Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Molecularly Imprinted Nanoparticle-Based Assay (MINA) – Detection of Leukotriene and Insulin

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1. Microplate inserts

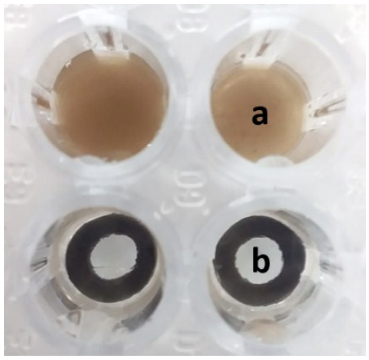

Figure S1. Microplate titter with 100 µL of MNP@LTE4 solution (0.4 mg/mL per well) (a) without and (b) with a magnetic insert. The MNP@LTE4 are suspended in solution (cloudy) without magnetic insert. When a magnetic insert is used, MNP@LTE4 are attracted to the bottom of the well and the solution is transparent.

2. Solid phase preparation.

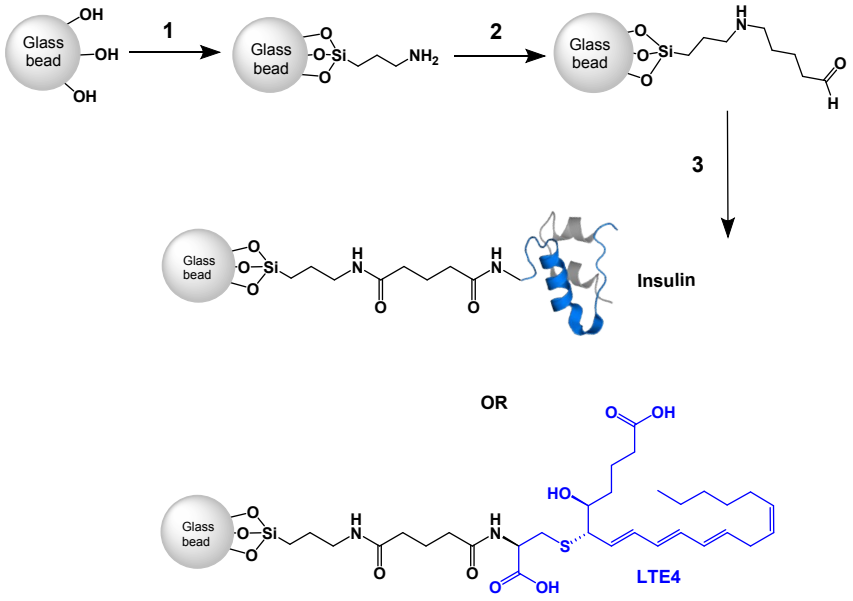

Figure S2. Preparation of the solid phase comprise activation, (1) silanization, (2) modification of glass beads using glutaraldehyde, and (3) analyte immobilization.

