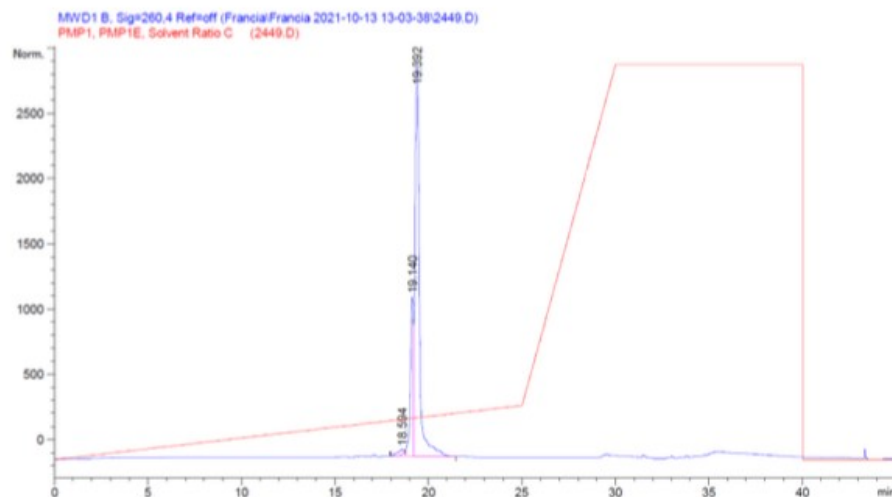
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(1 mod at 5' end)



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(g) AptaMIP-7 oligo

5'- ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG -3'
(1 mod at 3' end)



(h) AptaMIP-8 oligo

ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG
(1 mod in centre)

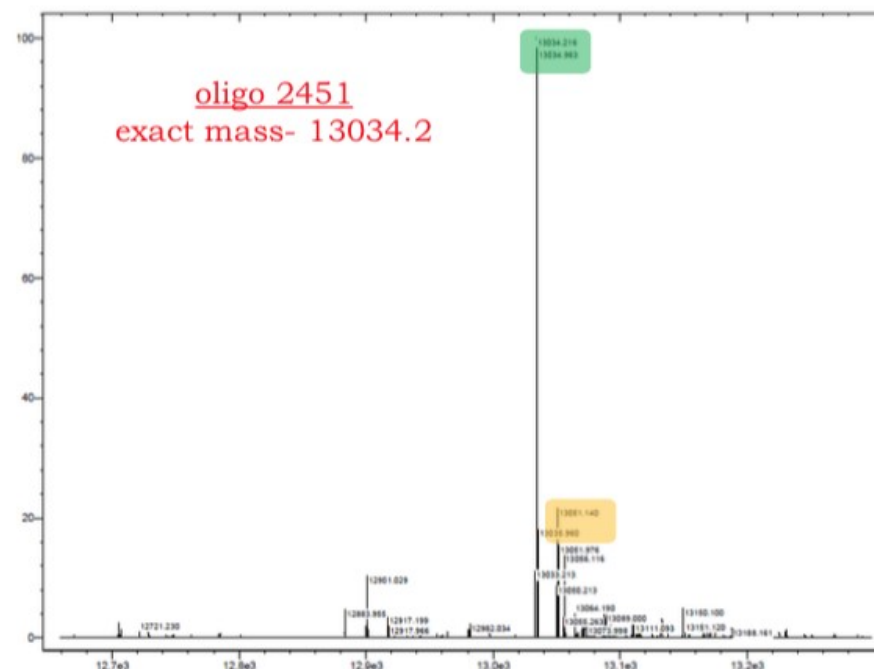
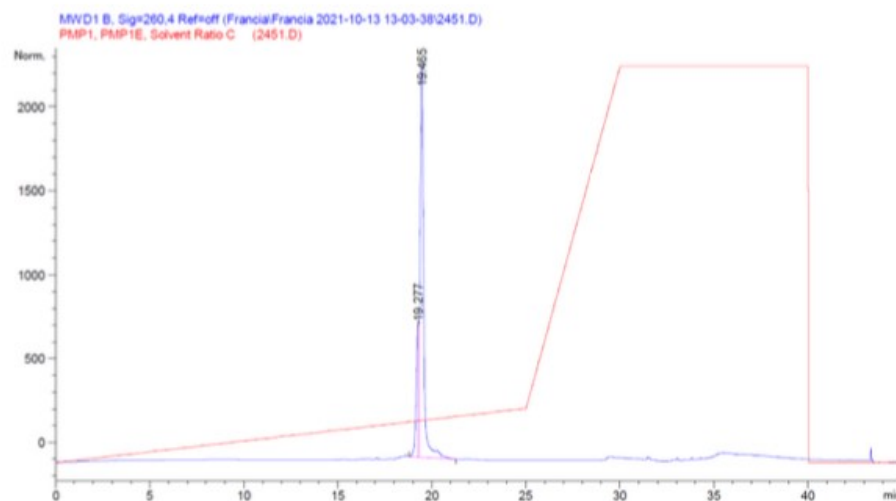


