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# Engineering immunomodulatory nanoplatforms from commensal bacteria-derived polysaccharide A

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## Supplementary Information (SI)

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# 1. EXPERIMENTAL

## 1.1 Equipment and Characterization

### 1.1.1 Ion exchange chromatography (IEX-HPLC)

Analytical IEX-HPLC was performed on a Thermo Ultimate3000 separation module with a variable wavelength detector (λ=206 nm, λ=254 nm) and employed a Tosoh Biosciences TSKgel DEAE-5PW HPLC column (7.5 cm × 7.5 mm, 10 µm, flow rate of 1 mL $min^{-1}$) at room temperature.

Preparative IEX-HPLC was performed using Thermo Ultimate3000 separation module with a variable wavelength detector (l=206 nm, l=254 nm) and employed a Tosoh Biosciences TSKgel DEAE-5PW HPLC column (15 cm × 21.5 mm, 13 µm particle size, flow rate 6 mL $min^{-1}$). Eluent fractions were collected every 30 seconds on a fraction collector.

The mobile phase consisted of eluents A (Tris buffer 25 mM, pH 7.3), eluent B (0.1 % CHAPS (w/v) in Tris buffer 25 mM, pH 7.3), eluent C (170 mM NaCl in Tris buffer 25 mM, pH 7.3) and D (500 mM NaCl in Tris buffer 25 mM, pH 7.3). Solvent D was substituted with 2000 mM NaCl solution for washing.

### 1.1.1 Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS)

MALDI-TOF mass spectrometry experiments were undertaken using a Bruker UltraFlextreme mass spectrometer equipped with a Smartbeam-II laser, (355 nm wavelength). The accelerating voltage was 20 kV. The polymer **(3)** was dissolved in DMF and10 µL of the solution was taken and mixed with 10 µL of the matrix solution. The matrix was a saturated solution of Sinapic acid in water/acetonitrile/TFA (70/30/0.1). The matrix solution was deposited first on the stainless steel MALDI plate and left to dry followed by deposition of the sample-matrix solution on top of the dry matrix spot. After drying, data collection and analysis was carried out using the Bruker Flex analysis software.

For PSA and the conjugates, aqueous solutions of the samples were prepared. Dithranol (20 mg/mL in THF) was used as the matrix. A solution of sodium trifluoroacetate (10 mg/mL) in water was used as a cationizing agent. The sample solution 5 µL was mixed with the matrix solution 15 µL and 0.5 µL of the cationization solution. The matrix solution containing the cationizing agent was deposited first on the stainless steel MALDI plate and left to dry followed by deposition of the sample-cationizing agent-matrix solution on

top of the dry matrix spot. After drying, data collection and analysis was carried out using the Bruker Flex analysis software.

### 1.1.4 Reverse Phase High-Performance Liquid Chromatography (RP-HPLC)

Analytical reverse-phase RP-HPLC was performed on an Ultimate 3000 SEC system equipped with a guard column attached to a Phenomenex Jupiter Proteo C18 column (250 x 4.6 mm column, 4 µm particle size, flow rate of 1 mL $min^{-1}$) and a variable wavelength UV-vis detector. The mobile phase consisted of eluents A (0.05% v/v TFA in water) and B (0.05% v/v TFA in acetonitrile) for all HPLC runs.

Preparative RP-HPLC was performed on an Ultimate 3000 SEC system equipped with a guard column attached to a Phenomenex Jupiter Proteo C18 column (250 x 4.6 mm column, 4 µm particle size, flow rate of 6 mL $min^{-1}$) and a variable wavelength UV-vis detector. The mobile phase consisted of eluents A (0.05% v/v TFA in water) and B (0.05% v/v TFA in acetonitrile) for all HPLC runs. Eluent fractions were collected every 30 seconds on a Varian 701 fraction collector.

### 1.1.5 Nuclear Magnetic Resonance (NMR)

NMR analyses were carried out on Bruker Ultra Shield Avance 500 or 800 spectrometers. NMR analyses, analyses were conducted in either deuterated DMSO (DMSO-$d_6$), deuterated chloroform or deuterated water (depending on the solubility of the product).

### 1.1.6 Fourier transform infra-red (FT-IR)

Solid and liquid samples were analyzed using a Bruker Tensor 27 FT-IR spectrometer equipped with an attenuated total reflectance (ATR) accessory. Spectra were averages of 256 scans, recorded at a resolution of 4 $cm^{-1}$ at room temperature.

### 1.1.8 Amino acid analysis (AAA)

AAA was conducted using a Hitachi 8800 Amino Acid Analyzer that is equipped with a strong-cation exchange separation via a TransGenomic (cat # AAA-99-6312) column. Amino acids were detected via a secondary post-column reaction with ninhydrin and observed/quantified in the visible spectrum (440 nm and 570 nm). Samples were prepared by transferring a known volume of sample into a vial which was subsequently placed a centrifugal evaporator to lyophilize the sample. The dry sample was hydrolyzed by treating with 6N HCl/1% phenol at 110 $^{o}$C for 24 hours followed by solvent removal. The residue was taken up NorLeu diluent and diluted to a known volume for analysis. NB: The analysis excludes tryptophan, methionine and cysteine units (which requires a different AAA procedure).

