SUPPORTING INFORMATION

Surface roughness influences the protein corona formation of glycosylated nanoparticles and alter their cellular uptake

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Experimental

Materials

Tripotassium phosphate (K_3PO_4), 3-mercaptopropionic acid, carbon disulfide, benzyl bromide, acetic anhydride, D-glucose, D-mannose, D-galactose, 2-hydroxyethyl acrylate (HEA), boron trifluoride diethyl etherate, magnesium sulfate, sodium chloride, sodium bicarbonate, sodium methoxide, potassium dihydrogenphosphate (KH_2PO_4), sulfuric acid (H_2SO_4), hydrochloric acid and sodium hydroxide were purchased from Sigma Aldrich and used as received. Deuterated dimethyl sulfoxide (DMSO-*d6*) and deuterated chloroform ($CDCl_3$) were purchased from Cambridge Isotope Laboratories. *n*-Butyl acrylate (BuA), styrene and 4-vinyl pyridine (4VP) were purchased from Sigma Aldrich and filtered over basic aluminium oxide before use. 2,2′-Azobis(2-methylpropionitrile) (AIBN) was purchased from Sigma Aldrich and recrystallised from methanol before use.

Analyses

FT-NIR Spectroscopy. A Bruker IFS\S Fourier transform spectrometer was used for the measurements, with a tungsten halogen lamp, a CaF_2 beam splitter and a liquid nitrogen cooled InSb detector. The spectral region of interest was between 7000 and 5000 cm^{-1} with a resolution of 4 cm^{-1}. Using a heating block the sample was maintained at 70 ± 0.5 °C.

Nuclear Magnetic Resonance (NMR). Either a Bruker Avance III 300 MHz, 5 mm BBFO probe (1H: 300.17 MHz) or a Bruker Avance III HD 400 MHz, 5 mm BBFO probe (1H: 400.13 MHz) spectrometer were used for NMR measurements. Solvents employed were

$CDCl_3$ and DMSO-*d6* and chemical shifts are expressed in ppm relative to residual solvent peaks. Measurements were performed at 25 °C. The raw data was processed using Bruker TOPSPIN 3.2 software.

Size Exclusion Chromatography (SEC). Experiments were run on a Shimadzu modular system with SIL-10AD autoinjector, LC-10AT pump, CTO-10A oven, 5.0-µm bead guard column (50 × 7.8 mm) followed by four 300 × 7.8 mm linear columns (Phenomenex) with 500, 103, 104, 105 Å pore size and 5 µm particle size. The solvent system was *N*,*N*-dimethylformamide (HPLC grade) with 0.05% w/v 2,6-dibutyl-4-methylphenol and 0.03% w/v LiBr. Flow rate was 1 mL min^{-1} at 50 °C and a refractive index detector was used (Shimadzu RID-10A). The calibration was performed using narrow polydispersity PMMA standards (0.5−1000 kDa) purchased from Polymer Laboratories.

Dynamic Light Scattering (DLS). A Malvern Zetasizer equipped with a He-Ne laser (4 mV) operating at 632 nm was used and the detection was at 173°. No filtration of the samples was necessary before the measurements. The temperature was kept at 25 °C and the polymer concentration varied between 0.2 and 1 mg mL^{-1}.

Transmission Electron Microscopy (TEM). A JEOL1400 TEM operating at 100 kV and equipped with a Gatan CCD camera was used. Samples were deposited on a 200 mesh copper grids coated with Formvar and carbon. 5 µL of sample at a concentration of 1 mg mL^{-1} were deposited on the grids and the excess liquid was immediately blotted away leaving the grid to air dry for at least one hour. If desired, a 2 wt. % solution of uranyl acetate in water was used to stain the samples for 5 min.

Quartz crystal microbalance with dissipation monitoring (QCM-D). A quartz crystal microbalance with dissipation monitoring system (Q-Sense Analyzer) was operated at 0.2 mL min^{-1} at a constant temperature of 37 ºC. Gold QCM-D crystals were mounted in the chambers then frequency (*f*) and dissipation (D) measurements versus time were recorded for the fundamental *f* (5 MHz) as well as the 3^{rd}, 5^{th}, 7^{th}, 9^{th} and 11^{th} overtones. PBS (0.01 M, pH 7.4) was passed over the crystals and allowed to stabilize before being replaced with 0.1 mg.mL^{-1} Con A in PBS (0.1 mg mL^{-1}) and allowed to adsorb to the gold sensor surface for 12 min before being replaced by bovine serum albumin (BSA, 0.1 mg mL^{-1}) in PBS that was exposed to the sensor surface for 5 min to bind to remaining areas of the gold sensor surface. The sensors were then rinsed using PBS buffer allowing for *f* and D stabilization. Next 0.2 mg mL^{-1} glycopolymer colloidal solution in PBS was added for 20 min, before rinsing

with PBS followed by the addition of DMEM supplemented with 10 vol% fetal bovine serum (FBS) for 20 min and then rinsing with PBS for 20 min. The QCM *f* and D measurements were modelled using the Voigt model to obtain mass of Con A, glycopolymers and serum proteins bound in each condition.

Protein corona analysis by liquid chromatography tandem mass spectrometry (LC/MS/MS). Samples were incubated with the same culture medium used for cytotoxicity and cellular uptake experiments at a concentration of 200 μg mL^{-1} for 2 hours at 37 °C. The particles with the adsorbed hard corona were pelleted through centrifugation (10 min, 20,000 rcf) and washed twice with 1 mL of 25 nM NH_4HCO_3. After removal of the supernatant 100 μL of 1 M dithiothreitol (DTT) solution were added to each sample and incubated at 80 °C for 30 min. 100 μL of iodoacetic acid (IAA) solution in 25 mM NH_4HCO_3 were added to each sample to a final concentration of 55 mM. The solutions were reacted for 20 minutes at room temperature in the dark. Trypsin was added to a concentration of 10 μg mL^{-1} in NH_4HCO_3 25 mM solution (100 μL). The sample tubes were sealed, wrapped in parafilm and incubated overnight at 37 °C before LC/MS/MS analysis. Digested peptides were separated by nanoLC using an Ultimate nanoRSLC UPLC and autosampler system (Dionex, Amsterdam, Netherlands). Samples (2.5 μl) were concentrated and desalted onto a micro C18 pre-column (300 μm × 5 mm, Dionex) with H_2O/CH_3CN (98:2, 0.1 % TFA) at 15 μl min^{-1}. After a 4 min wash the pre-column was switched (Valco 10 port UPLC valve, Valco, Houston, TX) into line with a fritless nano column (75 μ × ~ 15 cm) containing C18AQ media (1.9 μ, 120 Å Dr Maisch, Ammerbuch-Entringen, Germany). Peptides were eluted using a linear gradient of H_2O/CH_3CN (98:2, 0.1 % formic acid) to H_2O/CH_3CN (64:36, 0.1 % formic acid) at 200 nL min^{-1} over 30 minutes. High voltage (2000 V) was applied to low volume Titanium union (Valco) and the tip positioned ~ 0.5 cm from the heated capillary (T = 275 °C) of a Orbitrap Fusion Lumos (Thermo Electron, Bremen, Germany) mass spectrometer. Positive ions were generated by electrospray and the Fusion Lumos operated in data dependent acquisition mode (DDA). A survey scan m/z 350-1750 was acquired in the orbitrap (resolution = 120,000 at m/z 200, with an accumulation target value of 400,000 ions) and lockmass enabled (m/z 445.12003). Data-dependent tandem MS analysis was performed using a top-speed approach (cycle time of 2 seconds). MS^2 spectra were fragmented by HCD (NCE = 30) activation mode and the ion-trap was selected as the mass analyser. The intensity threshold for fragmentation was set to 25,000. A dynamic exclusion of 20 seconds was applied with a mass tolerance of 10 ppm. Peak lists were

