Electronic Supplementary Material (ESI) for Nanoscale Advances.

MOLECULARLY IMPRINTED NANOPARTICLES FOR BACTERIAL VISUALIZATION

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Supplement information

Tables

Table S1 Overview of publications on bacterial detection/targeting (information are obtained from www.webofknowledge.com - Key words: imprint* polymer*, bacteria) – imprinting of whole bacteria

			Whole bacteria imprinting	
Bacteria	**Year**	**Method**	**Detection**	**Ref.**
Staphylococcus aureus	2020	Bulk polymerization	Fluorescence microscopy	[1]
Escherichia coli	2020	Microcontact imprinting	SPR	[2]
Escherichia coli	2020	Bulk polymerization	Fluorescence spectrometry	[3]
Acinetobacter baumannii	2020	Electropolymerization	Impedance spectroscopy	[4]
Vibrio parahaemolyticus	2020	Microcontact imprinting	PCR/gel electrophoresis	[5]
Escherichia coli, ***Escherichia blattae***	2020	Microcontact imprinting	Pyroelectric detection	[6]
Escherichia coli	2019	Soft-lithography	Thermal resistance	[7]
Escherichia coli	2019	Microcontact imprinting	Time-dependent temperature measurement	[8]
Listeria Monocytogenes	2019	Pickering emulsion polymerization	Fluorescence microscopy	[9]
Enterococcus faecalis	2019	Emulsion polymerization	SPR	[10]
Escherichia coli	2019	Sol-gel imprinting	Impedimetric	[11]
Escherichia coli	2019	Electropolymerization	Impedimetric	[12]
Escherichia coli	2018	Microcontact imprinting	Thermal	[13]
Escherichia coli	2018	Nanoimprint lithography	Fluorescent microscopy	[14]
Escherichia coli*,** ***Listeria monocytogenes	2018	Sol–gel imprinting	Fluorescence spectrometry	[15]

Escherichia coli	2017	Electropolymerization	QCM	[16]
Escherichia coli	2017	Soft-lithography	Thermal	[17]
Staphylococcus epidermidis	2017	Electropolymerization	Impedimetric	[18]
Escherichia coli	2017	Soft-lithography	Heat-transfer method	[19]
Escherichia coli, ***Shewanella oneidensis,*** ***Staphylococcus aureus,*** ***Enterococcus faecium***	2017	Atomic transfer radical polymerization	Single-cell force spectroscopy	[20]
Escherichia coli	2017	Bulk polymerization	Cyclic voltammetry	[21]
Escherichia coli	2017	Microcontact imprinting	Cyclic voltammetry	[22]
Escherichia coli	2017	Nanoimprint lithography	QCM	[23]
Escherichia coli, ***Staphylococcus aureus***	2016	Microcontact imprinting	Heat-transfer method	[24]
Escherichia coli	2016	Sedimentation/stamp imprinting	QCM	[25]
Bacillus Cereus	2016	Sedimentation/stamp imprinting	QCM	[26]
Rhodobacter sphaeroides	2015	Microcontact imprinting	Raman spectrometry	[27]
Escherichia coli	2015	Microcontact imprinting	SPR/QCM	[28]
Escherichia coli, ***Staphylococcus aureus***	2014	Imprinted shells	Fluorescence microscopy	[29]
Methylomicrobium ***album, Methylosinus trichosporium***	2014	Stamp imprinting	Fluorescence microscopy	[30]
Escherichia coli, Micrococcus luteus	2014	Pickering emulsion polymerization	Fluorescence microscopy	[31]
Escherichia coli, ***Pseudomona aeruginosa,*** ***Bacillus subtilis,*** ***Staphylococcus aureus***	2014	Electropolymerization	Dielectrophoresis	[32]
Mycobacterium tuberculosis	2013	Sedimentation imprinting	Fluorescence microscopy	[33]
Bacillus subtilis	2013	Electropolymerization	Impedimetric	[34]
Escherichia coli, ***Klebsiella pneumoniae,*** ***Staphylococcus aureus,*** ***Staphylococcus epidermidis***	2012	Soft lithography	Confocal microscopy	[35]
Synechococcus elongatus	2012	Soft lithography	Inverted microscopy	[36]