### 1.1.9 Transmission electron microscopy (TEM)

TEM images were obtained using JEOL JEM 1011 electron microscope with an acceleration voltage of 100 kV. Samples (NPs **(5)**) were prepared by placing a drop of sample onto a carbon coated copper grid followed by negative staining with 2% uranyl

acetate. Samples were allowed to dry overnight before imaging. Templated samples were imaged without staining.

### 1.1.10 Dynamic Light Scattering (DLS)

Particle size measurements were carried out by dynamic light scattering (DLS) using a Malvern Instruments Zetasizer Nano series instrument. The system was allowed to equilibrate for 3 minutes before measurements were made and at least three replicate measurements were made for each sample.

### 1.1.11 Zeta potential

Zeta potential measurements were carried out using a Malvern Instruments Zetasizer Nano series instrument. An equilibration time of 3 minutes was allowed before each measurement and at least five replicate measurements were made for each sample.

### 1.1.12 Statistical Analysis

Statistical analyses were performed using Prism (Version 7, GraphPad, La Jolla, CA).

## 1.2 Results

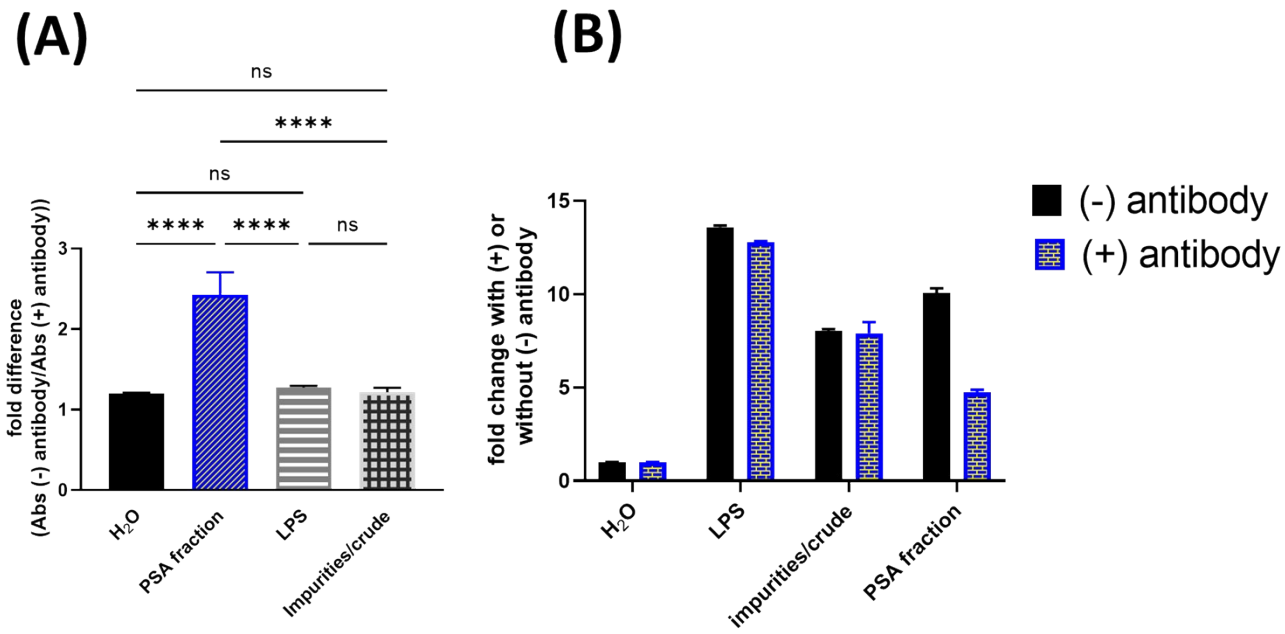



**Figure S1:** *In vitro* evaluation of the biological activity and specificity towards TLR2 (A) and extent of activation (B) of the purification fractions obtained from IEX-HPLC. LPS and water were the positive and negative controls respectively