generated using Mascot Daemon/Mascot Distiller (Matrix Science, London, England), and submitted to the database search program Mascot (version 2.5.1, Matrix Science). Search parameters were: precursor tolerance 4 ppm and product ion tolerances ± 0.5 Da, enzyme specificity was trypsin, 1 missed cleavage was possible and the Unirprot (2018, 556568 sequences; 199530821 residues) *Bos taurus* database was searched. For the semi-quantitative analysis, the significance threshold was limited to $p < 0.02$ and only proteins with an emPAI score greater than 2 were considered. Two independent experiments were conducted for each condition for statistical comparison using one-way ANOVA.

Sulforhodamine B (SRB) assay. In a 96-well plate RAW264.7 murine macrophages, MDA-MB-231 human breast cancer cells or HS 27 healthy fibroblasts were seeded at a concentration of 4000 cells per well and 200 µL of Dulbecco's modified Eagle's cell culture medium (DMEM) supplemented with 2.2 mg mL^{-1} GlutaMAX, 10 vol% fetal bovine serum (FBS), 100 µg mL^{-1} penicillin and 100 µg mL^{-1} streptomycin was added to each well. The plate was incubated at 37 °C with 5 % CO_2/ 95 % air atmosphere for 24 hours. The medium was then discarded and replaced with 200 µL of fresh medium containing different concentration of colloidal polymer solution and incubated at 37 °C for 72 hours. Cells were successively treated with 100 µL of trichloroacetic acid 10 % w/v solution and incubated at 4 ℃ for 30 minutes. After five washes with 200 µL of Milli-Q water per well, the TCA-fixed cells were stained for 30 min with 100 µL of 0.4 % w/v sulforhodamine B solution in 1% acetic acid. Excess dye was discarded and each well was washed five times with 200 µL of 1% acetic acid solution. The plate was air-dried for two hours and 100 µL of 10 mM Tris buffer were added to each well. After thorough mixing the absorbance at 570 nm was measured using a scanning spectrophotometer (BioTek's PowerWave™ HT Microplate Reader). Four wells with the same concentration of polymer particles were averaged and compared to the control to calculate cell viability following the equation:

$$Viability\ (\%) = (ABS_{sample} - ABS_{blank})/(ABS_{control} - ABS_{blank}) \times 100$$

where ABS_{blank} is the absorbance of a well without any seeded cells.

Flow cytometry. In a 6-well plate cells were seeded at a density of 5×10^5 cells per well together with 2 mL of DMEM medium supplemented as described above. The cells were incubated at 37 °C with 5 % CO_2/ 95 % air atmosphere and allowed to proliferate for 2 to 4 days until 80 % coverage was reached, after which the medium was discarded and replaced

by fresh medium containing the FITC-labelled polymer nanoparticles at a concentration of 200 μg mL^{-1}. The plate was incubated at 37 °C for 6 hours, the supernatant was discarded and each well was washed with 2 mL of cold DPBS three times. The cells were treated with trypsin and detached from the bottom of each well, centrifugated and resuspended in 1 mL of HBSS. Flow cytometry was performed with a BD FACSCanto II Analyzer (BD Biosciences, San Jose, USA). Each experiment was conducted in triplicate and fluorescence of the untreated cell sample was subtracted from the result. The statistical difference between the data was evaluated using a two-way ANOVA analysis with GraphPad Prism 7.

Confocal microscopy. Cells were seeded in 35 mm Fluorodishes (World Precision Instruments) with a density of 1×10^5 cells per well to a volume of 2 mL of DMEM medium. After incubation at 37 °C for 2 days under 5 % CO_2/ 95 % air atmosphere, the supernatant was discarded and replaced by 1 mL of fresh DMEM containing the particle solution at a concentration of 200 μg mL^{-1}. The plates were incubated at 37 °C for 2 hours and 24 hours. The cells were washed three times with cold PBS and fixed by adding 1 mL of 4 % paraformaldehyde solution to each well. The fixating agent was removed before imaging, the wells were washed with PBS and cells were stained with 2 μg mL^{-1} solution of Hoechst (Invitrogen) for 5 minutes. Each well was successively washed three times with PBS. A laser scanning confocal microscope system (Zeiss LSM 780) with a diode laser (excitation 405) connected to a Zeiss Axio Observer.Z1 inverted microscope (×20 magnification and 0.8 N.A objective) was used for observation. The Zen2011 imaging software (Zeiss) was used for image acquisition and processing.

Syntheses

Synthesis of 3-(benzylthiocarbonothioylthio)propionic acid (BSPA) RAFT agent. The compound was synthesised as previously reported.[1]

Synthesis of 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose (AcGlc). The synthesis of this same batch of monomer was already reported in the literature.[2]

Synthesis of 1,2,3,4,6-penta-O-acetyl-β-D-mannopyranose (AcMan). The synthesis of the protected mannose derivative followed a slightly different procedure than the glucose analogue.[3] A suspension of D-mannose (5 g, 28 mmol) in 25 mL of acetic anhydride was cooled down to 0 °C in an ice-bath and 5 drops of concentrated H_2SO_4 were added under vigorous stirring. After a few seconds the sugar appears to dissolve completely. The pale-

yellow solution was left stirring for 4 hours at room temperature and the colour became more intense. The reaction was interrupted by pouring the solution in 100 mL of chilled Milli-Q water and the product was extracted with dichloromethane three times (50 mL). The combined organic phase was washed with water (1 × 100 mL) and saturated $NaHCO_3$ solution (3 × 100 mL). Residual water in the organic phase was removed with magnesium sulphate and the product was collected as a colourless oil after removing the solvent under reduced pressure. Crystallisation of the mannose pentaacetate was observed a after drying under high vacuum. (yield: 8.94 g, 82 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 6.12 (1H, d, C*H*, *J* = 7 Hz), 5.35 (3H, m, C*H*), 4.20 (2H, m, CH_2), 2.11 (3H, s, CH_3), 2.08 (3H, s, CH_3), 2.03 (3H, s, CH_3), 2.03 (3H, s, CH_3), 2.01 (3H, s, CH_3).