3. **Solid phase synthesis of fluorescent nanoMIP**

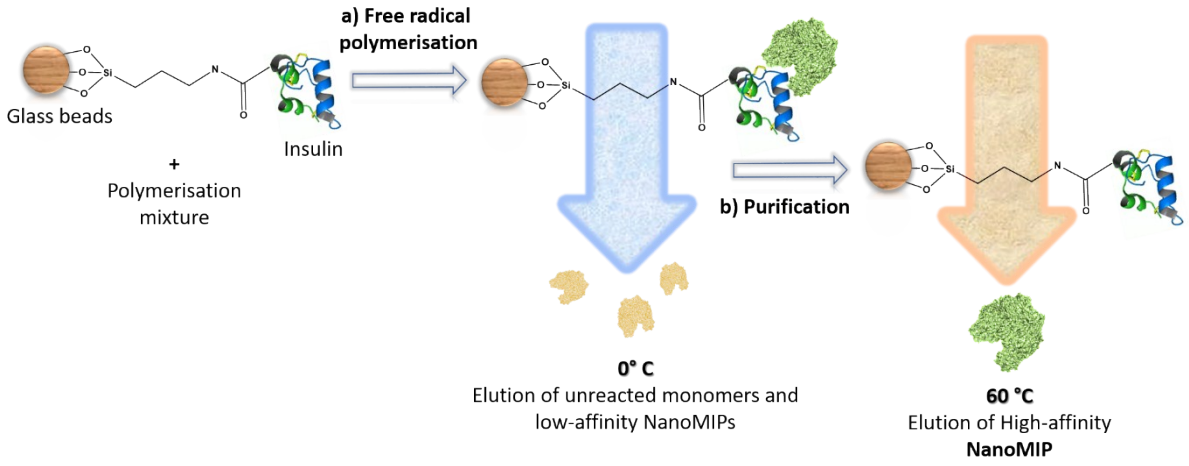

Figure S3. Solid phase synthesis of nanoMIPs, a) the polymerization take place in presence of the immobilized target on glass beads. b) The purification is performed by eliminating the residues at low temperature elution and nanoMIPs are eluted at high temperature.

4. **Synthesis and functionalization of MNP.**

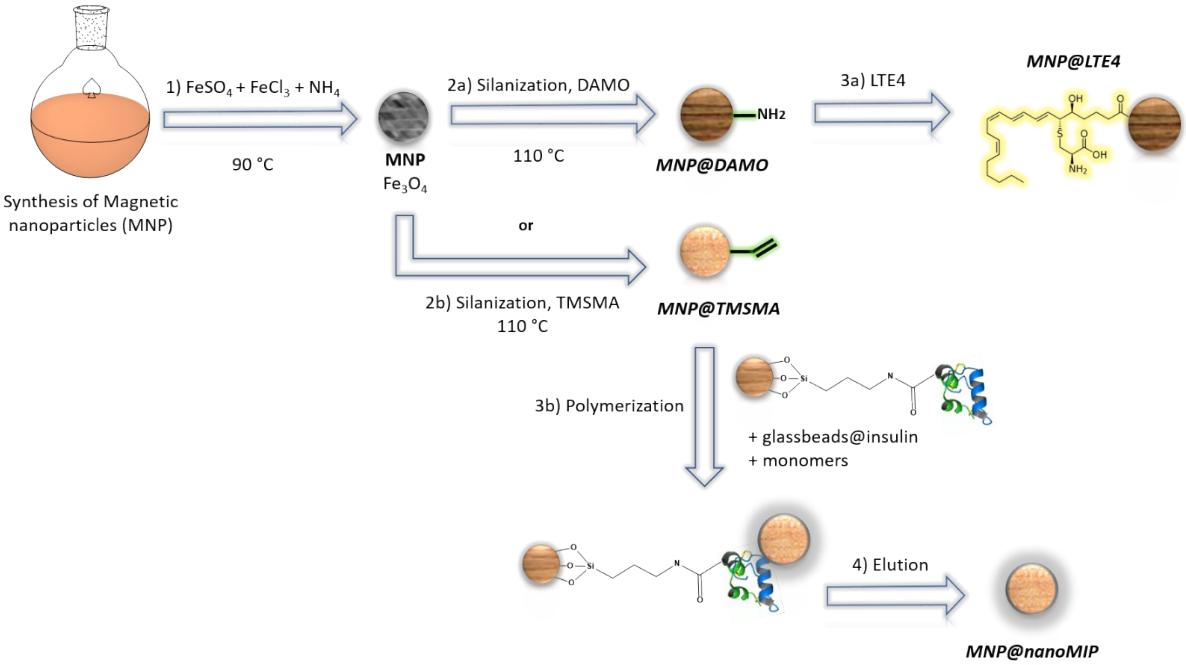

Figure S4. Super-magnetic nanoparticles (MNP) are 1) synthesized chemically and after purified by magnetic decantation. MNP are functionalized using silanes, allowing to modified the surface with 2a) amino groups, MNP@DAMO or 2b) allyl groups, MNP@TMSMA. 3a) The MNP@LTE4 are synthetized by coupling LTE4 with MNP@DAMO. The synthesis of MNP@nanoMIP specific to insulin are synthetized trough the polymerization of MNP@TMSMA in the presence of glassbeads@insulin and monomers.