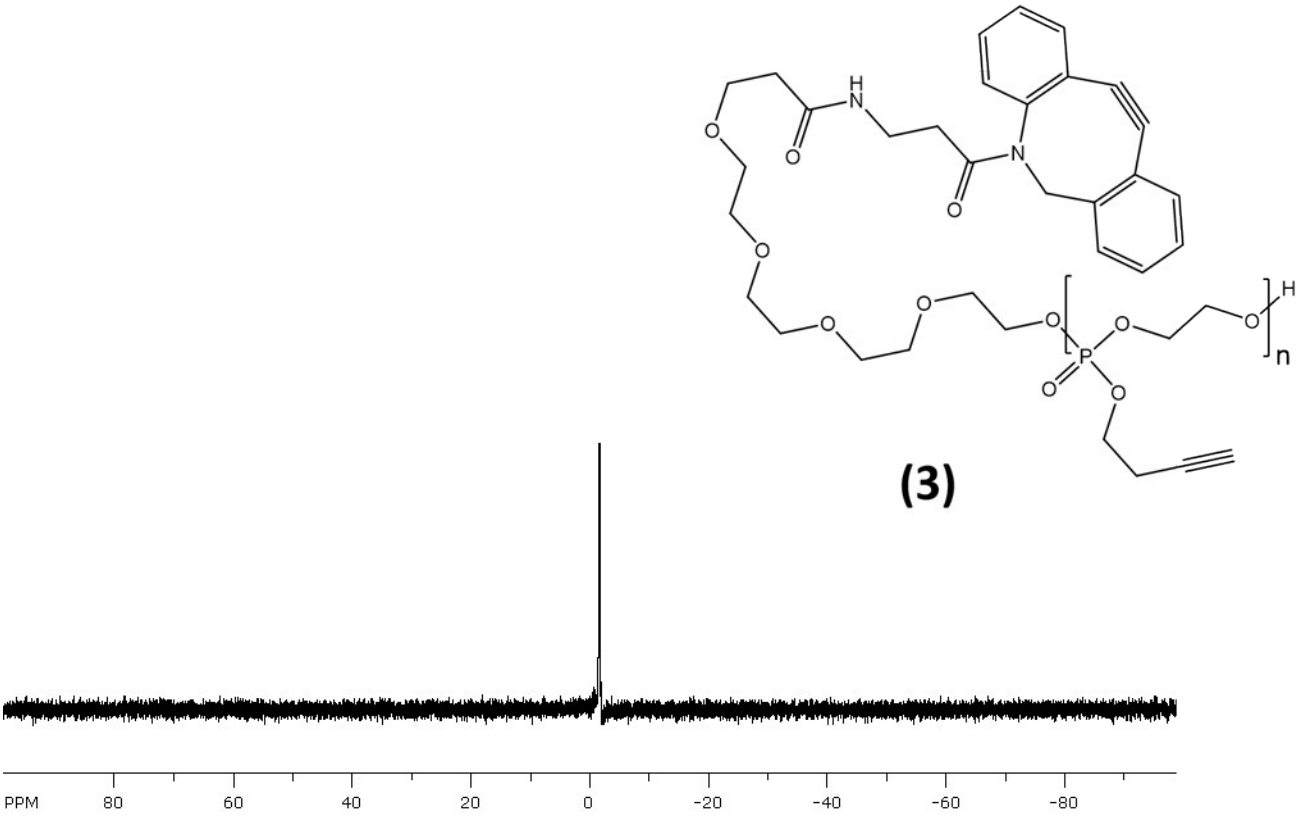



**Figure S2:** $^{31}P$ NMR spectrum (500 MHz, $CDCl_3$, ppm) of the polymer **(3).**

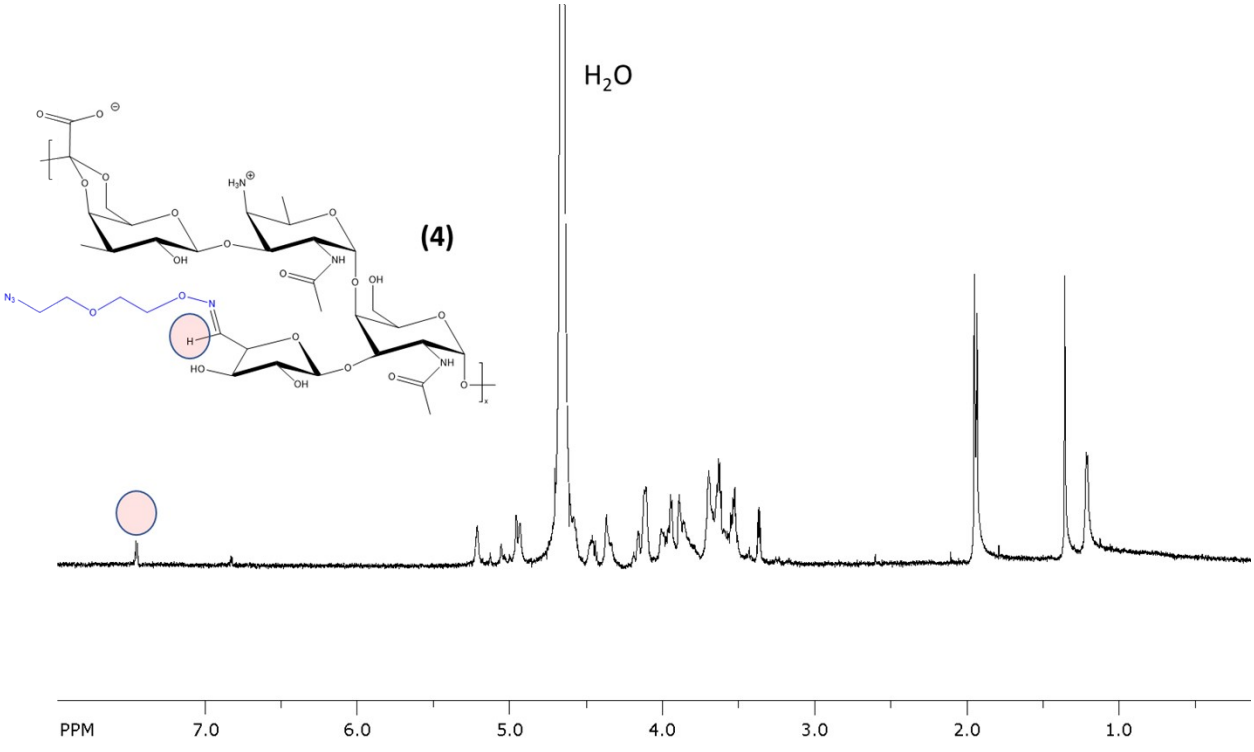


**Figure S3:** $^{1}H$ NMR spectrum (600 MHz, $D_2O$, ppm) of azide modified PSA **(4).