Synthesis of 1,2,3,4,6-penta-O-acetyl-β-D-galactopyranose (AcGal). In a similar way as the synthesis of pentaacetylated glucose, 2.5 g (31 mmol) of sodium acetate were dissolved in acetic anhydride (25 mL) and heated to 140 °C. D-galactose (5 g, 28 mmol) was added slowly to the boiling solution under vigorous stirring during a period of 20 minutes. Dissolution of the sugar was complete after a few minutes and the yellowish solution was left to cool down for 1 hour. The mixture was poured into 100 mL of ice-cold water and precipitation of the product was observed. The colourless powder was collected by suction filtration and washed with cold water. The crude product was purified by crystallisation, after dissolution in about 10 mL of hot ethanol colourless needles formed when the solution was cooled down to 4 °C overnight. The product was recovered by filtration (yield: 4.70 g, 43 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 5.69 (1H, d, C*H*, *J* = 7 Hz), 5.42 (1H, d, C*H*, *J* = 7 Hz), 5.33 (1H, m, C*H*, *J* = 9 Hz), 5.10 (1H, m, C*H*), 4.21-4.15 (2H, m, CH_2) 4.07 (1H, m, C*H*), 2.18 (3H, s, CH_3), 2.14 (3H, s, CH_3), 2.06 (3H, s, CH_3), 2.06 (3H, s, CH_3), 2.01 (3H, s, CH_3).

*Synthesis of 2-(2',3',4',6'-tetra-O-acetyl-D-*sugar*) ethyl acrylate (Ac*Sug*EA).* The procedure and the characterisation have been reported elsewhere for the glucose derivative.[2] Protected mannose and galactose compounds were reacted with HEA following the same procedure. The characterisation is reported below:

AcManEA. The eluent used for chromatographic purification was *n*-hexane/ethyl acetate 55:45 mixture. Yield: 2.8 g (27 %). (**Fig. S1**) ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 6.44 (1H, d, *J* = 17 Hz, CH_2-CH), 6.15 (1H, dd, *J* = 17, 10 Hz, CH_2-C*H*-C), 5.87 (1H, d, *J* = 10 Hz, CH_2-CH), 5.22-5.37 (3H, m, C*H*), 4.85 (1H, s, CH-CHO-C$H_{anomeric}$), 3.72-4.40 (7H, m, C*H*-CH_2), 2.15 (3H, s, CH_3), 2.08 (3H, s, CH_3), 2.03 (3H, s, CH_3), 1.98 (3H, s, CH_3).

AcGalEA. A 2:3 mixture of *n*-hexane and ethyl acetate was used for column chromatography and after removal of the solvent, a colourless solid was obtained (yield: 3.2 g, 60 %). (**Fig. S1**) ^{1}H NMR (300 MHz, $CDCl_3$) δ_H = 6.42 (1H, d, *J* = 17 Hz, C*H*$_2$-CH), 6.13 (1H, dd, *J* = 17, 10 Hz, CH_2-C*H*-C), 5.85 (1H, d, *J* = 10 Hz, C*H*$_2$-CH), 5.42 (1H, t, *J* = 9 Hz, CHO-C*H*), 5.24 (1H, m, CH-C*H*O-CH), 5.04 (1H, dd, *J* = 9, 7 Hz, CH-C*H*O-$CH_{anomeric}$), 4.56 (1H, d, *J* = 7, O-C*H*$_{anomeric}$O-CH), 3.82-4.35 (7H, m, C*H*$_2$), 2.17 (3H, s, C*H*$_3$), 2.07 (3H, s, C*H*$_3$), 2.05 (3H, s, C*H*$_3$), 2.01 (3H, s, C*H*$_3$).

*Synthesis of poly(2-(2',3',4',6'-tetra-O-acetyl-D-*sugar*) ethyl acrylate (PAc*Sug*EA).* The procedure described in a previous publication for PAcGlcEA[2] was followed for the RAFT polymerisation of the other two sugar-based acrylates. Ac*Sug*EA, BSPA CTA and AIBN initiator were dissolved in toluene (1.2 mL) in molar ratio respectively 100:1:0.1 and a monomer concentration of 1 M. The mixture was degassed bubbling nitrogen for 20 minutes inside an FT-NIR glass cuvette and the polymerisation was conducted at 70 °C. IR spectra were acquired during reaction and monomer conversion was monitored by integrating the vinylic peak over time (**Fig. S2**). The polymerisation was interrupted once the desired conversion was reached (approximately 60 %) by cooling down the mixture to 0 °C and introducing air. ^{1}H NMR of the polymerisation crude was used to calculate monomer conversion from the ratio between the integrals of the polymer and remaining monomer. Finally, to isolate the polymer from the mixture, the crude was added dropwise to cold diethyl ether (40 mL). The precipitate was collected and dried obtaining a glassy yellow solid while the supernatant was discarded. Conversion: PAcGlcEA 60 %, PAcManEA 50 %, PAcGalEA 70 %.

*Chain extension of macro-RAFT PAc*Sug*EA with n-butyl acrylate (PAc*Sug*EA-PBuA).* The purified macro-RAFT agents PAc*Sug*EA were chain extended with *n*-butyl acrylate in toluene as described for the glucose-based homopolymer elsewhere.[2] The molar ratio between BuA:macro-RAFT:AIBN was 300:1:0.1 and the monomer concentration was 2 M. The polymerisations were conducted at 70 °C in sealed FT-NIR glass cuvettes after purging with nitrogen for 20 minutes. After 1.5 hours the conversion had reached between 60 and 65 % in all cases and the polymerisations were stopped by quenching to 0 °C and introducing air. Monomer conversion and theoretical molecular weight were calculated by ^{1}H NMR as described above. The polymers were recovered by precipitation in cold *n*-hexane (50 mL) and dried under high vacuum for 1 hour obtaining yellow gelatinous solids. Conversion: PAcGlcEA-PBuA 65 %, PAcManEA-PBuA 65 %, PAcGalEA-PBuA 60 %.

*Chain extension of diblock macro-RAFT PAc*Sug*EA-PBuA with 4-vinyl pyridine (PAc*Sug*EA-PBuA-P4VP).* Following the same procedure described in the literature for the glucose-functionalised polymer,[2] the diblock macro-RAFT agents were extended again with 4VP in *N*,*N*-dimethylacetamide (1.5 mL). The same ratio of 500:1:0.2 (4VP:macro-RAFT:AIBN) was used for all three polymerisation and the monomer concentration was 0.5 M. The pale-yellow solution was degassed by three freeze-pump-thaw cycles and reacted under nitrogen atmosphere in a pre-heated oil bath at 70°C for 20 hours. The polymerisation was stopped cooling the solution with ice and introducing air. ^{1}H NMR was used to calculate the conversion. PAcGlcEA-PBuA-P4VP 32 %, PAcManEA-PBuA-P4VP 30 %, PAcGalEA-PBuA-P4VP 34 %.

*Chain extension of diblock macro-RAFT PAc*Sug*EA-PBuA with styrene (PAc*Sug*EA-PBuA-PS).* For the chain extension of all three glycopolymers 60 μL of styrene (55 mg, 0.53 mmol) were mixed with 0.5 mL of toluene and an appropriate amount of PAc*Sug*EA-PBuA macro-RAFT agent (93 mg of Glc-based, 85 mg of Man-based, 96 mg of Gal-based) was added to the solution in order to achieve a ratio of 300:1 (Sty:macro-CTA) based on the theoretical molecular weight. An appropriate volume of AIBN stock solution in toluene was added (0.2 mol of AIBN per macro-RAFT). The solution was transferred to a schlenk vial, sealed and degassed with three freeze-pump thaw cycles. The polymerisation was carried out at 70 °C and interrupted after 20 hours by quenching at 0 °C and introducing air. The conversion was measured by ^{1}H NMR.