Table S2 Overview of publications on bacterial detection/targeting (information are obtained from www.webofknowledge.com - Key words: imprint* polymer*, bacteria) – imprinting of proteins or epitopes

Epitope/ Protein imprinting

Bacteria	Target	Year	Method	Detection	Ref.
Mycobacterium leprae	Peptide	2019	Electropolymerization	QCM	[37]
Neisseria Meningitidis	Peptide	2018	Bulk polymerization	QCM	[38]
Staphylococcus aureus	Surface protein	2016	Electropolymerization	Cyclic voltammetry	[39]
Pseudomonas aeruginosa	Lipopoly-saccharide	2016	Precipitate polymerization	Fluorescence polarization	[40]
Staphylococcus aureus	Surface protein	2009	Bulk polymerization	UV spectrophotometry	[41]

Table S3 Summary of publications focused on the imprinted polymers of saccharides

Saccharide	Cell	Year	Ref.
Sialic acid	DU145 cell, PC3 cell, Jurkat cell	2015	[42]
Glucuronic acid	Human keratinocytes	2015	[43]
Sialic acid, Fucose, Mannose	HepG-2 cell, L-02 cell, MCF-7 cell, MCF-10A cell	2016	[44]
Sialic acid	DU 145 cell, HeLa cell	2017	[45]
Hyaluronan, Sialic acid	HaCaT cell	2017	[46]
Hyaluronan	HaCaT cell	2017	[47]
Hyaluronic acid	HaCaT cell	2018	[48]
Hyaluronic acid	HaCaT cell	2019	[46]
Sialic acid, Fucose, Mannose	HepG-2 cell, L-02 cell, MCF-10A cell, A-431 cell, HaCat cell, HK-2 cell, HeLa cell	2017	[49]

Results

Dynamic light scattering (DLS) analysis was performed on nanoMIPs after 2 min sonication and 30 min vortexing. The data show a single peak, indicating an average hydrodynamic diameter of 55.56 ± 6.1 nm for Man nanoMIPs (a) and 111 ± 7.5 for GlcNAc nanoMIPs (b).