** The oxime peak is highlighted.

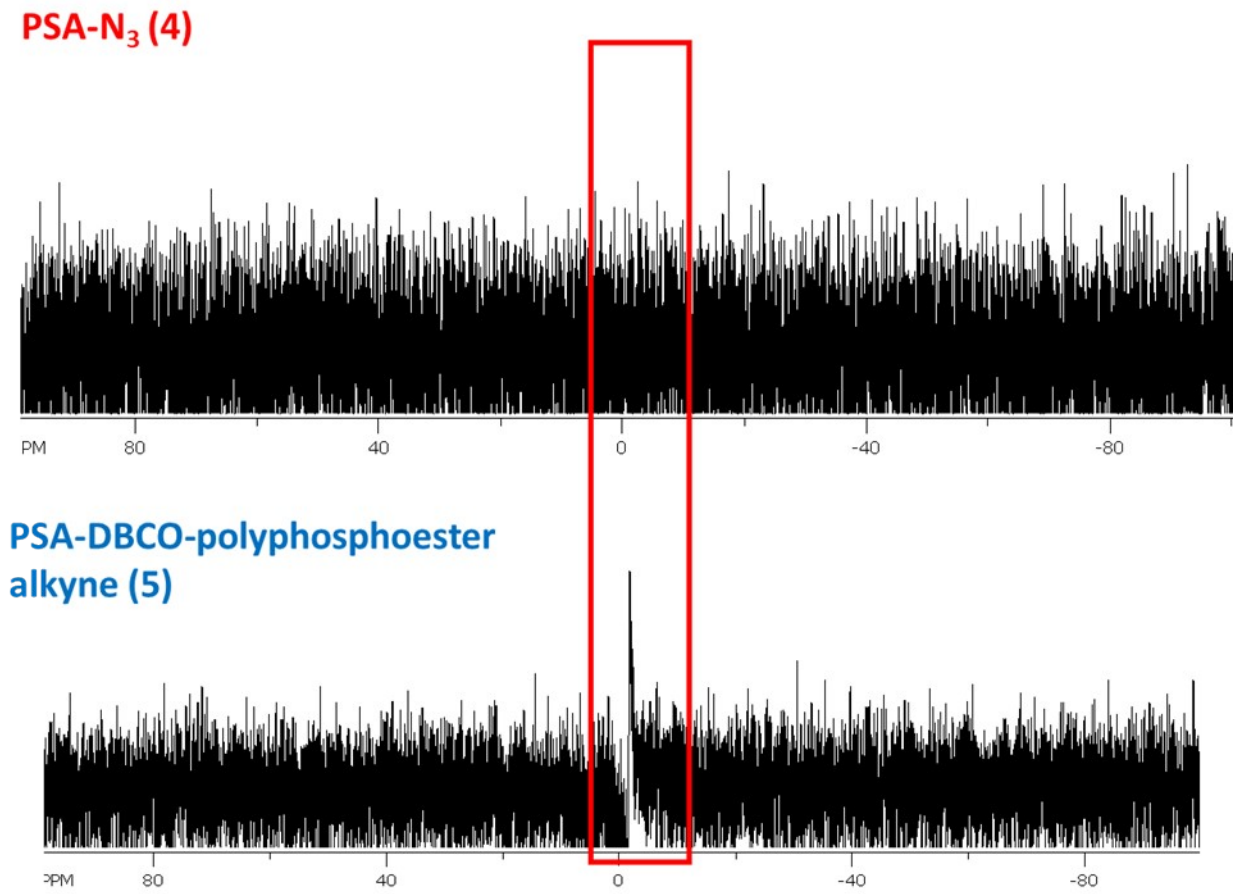



**Figure S4:** A comparison of the $^{31}P$ NMR spectra of the azide functionalized PSA (4) (500 MHz, $D_2O$, ppm) and the PSA polymer conjugate **(5)** (500 MHz, $D_2O$ + DMF, ppm)**.**