Figure S2: Reversed phase HPLC and ES-MS spectra of APTA-MIP oligonucleotide sequences. The MS spectra show highlighted peaks signals for the expected mass (full conversion to amide in green) and selected higher masses (one unconverted ester in orange and more than one unconverted ester in red). The authors are aware that the images are not the highest quality (due to the size of image and limitations of the supplementary docx. They are provided here as reference, hence the colour coding. Our lab runs an open data policy and, upon reasonable request, the data files can be requested via the corresponding author.

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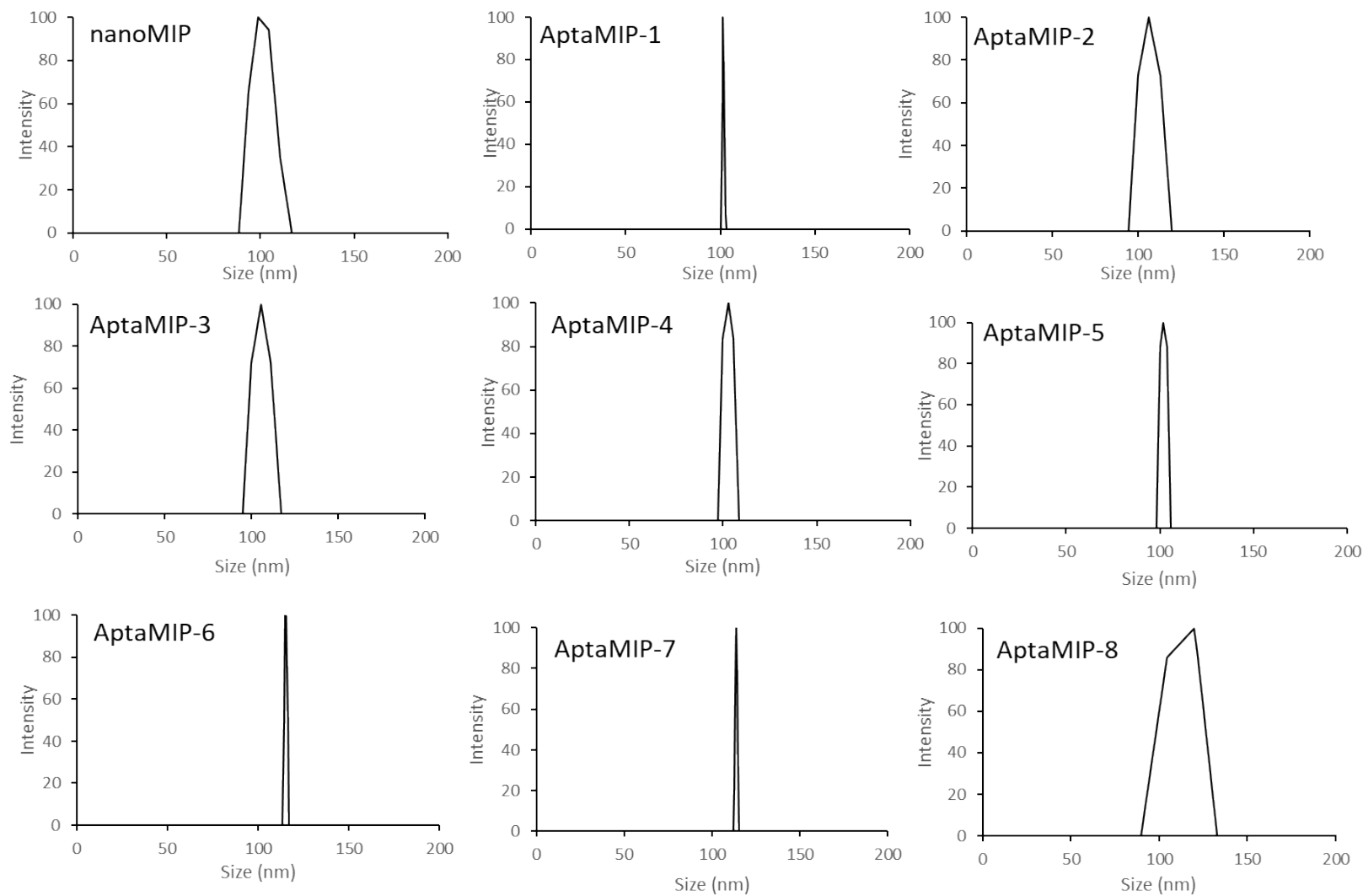