5. SPR experiments

The SPR response of specific LTE4-nanoMIP was evaluated against LTE4, LTD4 and LTB4 and the Kd calculated (Figure S3). The concentrations range of the injected leukotrienes ranged from 0.023 nM to 297 nM. The Kd for LTE4 was found 26 nM, which signifies a high affinity. Besides, LTB4 present a Kd of 1.6 µM (low affinity) and for LTD4 was 67nM (weak affinity). Moreover, the LTB4 relative cross-reactivity was estimated at 1.6 %. Therefore, the interaction between the nanoMIP towards LTB4 can be neglected (as shown in Figure S3). The LTD4 relative cross reactivity was found 39%. In summary, the LTE4 nanoMIPs have much higher affinity compare to LTB4 and LTD4.

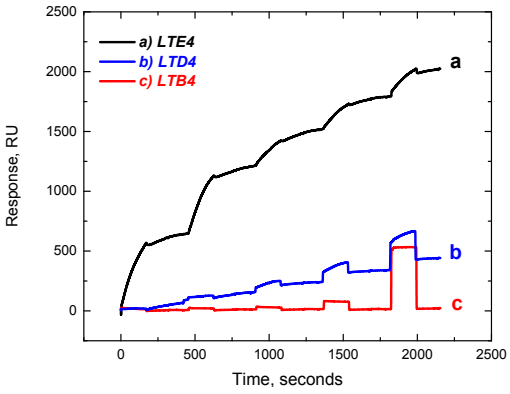

Figure S5. SPR response from NanoMIP towards leukotrienes (LTE4, LTD4 and LTB4). (a) High affinity is measured for the LTE4, (b) low response is observed for LTD4 and almost no response for LTB4.

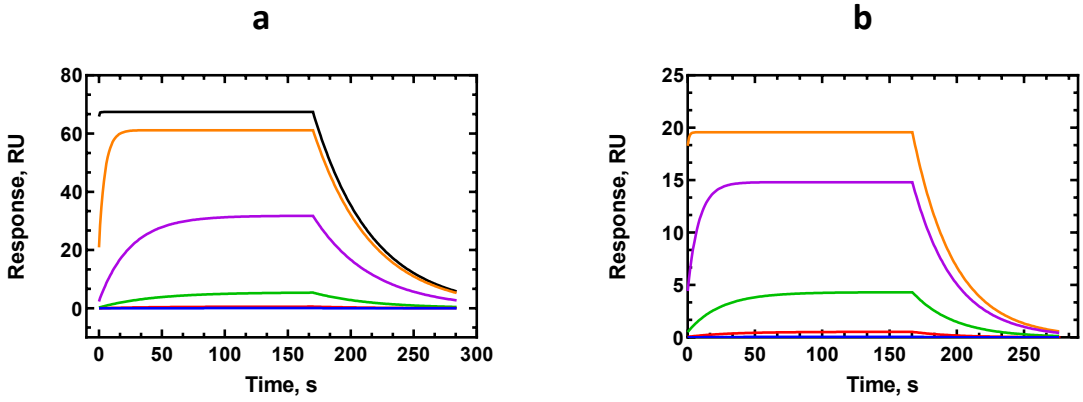

Figure S6. SPR response of corresponding nanoMIPs for (a) LTE4 and (b) insulin. LTE4 solutions were injected in the concentration rage 0.023-2300 nM) and insulin in 0.044-440 nM. All SPR experiments were performed in PBS buffer pH 7.4 at 25 °C.