*Deprotection of the triblock terpolymers (P*Sug*EA-PBuA-P4VP).* The P4VP-based triblock terpolymers were treated with sodium methoxide in methanol/chloroform in order to deacetylate the sugar-based side chains. As described in a previous publication,[2] the crude solutions were diluted with 8 mL of methanol/chloroform 1:1 mixture and a 2.5 wt. % solution of sodium methoxide in methanol was added dropwise under vigorous stirring. The volume added was chosen in order to have about 1 mole of sodium methoxide for every mole of sugar. The solutions were stirred for 1 hour and chloroform was removed under reduced pressure. The remaining cloudy solutions were dialysed against 2 L of Milli-Q water for 24 hours (MWCO 3500 Da). The polymers were recovered by lyophilisation as colourless solids and characterised by NMR (**Fig. S3**).

*Deprotection of the triblock terpolymers (P*Sug*EA-PBuA-PS).* The styrene-based triblock terpolymers were deprotected following a similar procedure. The polymerisation

crude was diluted with 4 mL of methanol/chloroform solution (1:1) and an appropriate volume of 2.5 wt. % solution of sodium methoxide in methanol was added dropwise. After reacting for 1 hour the mixtures were dialysed against 1 L of methanol for 6 hours and successively against 2 L of Milli-Q water for 24 hours (MWCO 3500 Da). The polymers were recovered by lyophilisation.

Self-assembly

General procedure of self-assembly of 4VP-based triblock terpolymers into compartmentalised micelles. Typically, 2 mg of triblock terpolymer were dissolved in 200 μL of DMF heating gently with a heat gun (100 °C). 10 μL of a 1.2 mg mL^{-1} solution of FITC fluorescent tag in DMF were added to the solution and reacted at room temperature overnight in the dark. To the well stirred solution 1.8 mL of methanol were added dropwise over a period of approximately 20 minutes to form core-shell micelles. This 1 mg mL^{-1} solution in methanol/DMF 9:1 was used as a starting point for the second step of the assembly. The solution was transferred in a 3500 Da MWCO dialysis bag and dialysed against 1 L of 5 mM K_2HPO_4/KH_2PO_4 buffer at pH 7.4 for about 20 hours in the dark. Particles were characterised by DLS and TEM.

General procedure for of the self-assembly of styrene-based triblock terpolymers into spherical micelles. Typically, 2 mg of triblock terpolymer were dissolved in 200 μL of DMF and heated gently with a heat gun (100 °C). 10 μL of a 1.2 mg mL^{-1} solution of FITC fluorescent tag in DMF were added to the solution and reacted at room temperature overnight in the dark. To the well stirred solution, 1.8 mL of Milli-Q water were added dropwise over a period of 20 min. Finally, the solution was dialysed against 1 L of 5 mM K_2HPO_4/KH_2PO_4 buffer at pH 7.4 for about 20 hours in the dark (3500 Da MWCO). Particles were characterised by DLS and TEM.

Additional figures

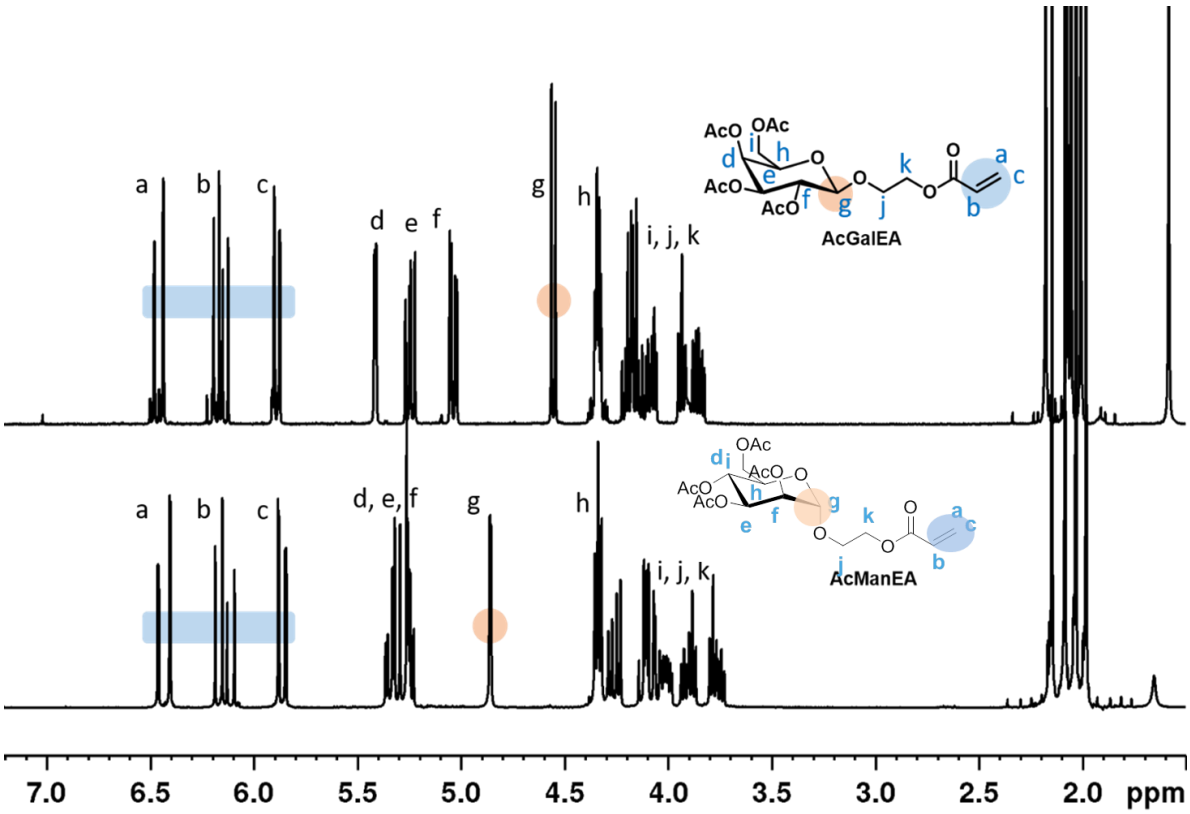

Fig. S1 ^{1}H NMR of the purified galactose and mannose-based monomers in $CDCl_3$ at 25 °C. The main differences between the sugars are in the chemical shift of the anomeric proton (orange) and the protons in the ring (5.0-5.5 ppm). Characterisation of the glucose-based monomer can be found in the literature.[2]