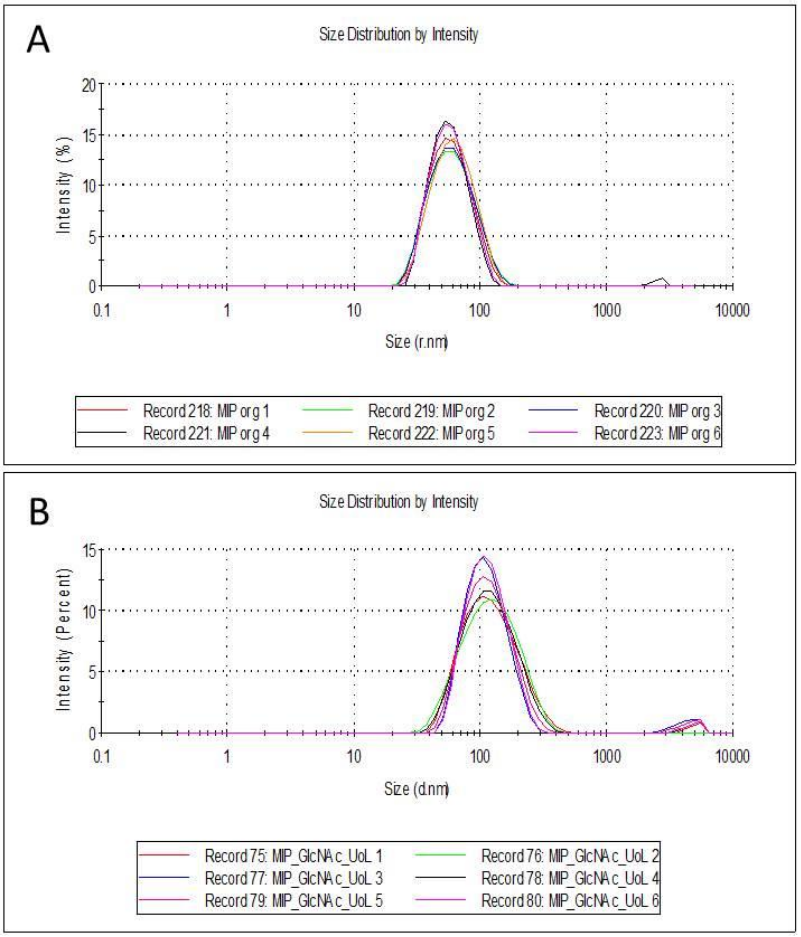

Figure S1 DLS analysis of Man-nanoMIPs (a), GlcNAc-nanoMIPs (b).

Flow cytometry analysis

Different concentrations of GlcNAc-nanoMIPs (0.00015 - 0.40000 mg/mL) and Man-nanoMIPs (0.0125 - 0.40000 mg/mL) were incubated respectively *E.Coli* (Figure S2) and *D39_S. Pneumoniae* (Figure S3). In the graphs reported below, the shift in the fluorescent signal indicates binding between fluorescent nanoMIPs and respectively *E.Coli* or *D39_S.Pneumoniae*. Both bacteria were run without nanoMIPs and the autofluorescence subtracted. NanoMIPs imprinted for a small non-related compound were incubated with both bacteria and used as controls.

