Supplementary Information

1. Theoretical Calculations

Theoretical Calculations for 10 min ramp time particles:

The MNP@CHO (120 nm), MNP@protein (330 nm) and nanoMIPs (125 nm) are modelled as spheres and their diameters are taken from DLS measurements. Assume protein has diameter 5 nm (BHb).

Theoretical/ Particle Geometry considerations:

Assuming protein attachment to MNP@CHO gives a 100% monolayer, we can determine the number of protein molecules attached to each MNP surface

Surface area (SA) of MNP@CHO (120 nm diameter with radius r_1):

$SA = 4\pi r_1^2$

Area (A) of protein (radius r_2 = 2.5 nm): πr_2^2

Total number of protein molecules = $SA/A = 4r_1^2/r_2^2 = 4 \times 900 = 2304$

Therefore, theoretically, we can have 2304 protein molecules packed tightly on one MNP@CHO particle.

Our DLS data indicates that the hydrated MNP particle size increases to 330 nm upon protein attachment (MNP@protein; radius r_3 = 165 nm). NanoMIPs with particle size 125 nm (radius r_4 = 63 nm). Therefore, we can theoretically determine how many nanoMIP particles each MNP@protein can produce:

$SA = 4\pi r_3^2$

Area (A) of nanoMIP (radius r_4 = 130 nm): πr_2^2

Total number of protein molecules = $SA/A = 4r_3^2/r_4^2 = = 27.4$

Therefore, theoretically, we can have 27.4 nanoMIP particles produced for every one MNP@protein particle.

Experimental considerations:

Magnetic nanoparticle volume in a 10 mg MNP batch:

From DLS (r_1 for MNP@CHO = 60 nm)

Volume per MNP@CHO particle: $V_1 = 4/3\pi r_1^3 = 2.16 \times 10^{-22}$ m^3 $= 2.16 \times 10^{-16}$ cm^3

Assuming the density of an iron oxide core nanoparticle to be 5.15 g/cm^3, then 10 mg of MNP@CHO can be approximated to have a total volume of V_2:

$V_2 = 0.01/5.15 = 0.001942$ cm^3

Therefore estimated no. of MNP@CHO particles in 10 mg

$= V_2/V_1$ $0.001942/2.16 \times 10^{-16} = 8.990 \times 10^{12}$ particles.

With 27.4 nanoMIP particles theoretically being produced per MNP particle, we can then say that 10 mg in 1 mL suspension can generate: $27.4 \times 8.990 \times 10^{12} = 2.463 \times 10^{14}$ nanoMIP particles/ mL.

However, our experimental data (Fig. 3) suggests that only 0.6 mg of protein is taken up by 10 mg of MNP@CHO.

No of protein molecules in 0.6mg of BHb = $((0.6 \times 10^{-3}) / 64500) \times 6.022 \times 10^{23}$

$= 5.60279 \times 10^{15}$ protein molecules

Therefore, actual number of protein molecules adsorbed per particle

$= 5.60279 \times 10^{15} / 8.990 \times 10^{12} = 623.25$ protein molecules per MNP@CHO particle

Note: our geometric calculation for a tightly packed MNP sphere suggested: 2304 protein molecules per particle.

Therefore % actual protein coverage per MNP@CHO particle = (623.25/2304) x 100 = 27.05%

It is not unreasonable that each MNP is only 27% decorated with protein, when taking into account steric hindrance during protein conjugation reaction with MNP@CHO.

Further, with 27.4 nanoMIP particles being theoretically produced per MNP@protein particle, we can say that each nanoMIP particle theoretically has 623.25/27.4 = 23 protein recognition (binding) sites.

Therefore, during protein rebinding on nanoMIP:

Ratio of protein molecules bound : nanoMIP particles= 25:1.

2. Figures

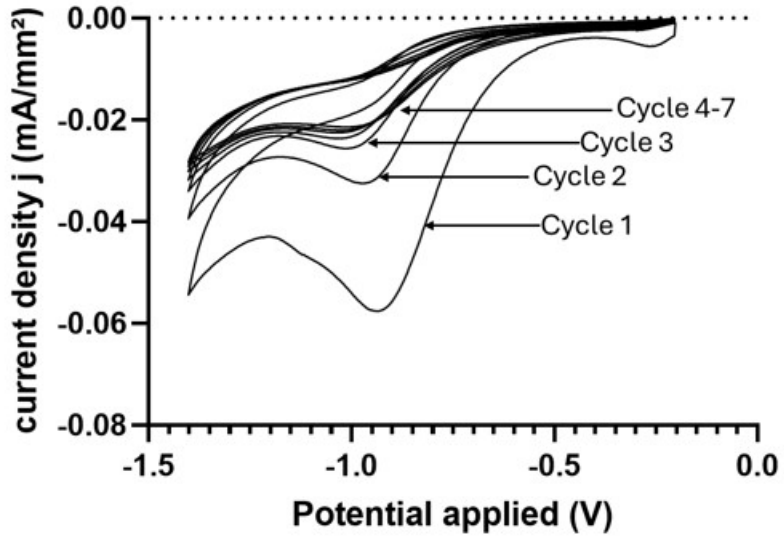

Fig S1a Entrapment of nanoMIPs within an electropolymerized layer (E-layer) on a BT-Au screen-printed electrode (SPE). Cyclic voltammetry was employed, utilizing a potential range of -0.2 V to -1.4 V vs the Ag/AgCl reference (7 cycles) at a scan rate of 50 mV s^{-1}. The peak cathodic peak decreases with each cycle representing progressive E-layer formation during nanoMIP entrapment.