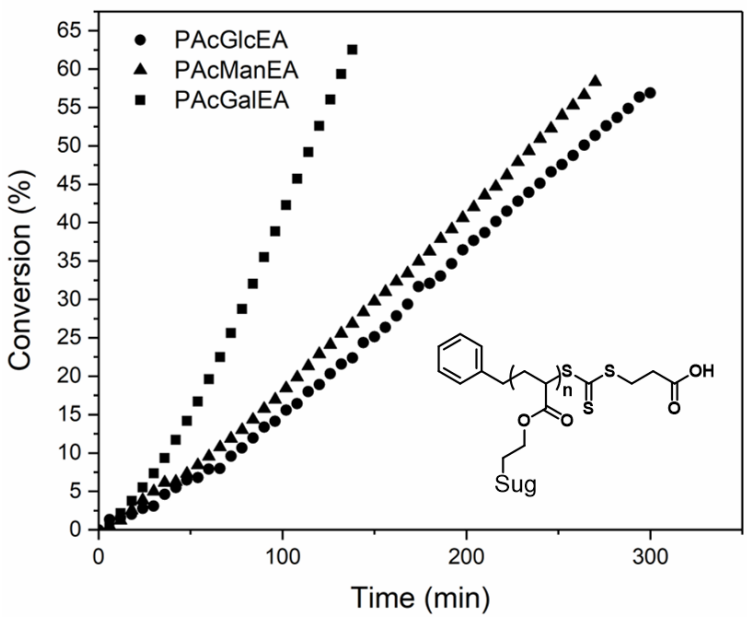

Fig. S2. Conversion over time during the polymerization of different sugar monomers measured by FT NIR. The integral of the vinylic peak at different time intervals was compared to the same peak at t = 0. The galactose functionalised monomer appears to polymerise faster than the others.

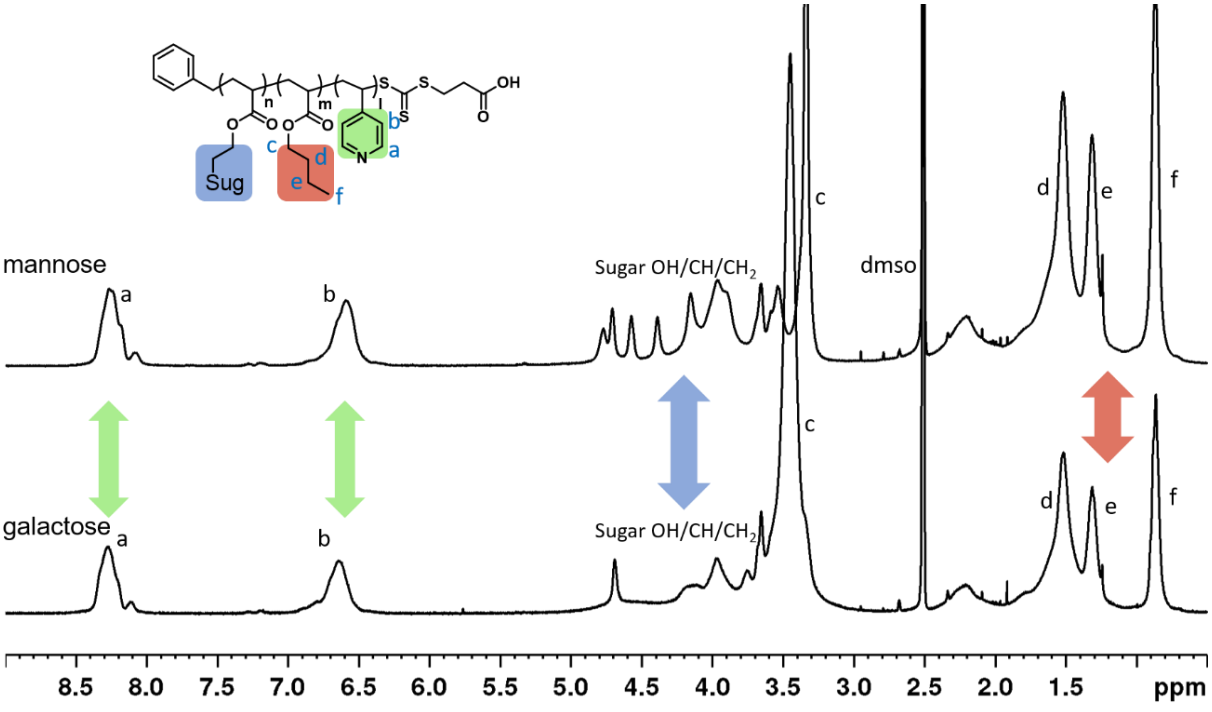

Fig. S3. [1]H NMR of purified poly(4-vinyl pyridine)-based triblock glycopolymers after deprotection of the sugar side chains. Experiments were run at 25 °C in DMSO-*d6*. Characterisation of the glucose-based triblock is reported in a previous publication.[2]

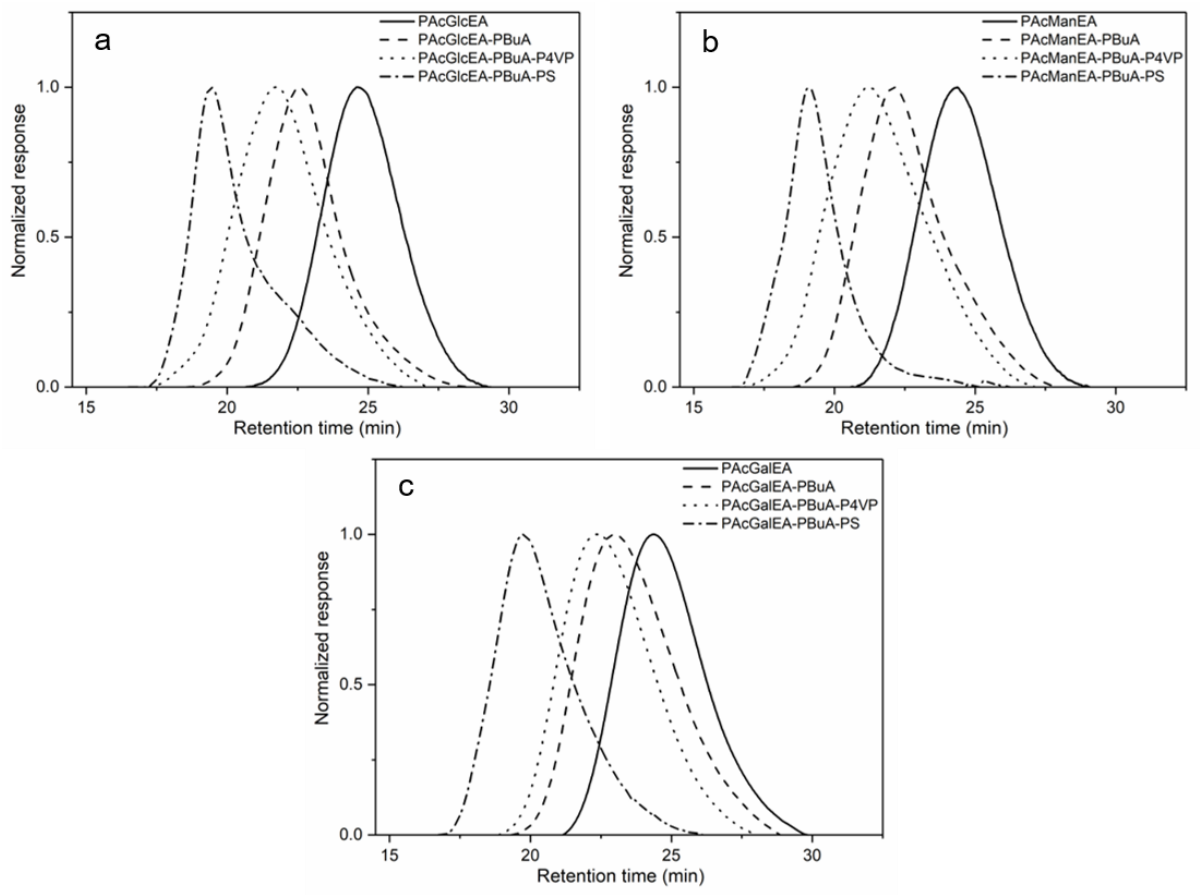

Fig. S4. SEC curves of the glucose (a), mannose (b) and galactose-based polymers (c) in DMF. The distribution shows a clear shift to shorter retention time after chain extension. Some tailing indicates the presence of a small amount of homopolymer or diblock copolymer.