The interaction of NanoMIP to insulin was found at 100 % with a Kd= 0.74 nM (Chi^2=3.3). The cross reactivity towards the Insulin-like growth factor 1 (IGF-1) was found at 25% interference with a Kd =2.96 nM (Chi^2= 3.2). Similarly, the Human Proinsulin C-peptide (HPC) cross reactivity was found at 0.7 % with a Kd =110 nM (Chi^2= 1.7) as shown in Figure S5. Interaction between the NanoMIP and IGF1 was caused by some degree of sequence homology (at 49%) between IGF1 and the insulin. The sequence homology with IGF1 are located in the A (at 62%) and B (at 40%) chain from insulin.

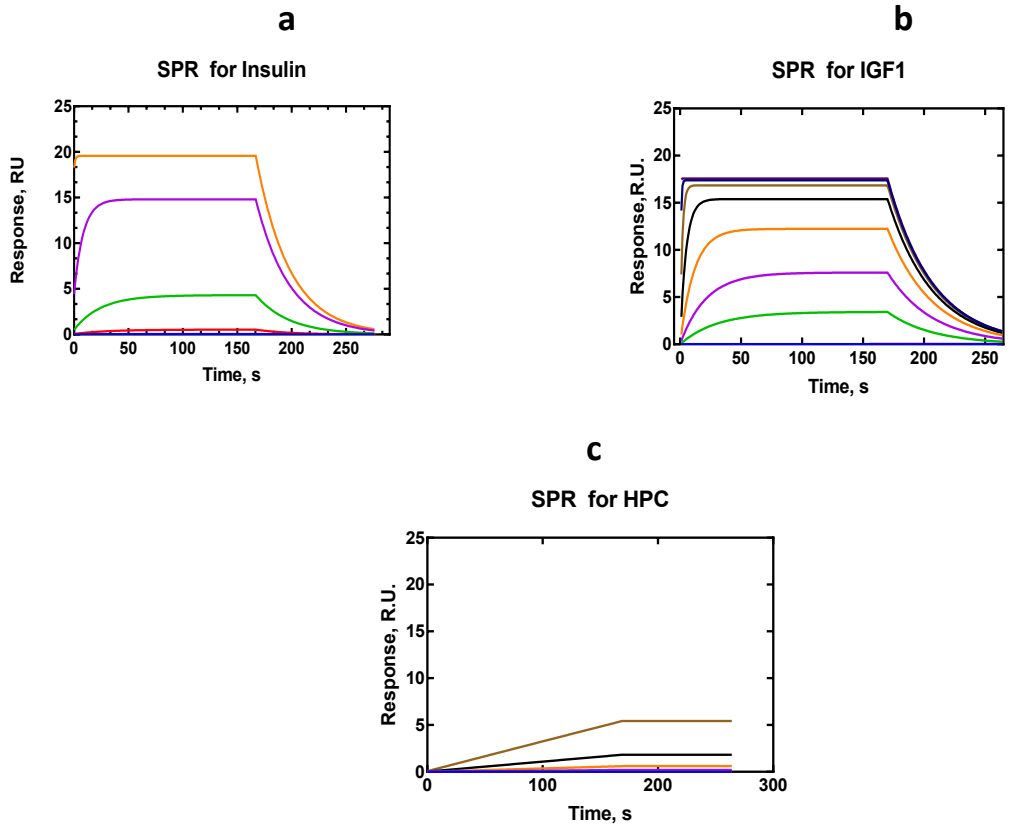

Figure S7. SPR response for NanoMIP to a) insulin, b) IGF and c) HPC in a concentration range of (0.044-440 nm) in PBS.