Figure S3a: DLS particle sizing for synthesised MIPs. Representative Curves. Experimental ran n=3 to acquire SD's.

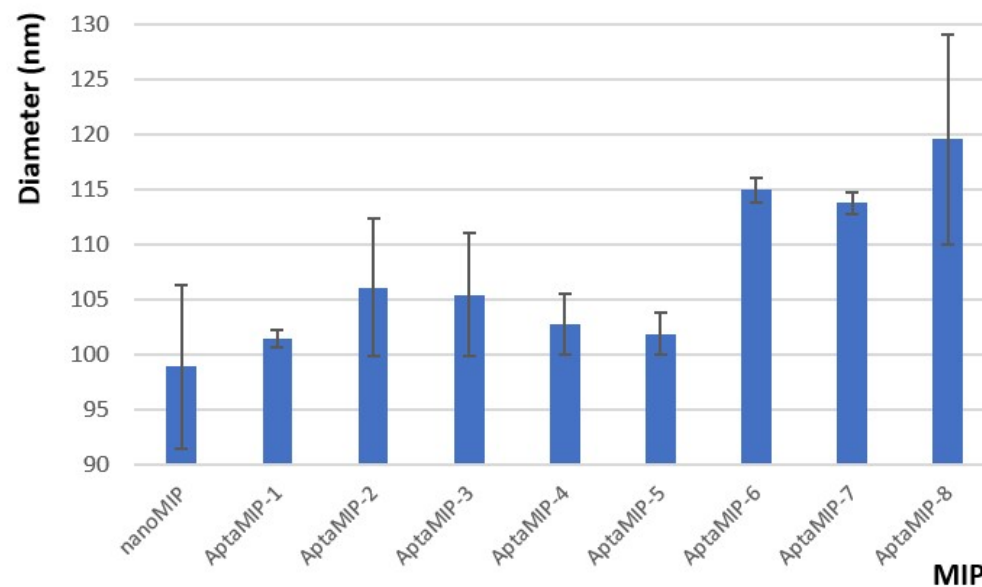


Figure S3b: DLS particle sizing for the synthesised MIPs. N = 5. Error bars highlight sample particle size distribution (Supplementary Figure S3). Truncated y-axis for clarity.