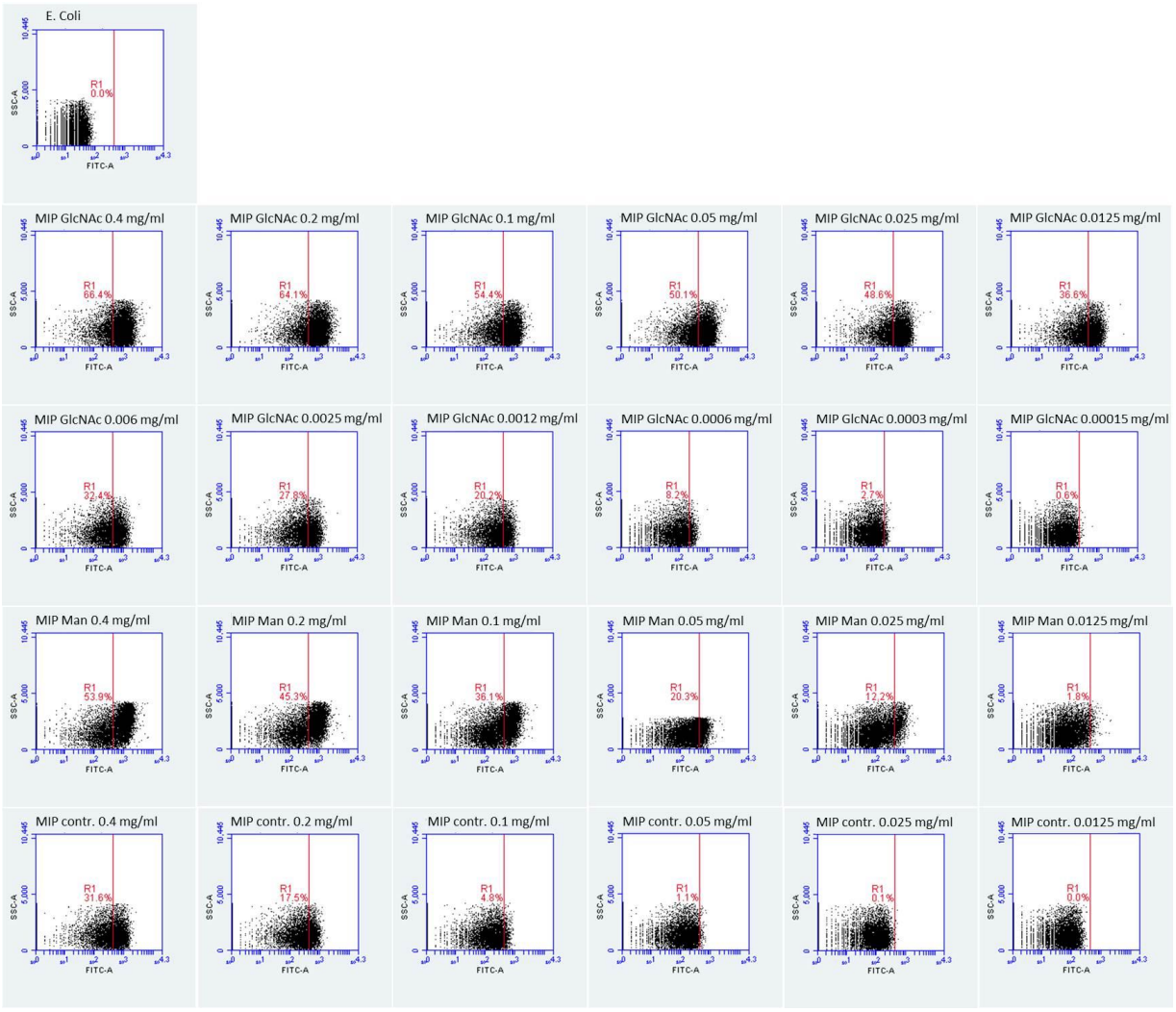

Figure S2 Flow cytometry analysis of control *E. Coli (no nanoMIPs)*, GlcNAc nanoMIPs (0.00015 – 0.40000 mg/mL) binding *E. Coli;* Man NanoMIPs (0.00125 – 0.4000 mg/mL) binding *E.Coli* and control nanoMIPs binding *E.Coli*