6. **Microscopy characterization.**

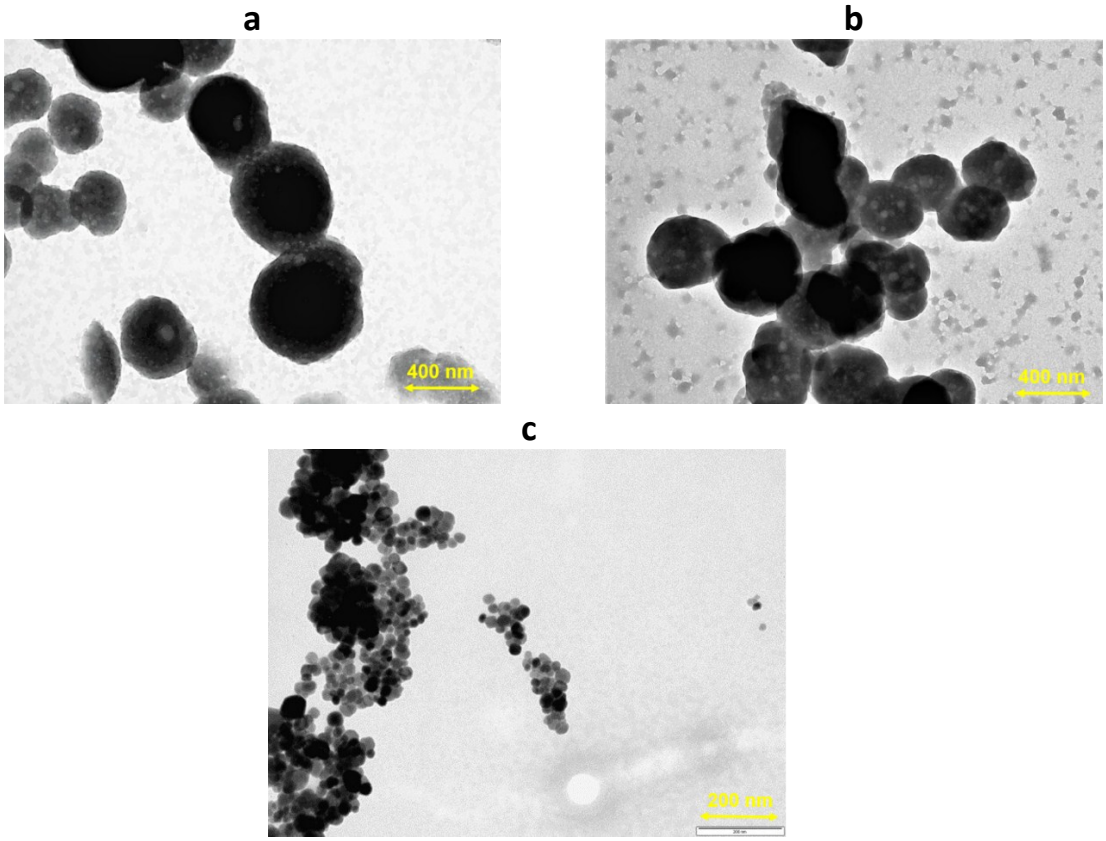

Figure S8. TEM images obtained for NanoMIP specific for (a) LTE4 and (b) Insulin at 400 nm scale. In (c) image from MNP scale at 200 nm. Samples were viewed on a JEOL JEM-1400 TEM with an accelerating voltage of 100kV. Digital images were collected with a Megaview III digital camera with iTEM software.