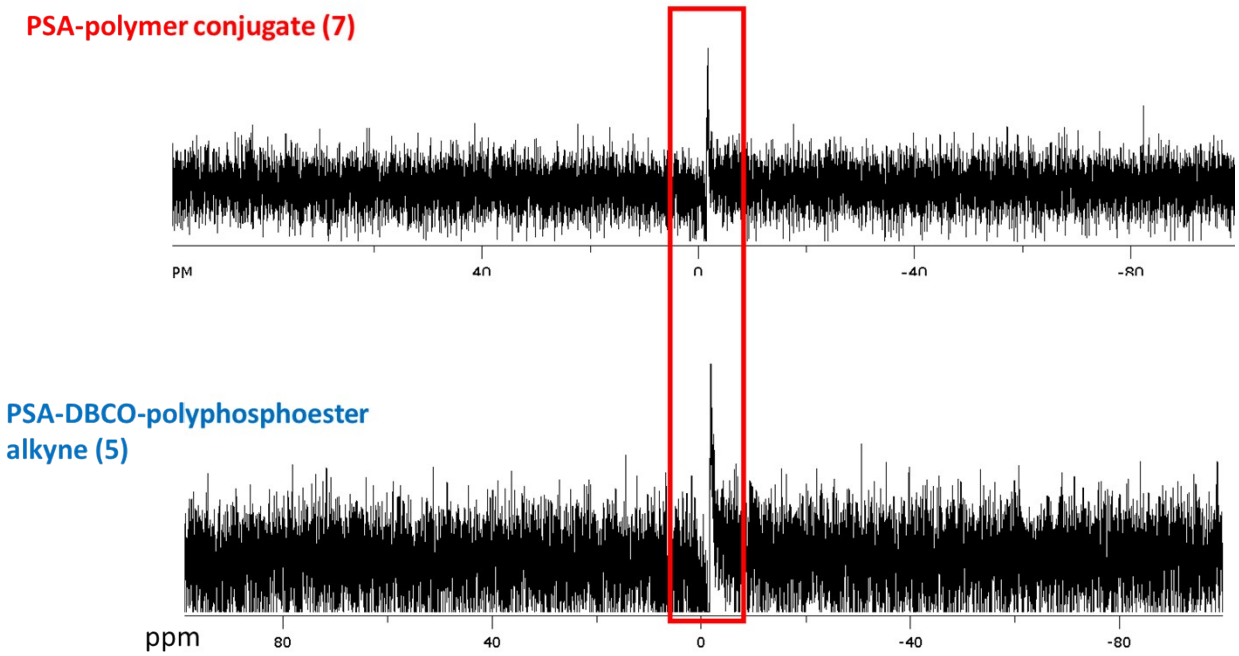
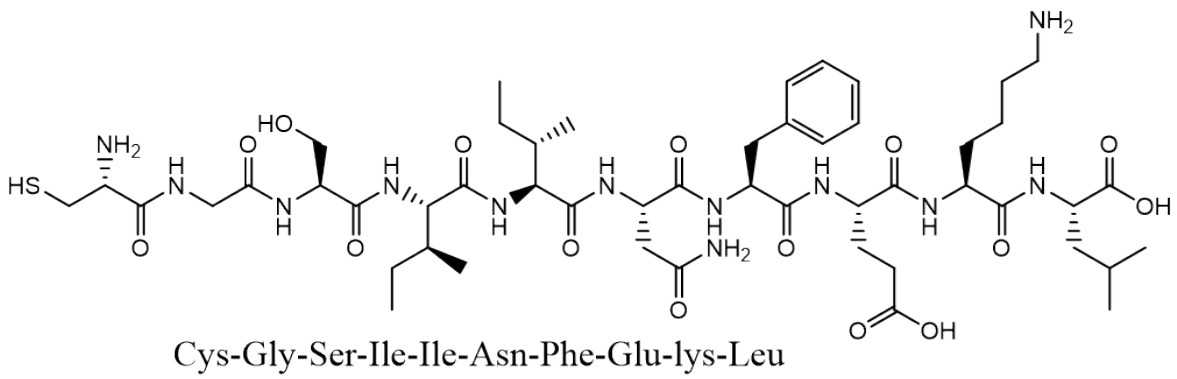



**Figure S5:** A comparison of the $^{31}P$ NMR spectra of the PSA polymer conjugate **(5)** (500 MHz, $D_2O$ + DMF, ppm) and the polymer conjugate **(7)** obtained after modification of **(5)** with 2-aminoethanethiol**.**