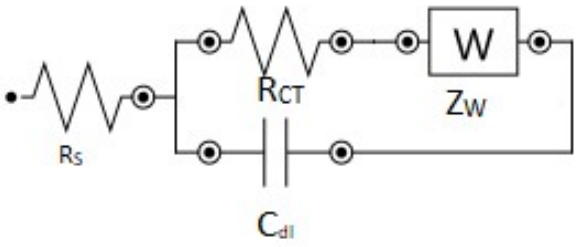

Fig. S1b Randles Equivalent Circuit model used to determine charge transfer resistance (R_{CT}) from electrochemical impedance spectra of bare and nanoMIP-loaded disposable BT-Au SPCEs.

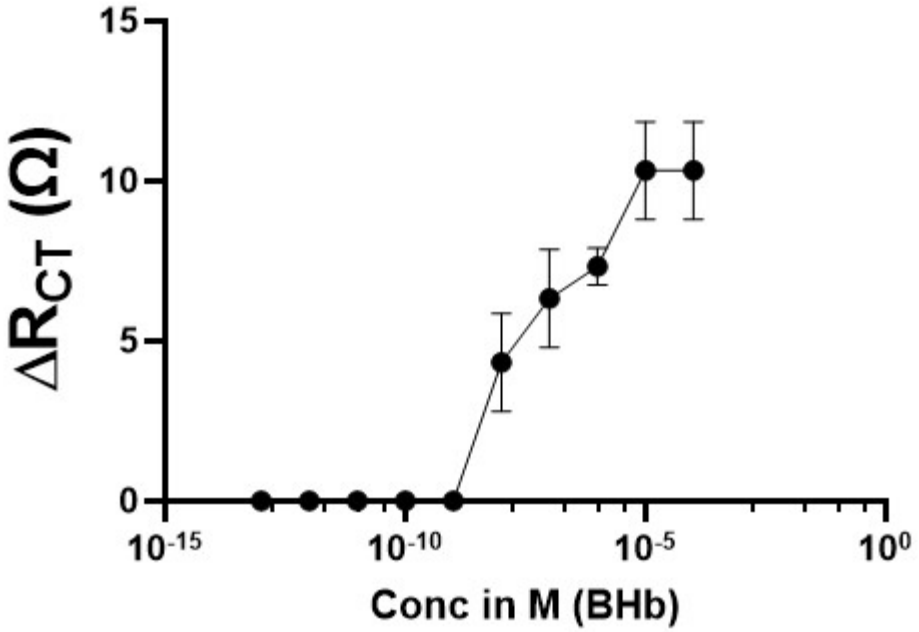

Fig. S2 NanoNIP islands linear range (this is control polymer (nanoNIP) and gives very little signal; a few ohms compared to 100s of ohms with nanoMIP)

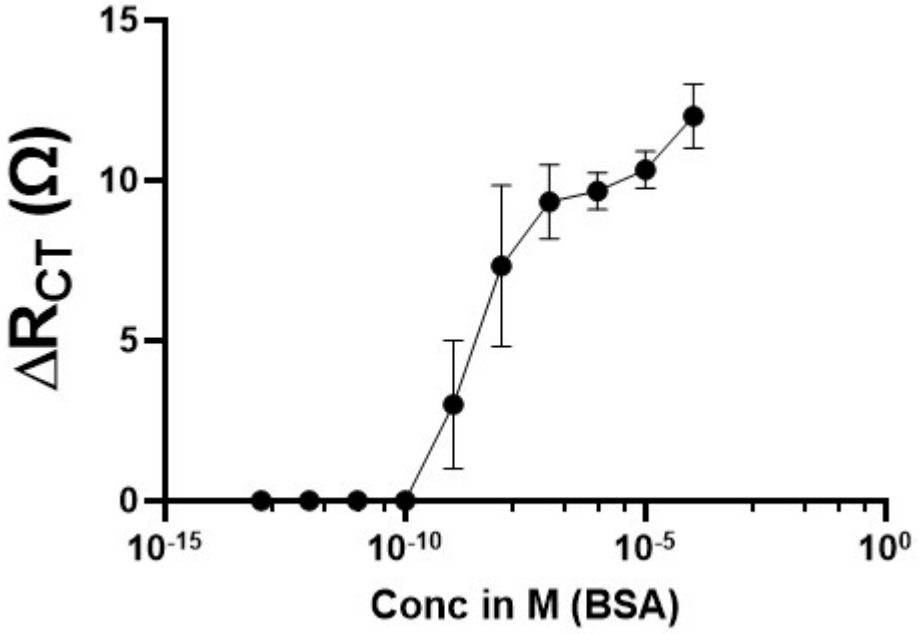

Fig S3 BHb nanoMIP islands cross-bound with bovine serum albumin