Table S1 Summary of the characterisation of the polymers.

Entry	Polymer	DP[a]	M_n (theo)[a] [g/mol]	SEC[b]	
				M_n [g/mol]	$Đ$
1	PAcGlcEA	60	27,000	15,500	1.31
2	PAcManEA	50	22,600	17,600	1.29
3	PAcGalEA	70	31,500	15,100	1.27
4	PAcGlcEA-*b*-PBuA	60-200	52,600	30,800	1.29
5	PAcManEA-*b*-PBuA	50-200	48,200	33,100	1.33
6	PAcGalEA-*b*-PBuA	70-180	54,500	21,800	1.33
7	PAcGlcEA-*b*-PBuA-*b*-P4VP	60-200-160	69,400	38,100	1.37
8	PAcManEA-*b*-PBuA-*b*-P4VP	50-200-150	63,900	39,600	1.43
9	PAcGalEA-*b*-PBuA-*b*-P4VP	70-180-170	72,400	33,200	1.29
10	PAcGlcEA-*b*-PBuA-*b*-PS	60-200-110	64,100	60,300	1.58
11	PAcManEA-*b*-PBuA-*b*-PS	50-200-90	57,500	49,900	1.68
12	PAcGalEA-*b*-PBuA-*b*-PS	70-180-75	62,300	59,800	1.69

(a) Obtained by ^{1}H NMR of the polymerisation mixture comparing the integrals of polymer and leftover monomer peaks.
(b) SEC in DMF

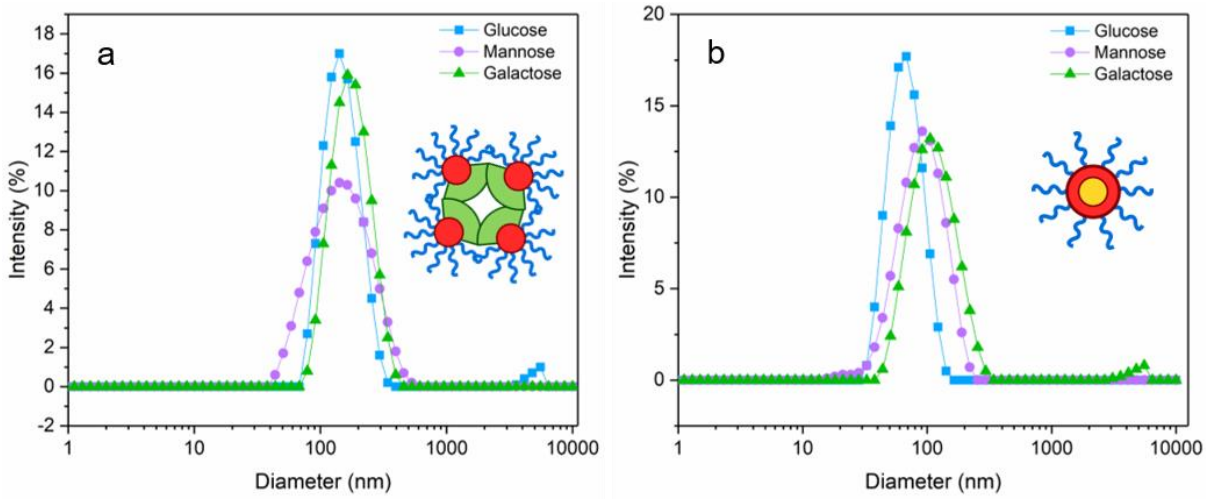

Fig. S5. Size distribution of patchy (a) and smooth (b) particles according to DLS in 5 mM buffer at pH 7.4. The hierarchical structures show a slightly larger hydrodynamic diameter and a broader distribution than the polystyrene-containing particles. Sample concentration is 1 mg mL^{-1}.