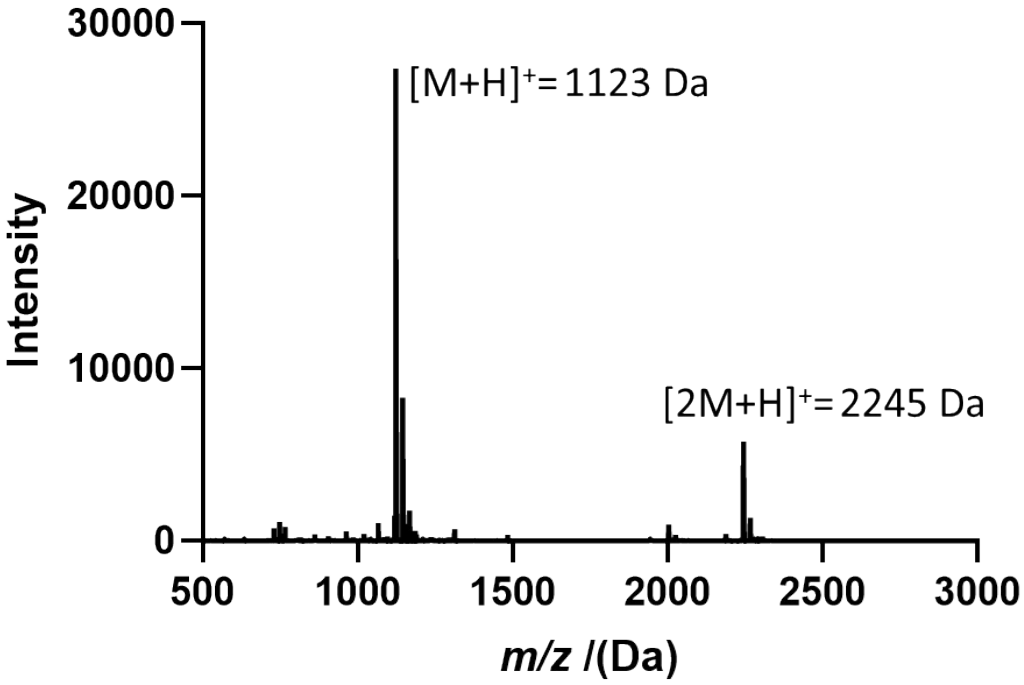




**Figure S6:** Chemical structure and MALDI-TOF analysis of the cysteine modified (SIINFEKL) peptide.

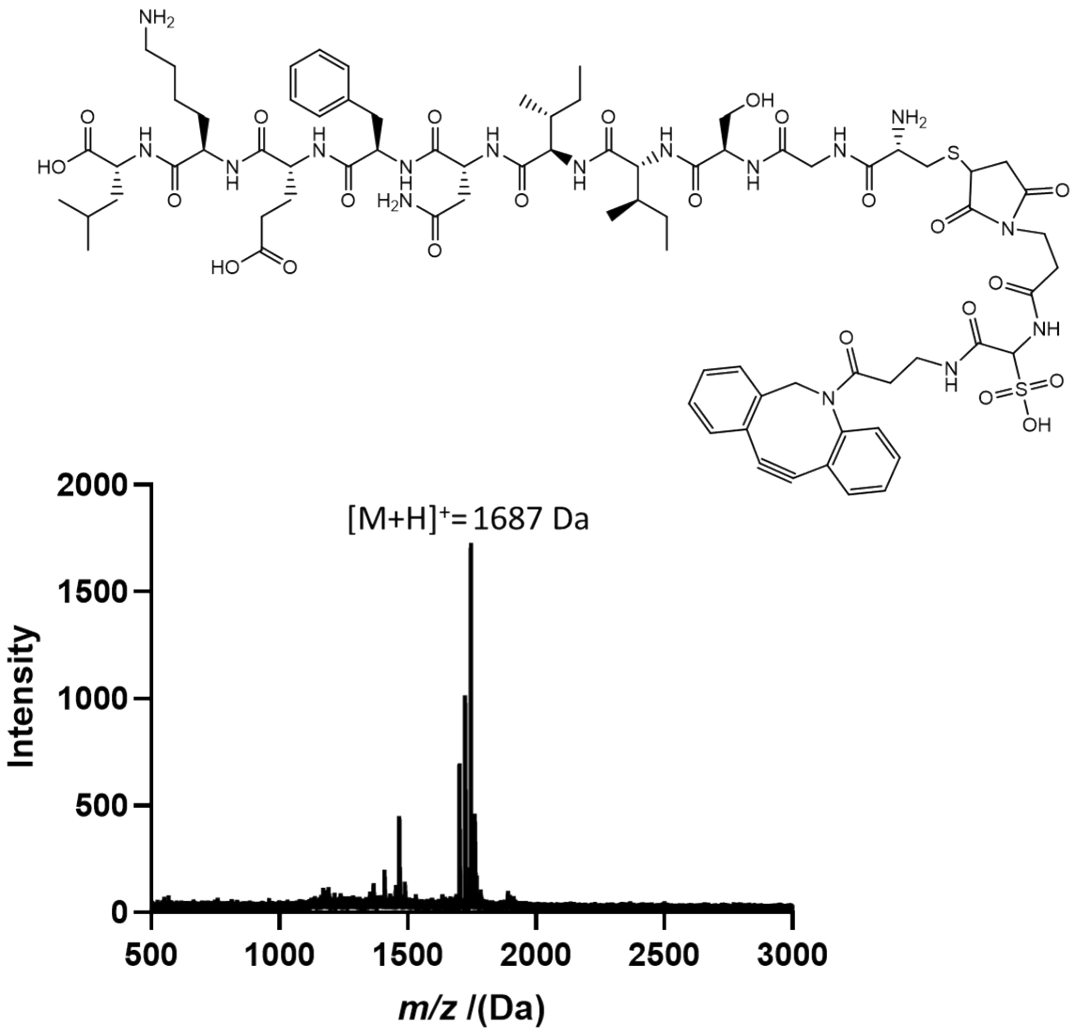



**Figure S7:** Chemical structure and MALDI-TOF analysis of the DBCO modified (SIINFEKL) peptide.

**Table S1:** Amino acid analysis of the PSA-CGSIINFEKL peptide conjugate. Results correspond with the peptide sequence minus cysteine. Cysteine is excluded from the analysis as it requires a different analytical procedure.

| Amino Acid | nmoles | nms/50 ul | mole % | N (data) | N (exp) |
|---|---|---|---|---|---|
| | | | | | |
| Asx | 1.547 | 1.531 | 11.67 | 1.1 | 1 |
| Thr | 0.000 | 0.000 | 0.00 | 0.0 | 0 |
| Ser | 1.544 | 1.528 | 11.64 | 1.0 | 1 |
| Glx | 1.711 | 1.693 | 12.90 | 1.2 | 1 |
| Pro | 0.000 | 0.000 | 0.00 | 0.0 | 0 |
| Gly | 1.953 | 1.933 | 14.73 | 1.3 | 1 |
| Ala | 0.000 | 0.000 | 0.00 | 0.0 | 0 |
| Val | 0.000 | 0.000 | 0.00 | 0.0 | 0 |
| Ile | 1.849 | 1.830 | 13.94 | 1.3 | 2 |
| Leu | 1.445 | 1.430 | 10.90 | 1.0 | 1 |
| Tyr | 0.000 | 0.000 | 0.00 | 0.0 | 0 |
| Phe | 1.721 | 1.703 | 12.98 | 1.2 | 1 |
| His | 0.000 | 0.000 | 0.00 | 0.0 | 0 |
| Lys | 1.490 | 1.475 | 11.24 | 1.0 | 1 |
| Arg | 0.000 | 0.000 | 0.00 | 0.0 | 0 |
| | | | | | |
| Cysteic | 0.000 | 0.000 | 0.00 | 0.0 | 0 |
| MetSO2 | 0.000 | 0.000 | 0.00 | 0.0 | 0 |
| Trp | 0.000 | 0.000 | 0.00 | 0.0 | 0 |
| | | | | | |
| **Totals:** | | 13.122 | 100.00 | | 9 |