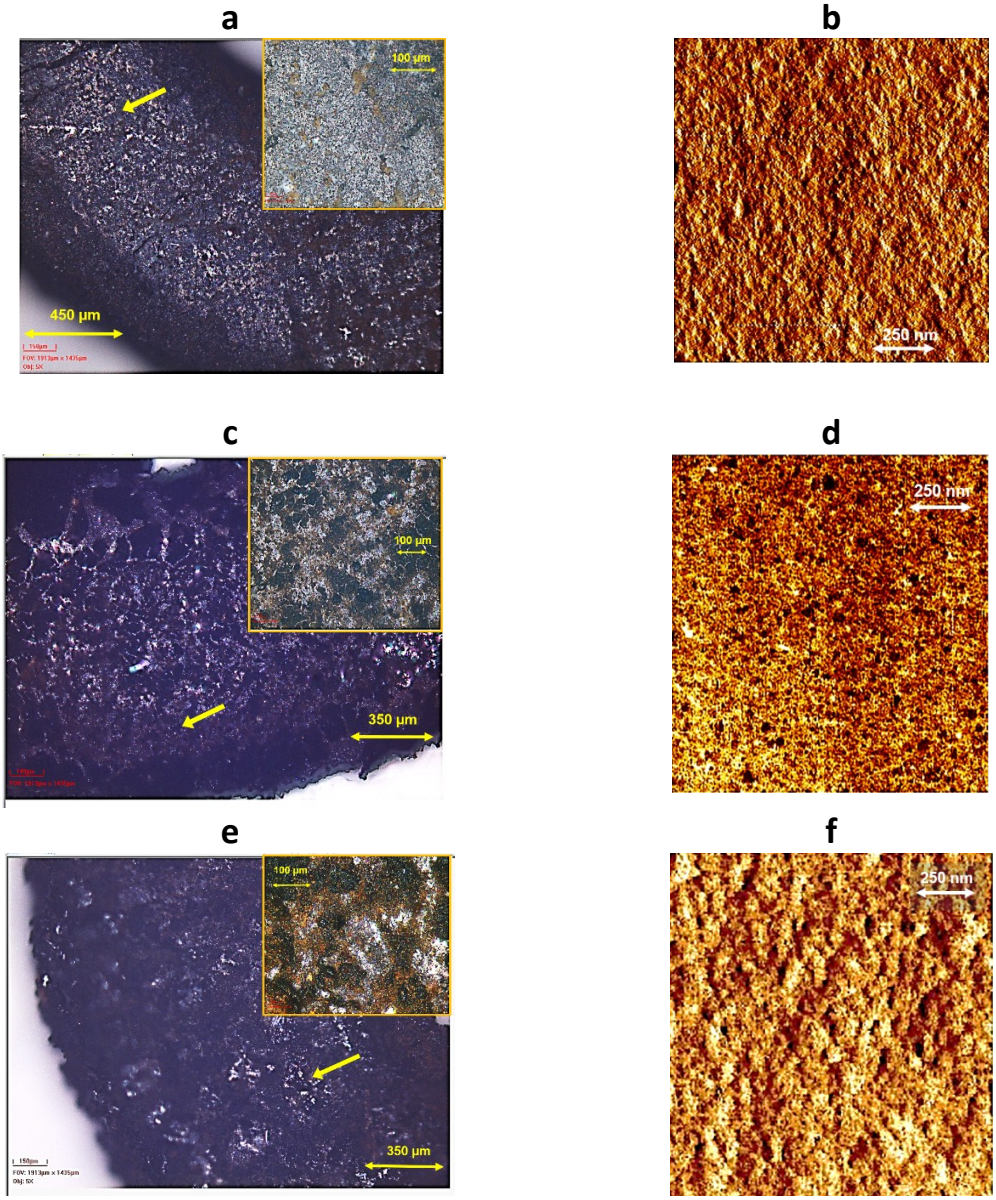

Figure S9. Optical microscopy and AFM images for a magnetic insert (a & b) naked, (c & d) with immobilized MNP@LTE4 and (e & f) obtained for the complex between NanoMIP and MNP@LTE4. These samples were previously dried under nitrogen at 30^{o}C.

5. Instrumentation

5.1 Characterisation, size and FTIR

The particle size was determined using Dynamic Light Scattering (DLS) using a Malvern instruments, Zetasizer Nano (Nano-S) particle-size analyser from Malvern Instruments Ltd (UK). DLS measurements were perform in a 10 times diluted stock solution (0.01 mg/mL) previously sonicated for 5 minutes. The FT-IR spectra was acquired in a PLATINUM Diamond ATR accessory and INVENIO FTIR spectrometer equipped with the BRUKER FM optical components.