Supplementary Information

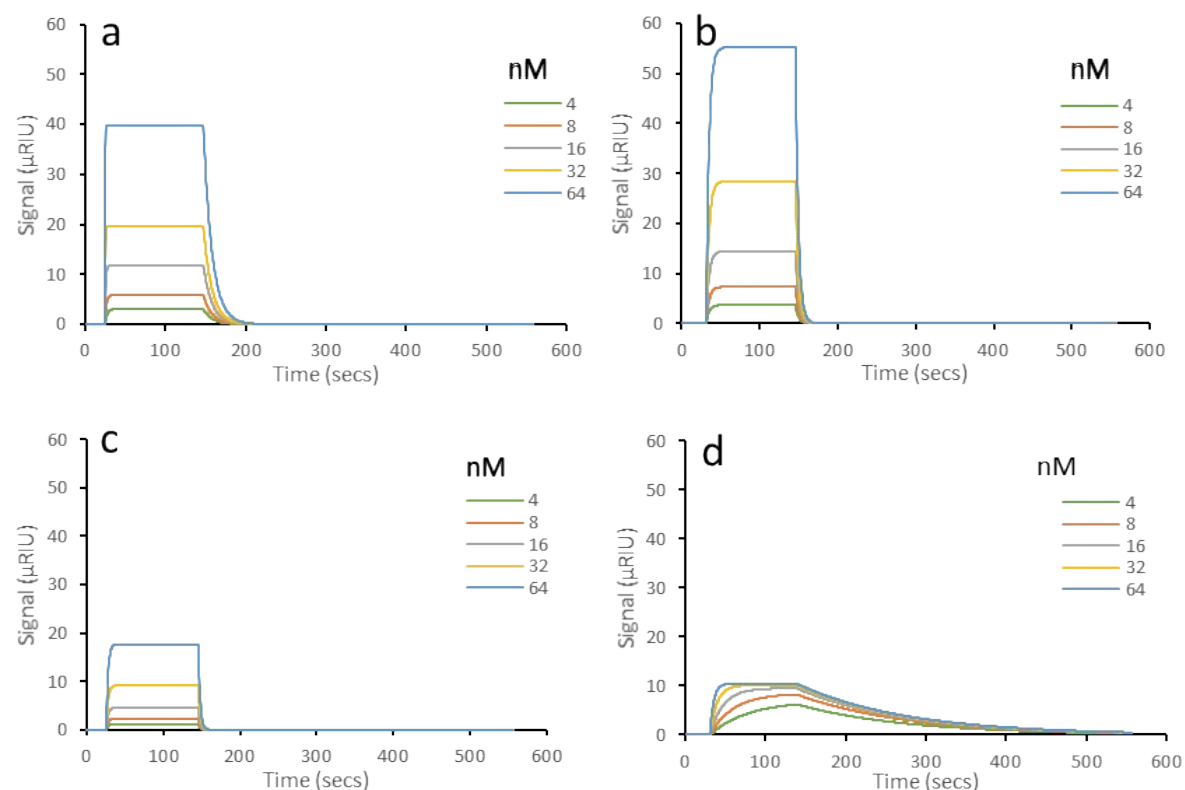


Figure S4a: Representative SPR sensorgrams of molecular interactions of imprinted nanoparticles immobilized on carboxymethyl dextran hydrogel coated Au chips. The SPR running buffer (PBST) was a phosphate buffered saline made at 10 mM, pH 7.4, supplemented with 0.01 % (v/v) Tween 20. Tween 20 is included to reduce non-specific binding during rebinding studies. Regeneration buffer was 10 mM Glycine-HCl at pH 2. All rebinding at 25 °C. All experiments in triplicate. Range of five concentrations (between 4 – 64 nM) for each protein. **(a)** Aptamimip-2 – Lysozyme. **(b)** Aptamimip-3 – Lysozyme. **(c)** Aptamimip-4 – Lysozyme. **(d)** Aptamimip-5 – Lysozyme. Related cross-reactivity studies (panels e – l) can be found in Figure S4.