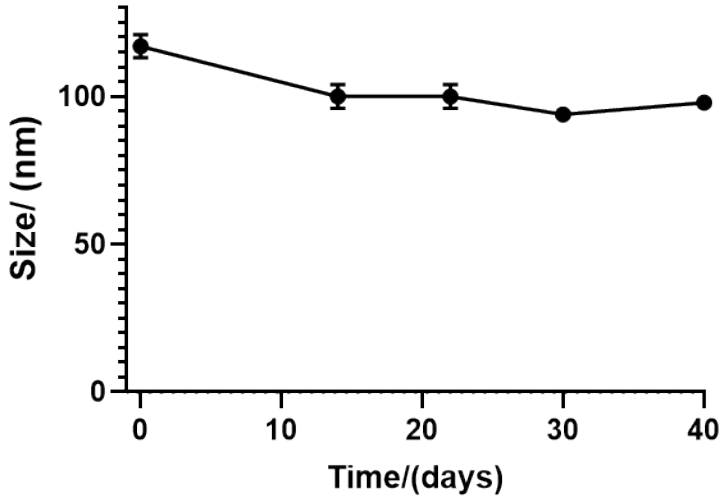



**Figure S8:** Particle size measured over time from NPs **(5)**.

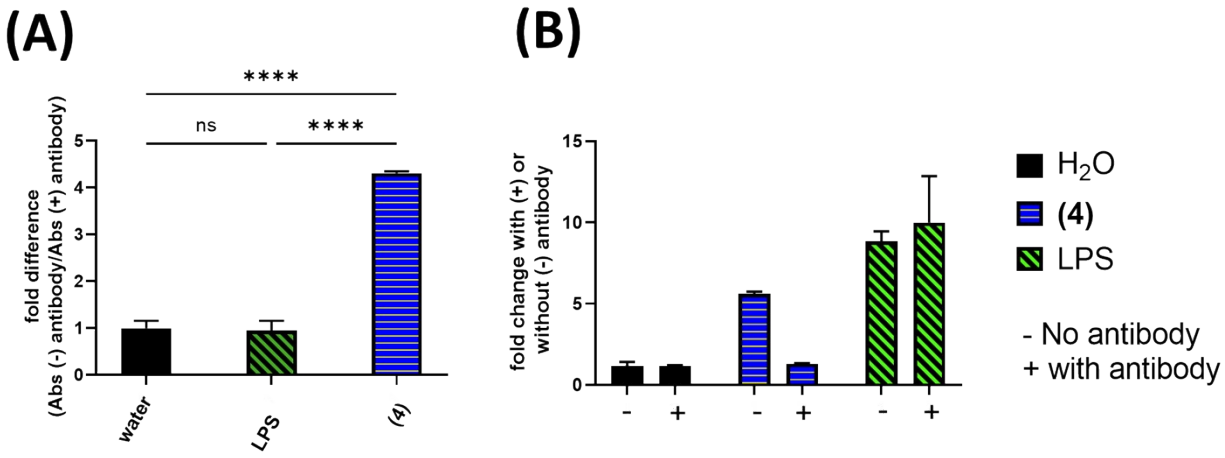



**Figure S9:** *In vitro* evaluation of the biological activity and specificity towards TLR2 of **(4)** and extent of activation (B) after azide modification, LPS and water were the positive and negative controls respectively