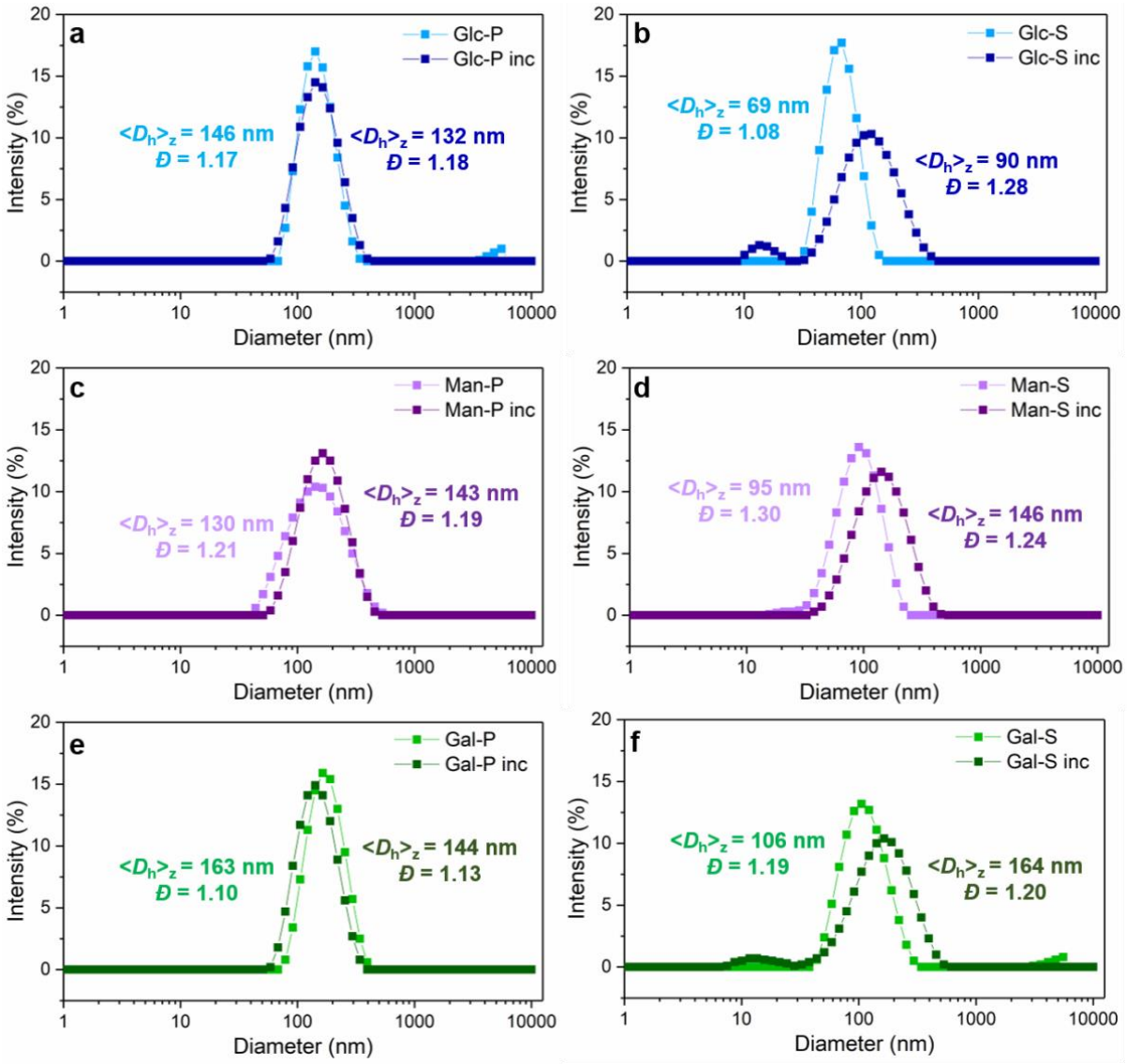

Fig. S6. Hydrodynamic size measured by DLS of the two sets of particles before and after incubation with DMEM supplemented with 10 vol% FBS at 37 °C for 2 hours. All particles with a patchy surface (a, c, e) show no change in diameter while smooth particles (b, d, f) undergo a significant size increase after incubation. Sample concentration after incubation was 200 μg mL^{-1}.

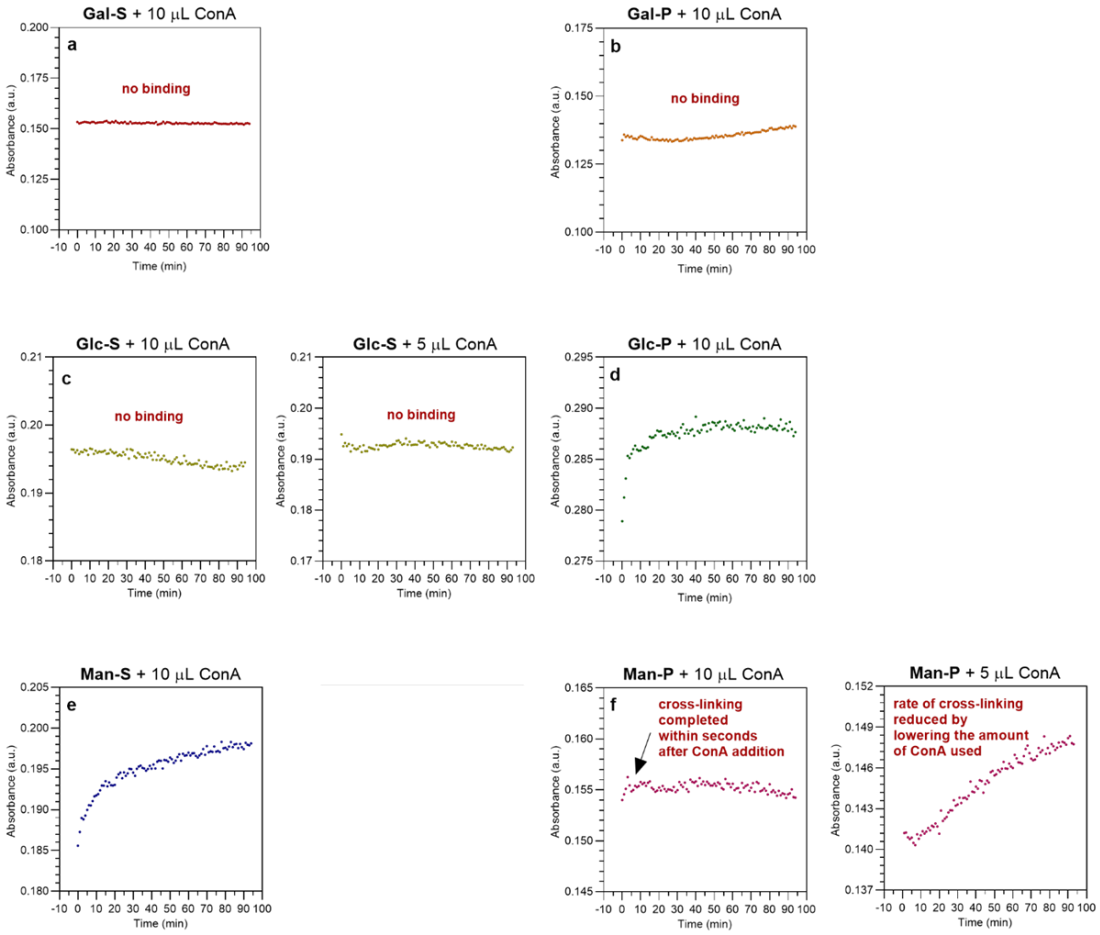

Fig. S7 Con A binding assay conducted on a UV-Vis instrument. The onset of Con A binding can be identified by an abrupt increase in the absorbance value at 492 nm, which corresponds to an increase in baseline scattering due to the formation of large irregular aggregates as a result of Con A cross-linking. (a) Gal-S + 10 µL Con A, (b) Gal-P + 10 µL Con A, (c) Glc-S + 5/10 µL Con A, (d) Glc-P + 10 µL Con A, (e) Man-S + 10 µL ConA, (f) Man-P + 5/10 µL Con A. The concentration of Con A was 3 mg/mL in all cases. Briefly, binding events were not observed in both Gal-S and Gal-P because galactose is known to not bind to Con A. In the case of Man-P, cross-linking occurred too rapidly when 10 µL of Con A was used. A two-fold reduction in volume of Con A was therefore necessary to slow down the cross-linking rate. As further negative control for Glc-S, we introduced the same (two-fold reduced) volume of Con A to Glc-S, but found no occurrence of cross-linking.

The figure was arranged in a way that similar nanoparticles are placed in the same row or column