Supplementary Information

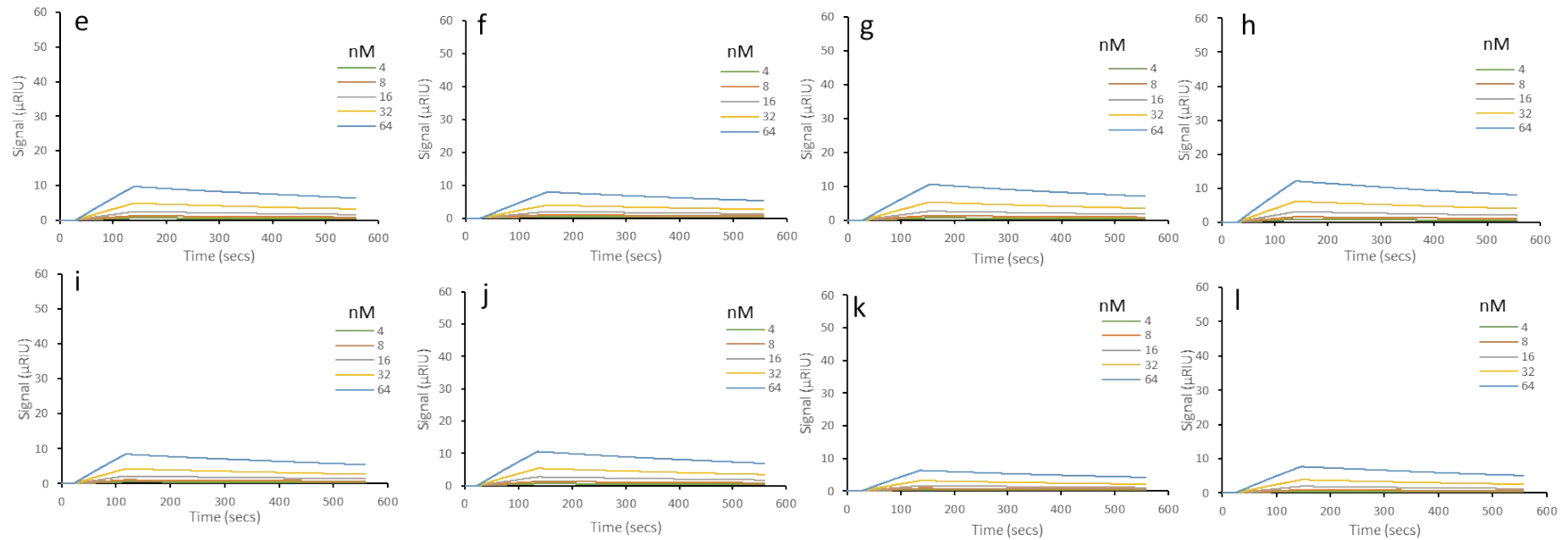


Figure S4b: Representative SPR sensorgrams of molecular interactions of imprinted nanoparticles immobilized on carboxymethyl dextran hydrogel coated Au chips. **Left Column:** Aptamimetic Imprinted Particles (AptMIP)-2. **Centre Left Column:** Aptamimetic Imprinted Particles (AptMIP)-3. **Centre Right Column:** Aptamimetic Imprinted Particles (AptMIP)-4. **Right Column:** Aptamimetic Imprinted Particles (AptMIP)-5. The SPR running buffer (PBST) was a phosphate buffered saline made at 10 mM, pH 7.4, supplemented with 0.01 % (v/v) Tween 20. Tween 20 is included to reduce non-specific binding during rebinding studies. Regeneration buffer was 10 mM Glycine-HCl at pH 2. All rebinding at 25 °C. All experiments in triplicate. Range of five concentrations (between 4 – 64 nM) for each protein. **(e)** Aptamimetic Imprinted Particles (AptMIP)-2 – Trypsin. **(f)** Aptamimetic Imprinted Particles (AptMIP)-3 – Trypsin. **(g)** Aptamimetic Imprinted Particles (AptMIP)-4 – Trypsin. **(h)** Aptamimetic Imprinted Particles (AptMIP)-5 – Trypsin. **(i)** Aptamimetic Imprinted Particles (AptMIP)-2 – BSA. **(j)** Aptamimetic Imprinted Particles (AptMIP)-3 – BSA. **(k)** Aptamimetic Imprinted Particles (AptMIP)-4 – BSA. **(l)** Aptamimetic Imprinted Particles (AptMIP)-5 – BSA. Related to Figure 5 in main manuscript.