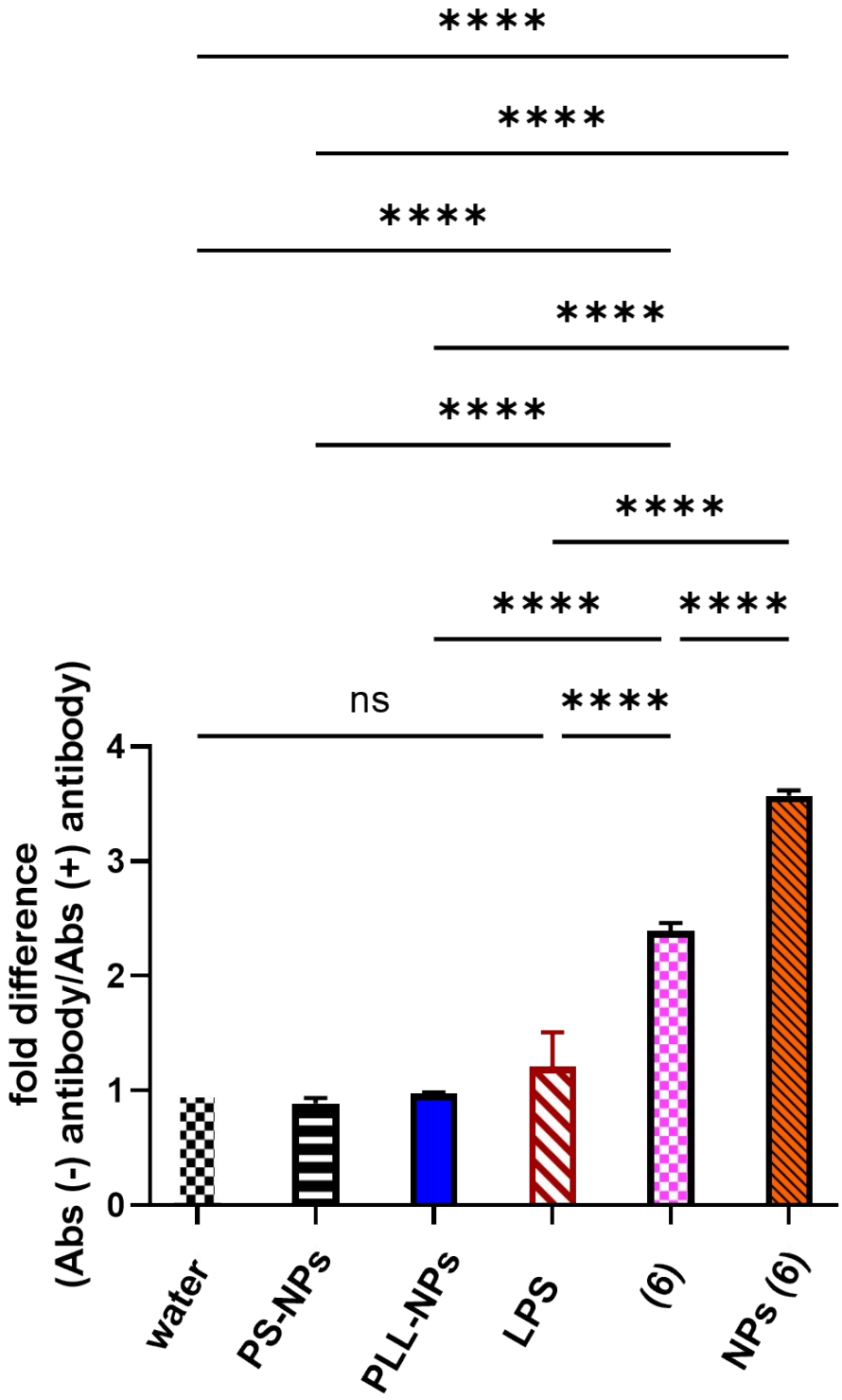



**Figure S10:** *In vitro* evaluation of the biological activity and specificity towards TLR2 of **(6)** and NPs **(6),** the template and the template (**PS-NPSs**) after coating with positiviely charged polylysine (**PLL-NPs**).

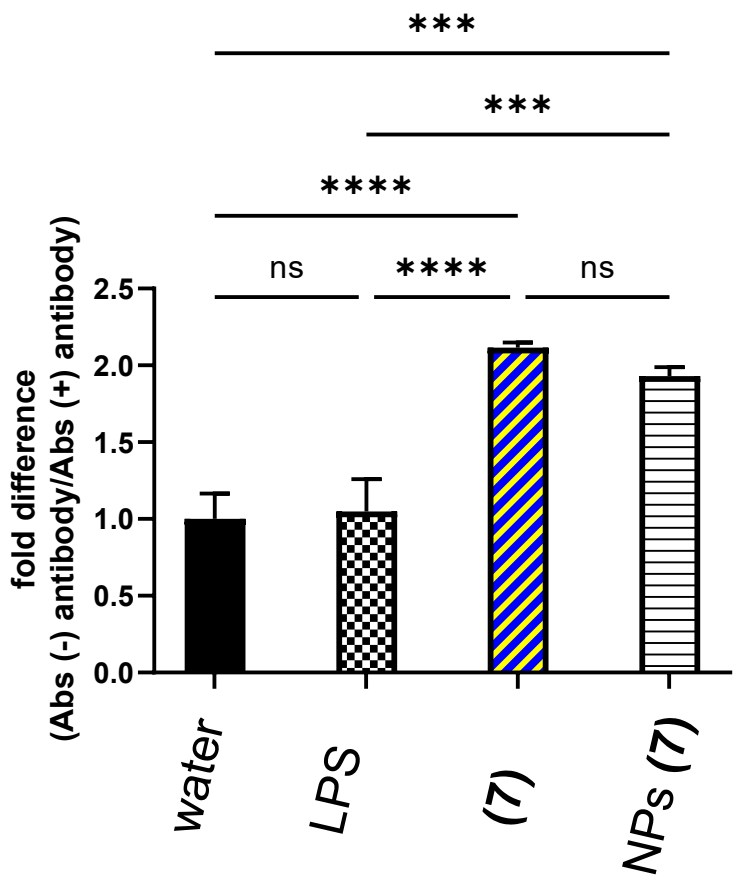



**Figure S11:** *In vitro* evaluation of the biological activity and specificity towards TLR2 of **(7)** and NPs **(7)**.