5.2 Microtiter plate preparation using magnetic inserts

96 well clear flat bottom polystyrene microtiter plates were purchased from Costar® and modified with magnetic disk inserts (3/6 mm internal and external diameter). Magnetic disk inserts were fabricated by laser cutting of magnetic sheets (0.5 mm thick, high magnetism=) (brown self-adhesive A4, 210 x 297 x 0.5 mm, with an adhesive force of 80 g/cm^2, purchased from Magnosphere) and placed manually on the bottom of each well.

5.3 Spectroscopy

The UV-Vis spectra of the nanoMIPs was analysed using a UV-1800 spectrophotometer SHIMADZU spectrometry in a sample diluted 10 times (0.01 mg/mL) before the analysis. Microtiter plate measurements were performed using Hidex Sense microtiter plate reader in Fluorescent mode with an excitation and emission at (485 ± 10) and (535 ± 20) nm, respectively, with number of flashes set to 30 and lamp power 200 (high), Flatbed laser model 4060 60W, TS-0034, QA8A-1105. NanoMIP fluorescence was measured using a 96-microtiter plate, 200 µL of nanoMIPs were dispensed in each well and dilutions were made from the stock solution (2.3 mg/mL).

5.4 Surface Plasmon Resonance (SPR)

NanoMIPs affinity and specificity was analysed using Biacore 3000 instrument (GE Healthcare Life Sciences, UK) at 25 °C using PBS (0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4) as the running buffer at flow 35 µL/min. The self-assembled gold sensor chip (SA) was cleaned using plasma and placed in a

solution of mercaptododecanoic acid in ethanol (2.2 mg/mL) where they were stored until use. Before assembly the sensor chip was rinsed with ethanol and dried in a stream of air.

The equilibrium association and dissociation constants K_A and K_D, respectively, were calculated by the Langmuir binding theory. The K_D is related to the rate of complex formation (described by association rate constant k_{on}) and the rate of breakdown (described by dissociation rate constant k_{off}) such that $K_D=k_{off}/k_{on}$. The association constant (K_A) can be then calculated as K_D^{-1}.

Dissociation constants (K_D) were calculated from plots of the equilibrium biosensor response using the BiaEvaluation v4.1.1 software using a 1:1 binding model with drifting baseline (DB) fitting. The calculation of the dissociation constant was also done using Langmuir Blodgett (LB) algorithm using the AB (absorption) component of the SPR response, which was obtained after the subtraction of the drift and bulk effect.

For leukotriene nanoMIPs analysis: Both leukotriene-specific nanoMIPs were diluted in PBS and then immobilized *in situ* on the chip surface containing carboxyl groups using the EDC/NHS coupling (0.4 mg and 0.6 mg/mL, correspondingly). The leukotriene solutions were diluted in PBS in the concentration range between 0.023 and 2300 nM. Sensorgrams were collected sequentially for all nanoparticles concentrations running in KINJECT mode (injection volume- 100 µL and dissociation time- 120 s). Dissociation constants (Kd) were calculated from plots of the equilibrium biosensor response using the BiaEvaluation v4.1 software using a 1:1 binding model with drifting baseline (DB) fitting. The calculation of the dissociation constant was also done using Langmuir Blodgett (LB) algorithm using the AB (absorption) component of the SPR response, which was obtained after the subtraction of the drift and bulk effect.

For insulin nanoMIPs analysis: The solution of insulin nanoMIPs in PBS (1 mg/mL) was injected and immobilised *in situ* on the chip surface containing carboxyl groups using the EDC/NHS coupling (0.4 mg/mL and 0.6 mg/mL, correspondingly). The insulin was diluted in PBS in the concentration range between 0.044 and 440 nM. Sensorgrams were collected sequentially for insulin concentrations running in KINJECT mode (injection volume- 100 µL and dissociation time- 120 s).