Supplementary Information

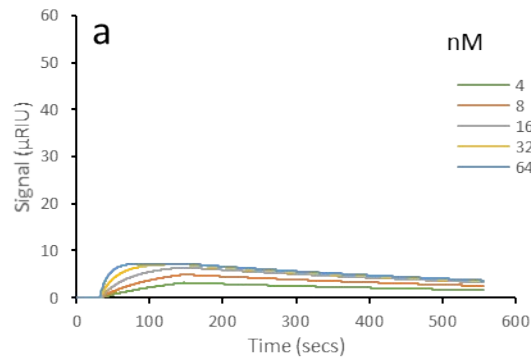
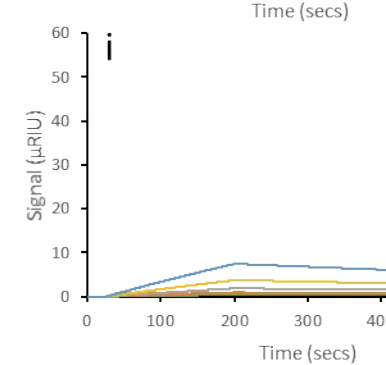
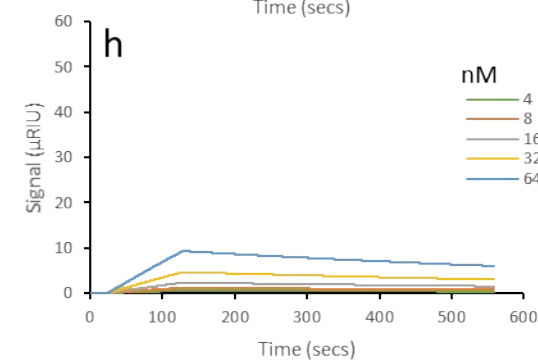
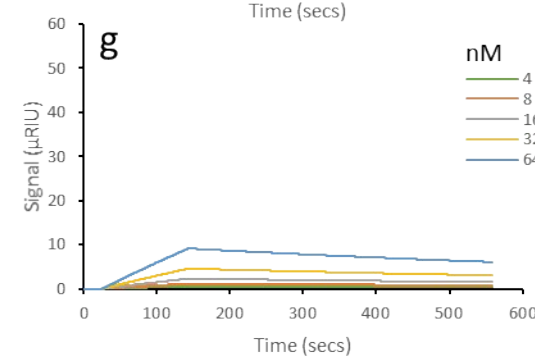
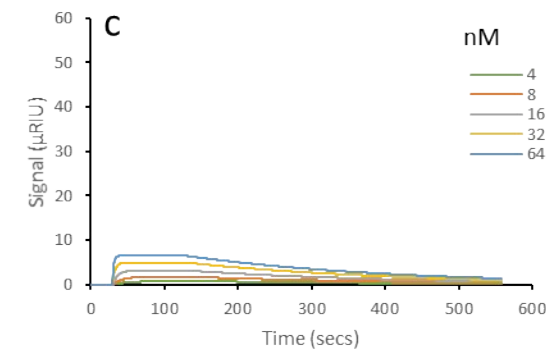
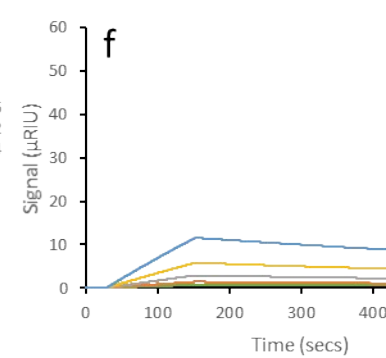
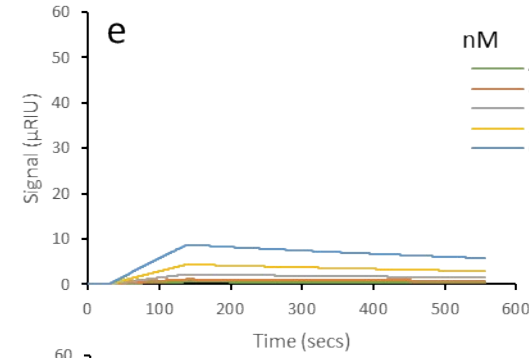
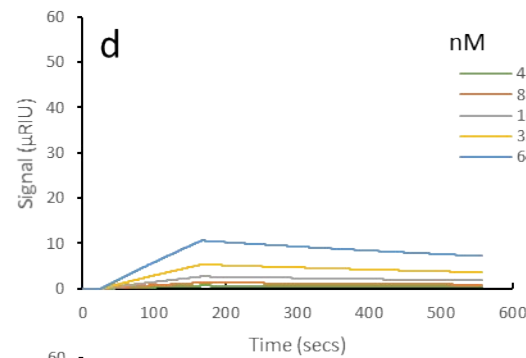
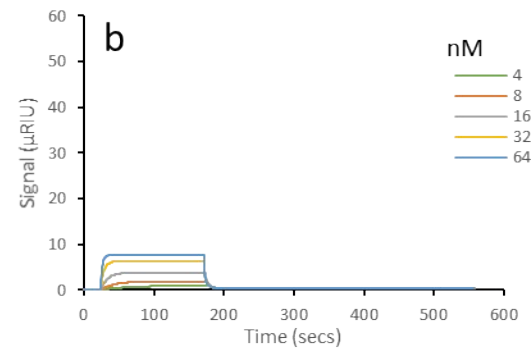