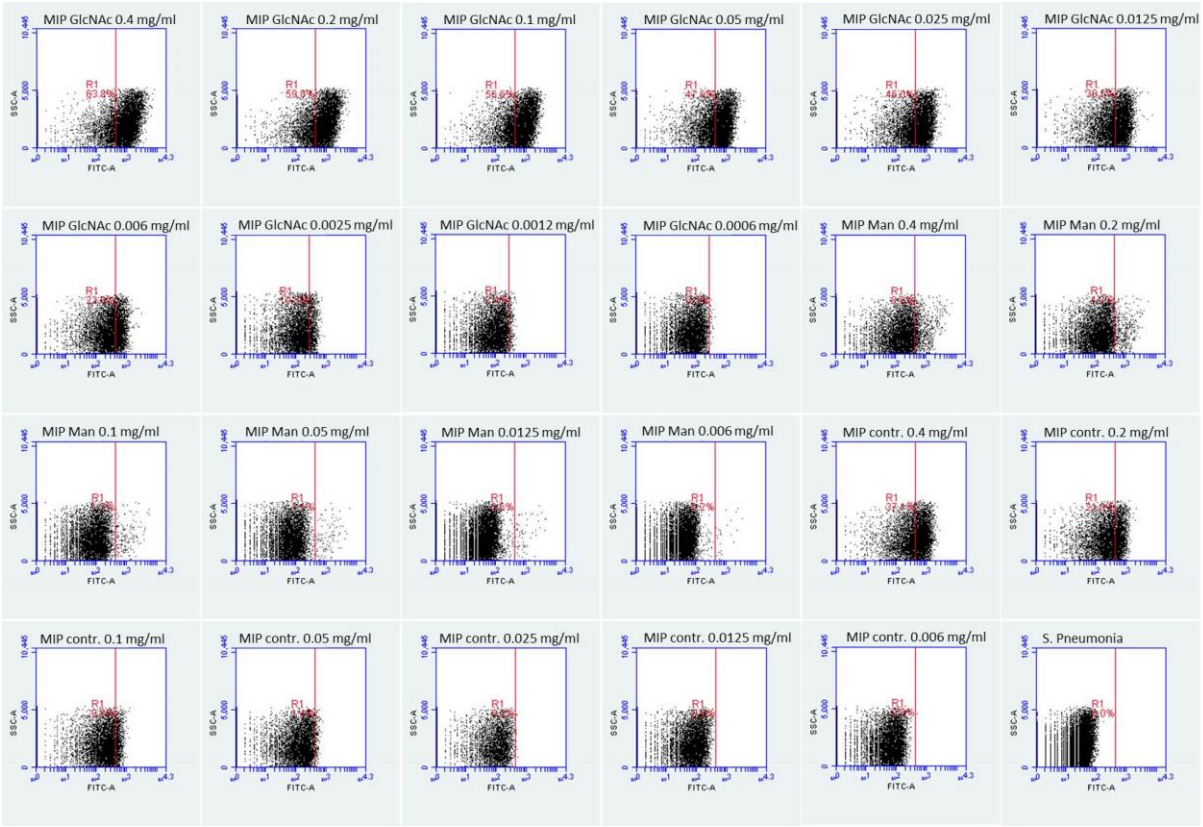

Figure S3 Flow cytometry analysis of GlcNAc nanoMIPs (0.0006 – 0.4 mg/mL) binding *S. Pneumoniae;* Man NanoMIPs (0.006 – 0.4 mg/mL) binding *S. Pneumoniae*; control nanoMIPs binding *S. Pneumoniae; Control S. Pneumoniae (no nanoMIPs)*

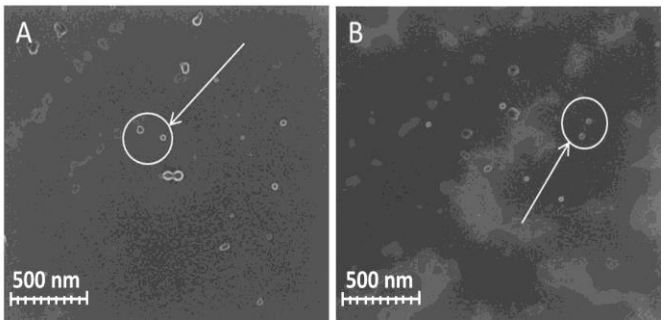

Figure S4 (A) Image of Man-nanoMIPs by SEM; (B) image of GlcNAc-nanoMIPs by SEM.

Comparison of binding

ConA was labelled with a fluorescent dye (Alexa 647) and binding between bacteria and ConA was assessed by using flow cytometry. From measured data (Fig S.4) it was observed that the amount of bound ConA was higher in the case of *E. Coli*. These data confirm that *E. Coli* bears higher amount of mannose molecule on their surface compared with *S. Pneumoniae*.

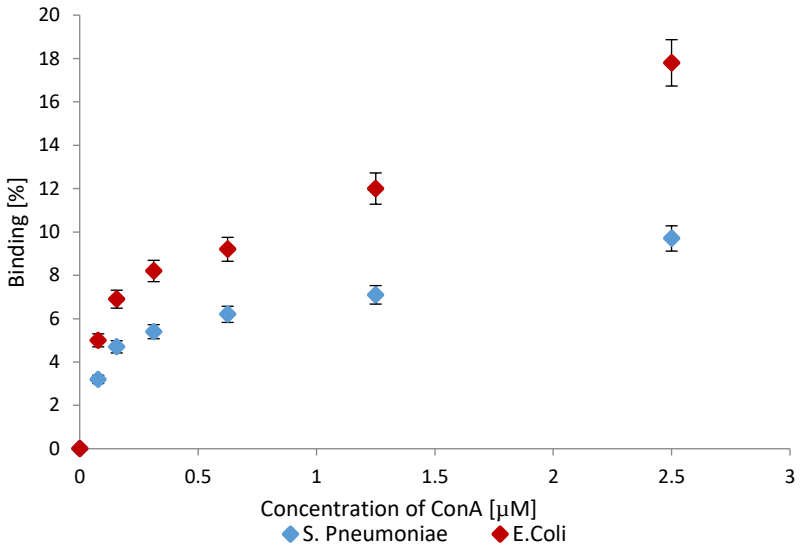

Figure S5 Binding of concanavalin A to gram-negative (red) and positive (blue) bacteria by flow cytometry.

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