Figure S5a Representative SPR sensorgrams of molecular interactions of imprinted nanoparticles immobilized on carboxymethyl dextran hydrogel coated Au chips. The SPR running buffer (PBST) was a phosphate buffered saline made at 10 mM, pH 7.4, supplemented with 0.01 % (v/v) Tween 20. Tween 20 is included to reduce non-specific binding during rebinding studies. Regeneration buffer was 10 mM Glycine-HCl at pH 2. All rebinding at 25 °C. All experiments in triplicate. Range of five concentrations (between 4 – 64 nM) for each protein. **(a)** Aptamim-6 – Lysozyme. **(b)** Aptamim-7 – Lysozyme. **(c)** Aptamim-8 – Lysozyme. Related cross-reactivity studies (panels d – i) can be found in Figure S5.



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Figure S5b: Representative SPR sensorgrams of molecular interactions of imprinted nanoparticles immobilized on carboxymethyl dextran hydrogel coated Au chips. The SPR running buffer (PBST) was a phosphate buffered saline made at 10 mM, pH 7.4, supplemented with 0.01 % (v/v) Tween 20. Tween 20 is included to reduce non-specific binding during rebinding studies. Regeneration buffer was 10 mM Glycine-HCl at pH 2. All rebinding at 25 °C. All experiments in triplicate. Range of five concentrations (between 4 – 64 nM) for each protein. **(d)** AptaMIP-6 – Trypsin. **(e)** AptaMIP-7 – Trypsin. **(f)** AptaMIP-8 – Trypsin. **(g)** AptaMIP-6 – BSA. **(h)** AptaMIP-7 – BSA. **(i)** AptaMIP-8 – BSA. Related to Figure 6 in main manuscript.

Supplementary Information

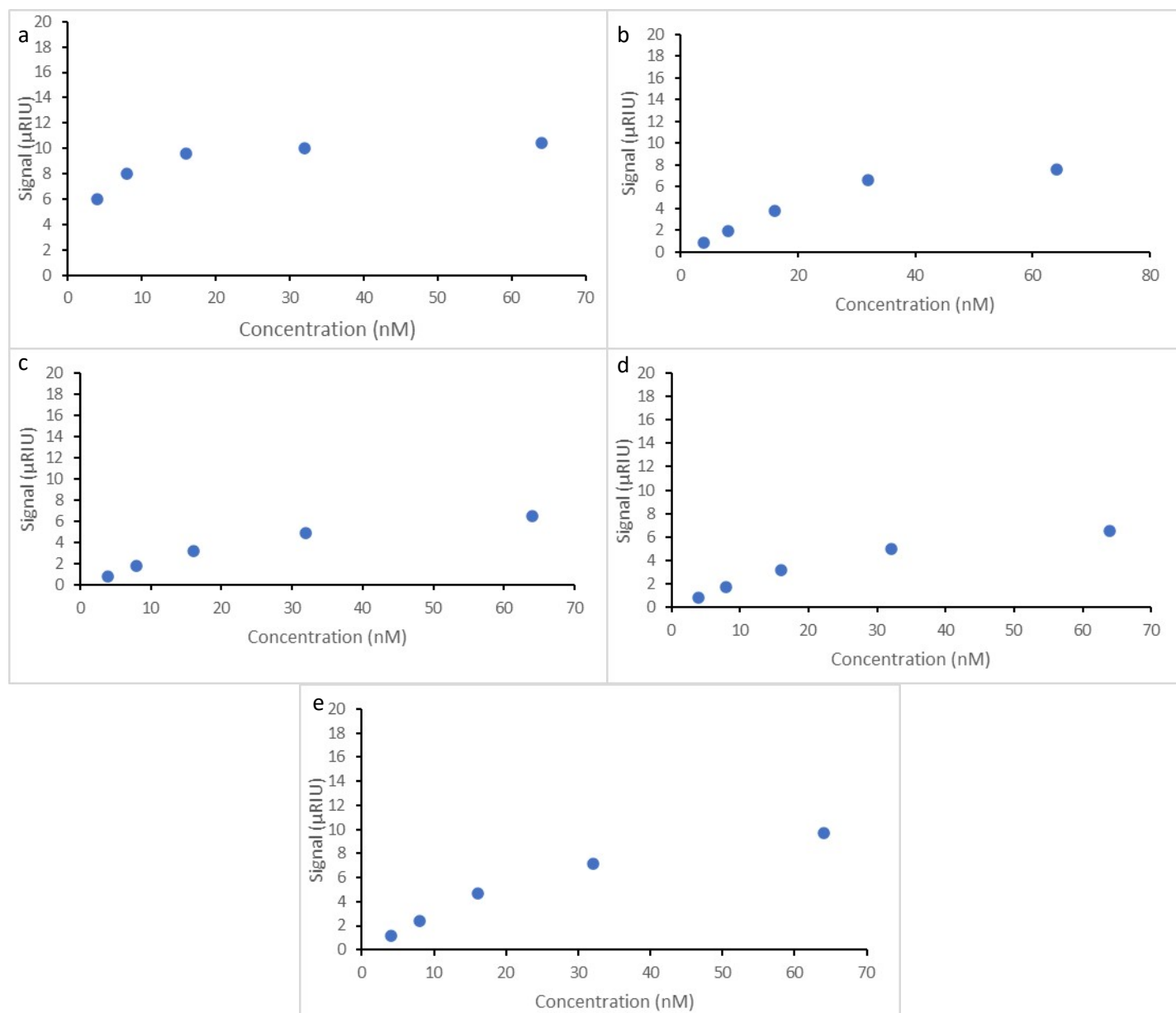


Figure S6: Elucidation of theoretic limit of detection for SPR sensor. Relative signal versus concentration. a) Lysozyme binding to AptaMIP 4, b) Lysozyme binding to AptaMIP 5, c) Lysozyme binding to AptaMIP 6, d) Lysozyme binding to AptaMIP 7, e) Lysozyme binding to AptaMIP 8.