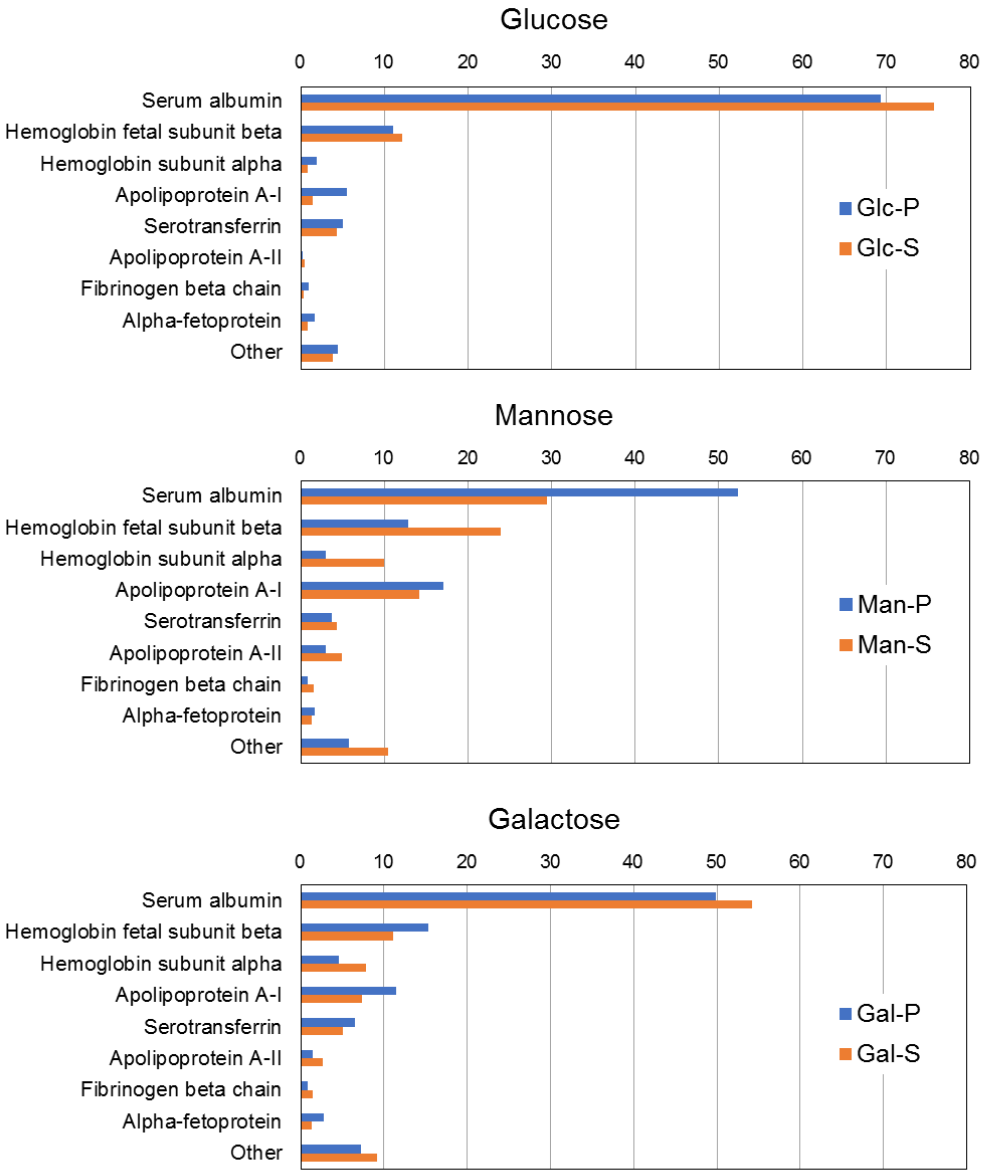

Fig. S8. Relative molar protein abundance determined by tandem mass spectroscopy using the emPAI score of each protein adsorbed on the smooth and the patchy nanoparticles after incubation with DMEM supplemented with 10 vol% FBS for 2 hours at 37 °C. As the absolute amount of absorbed protein on the patchy surface is very low the measured values here are likely unreliable.

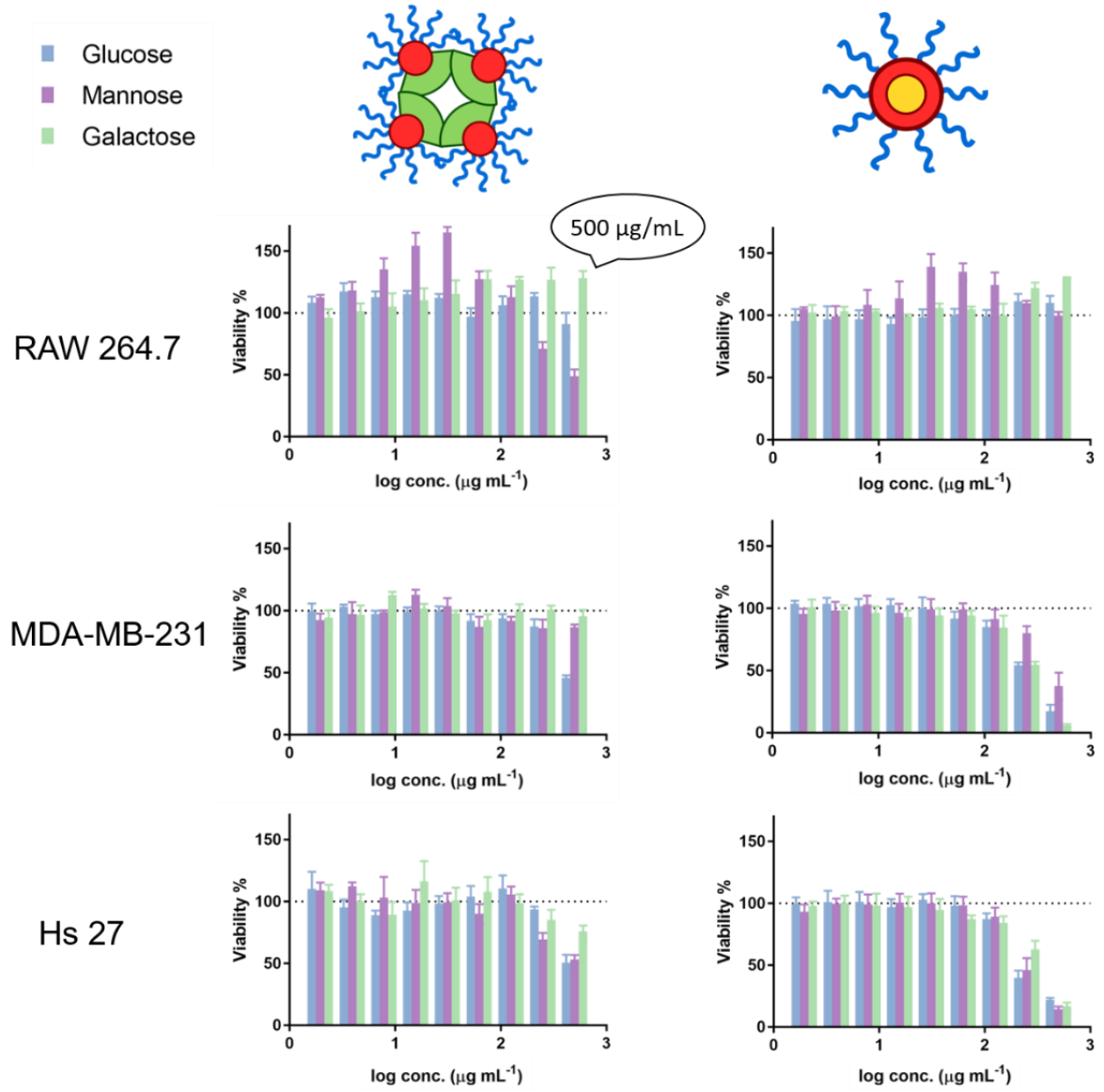

Fig. S9 Cell viability of three different cell lines determined with the SRB assay after incubation at 37 °C for three days with the six polymeric nanoparticles. The samples have only a low cytotoxic effect at high concentration (> 200 µg mL^{-1}).

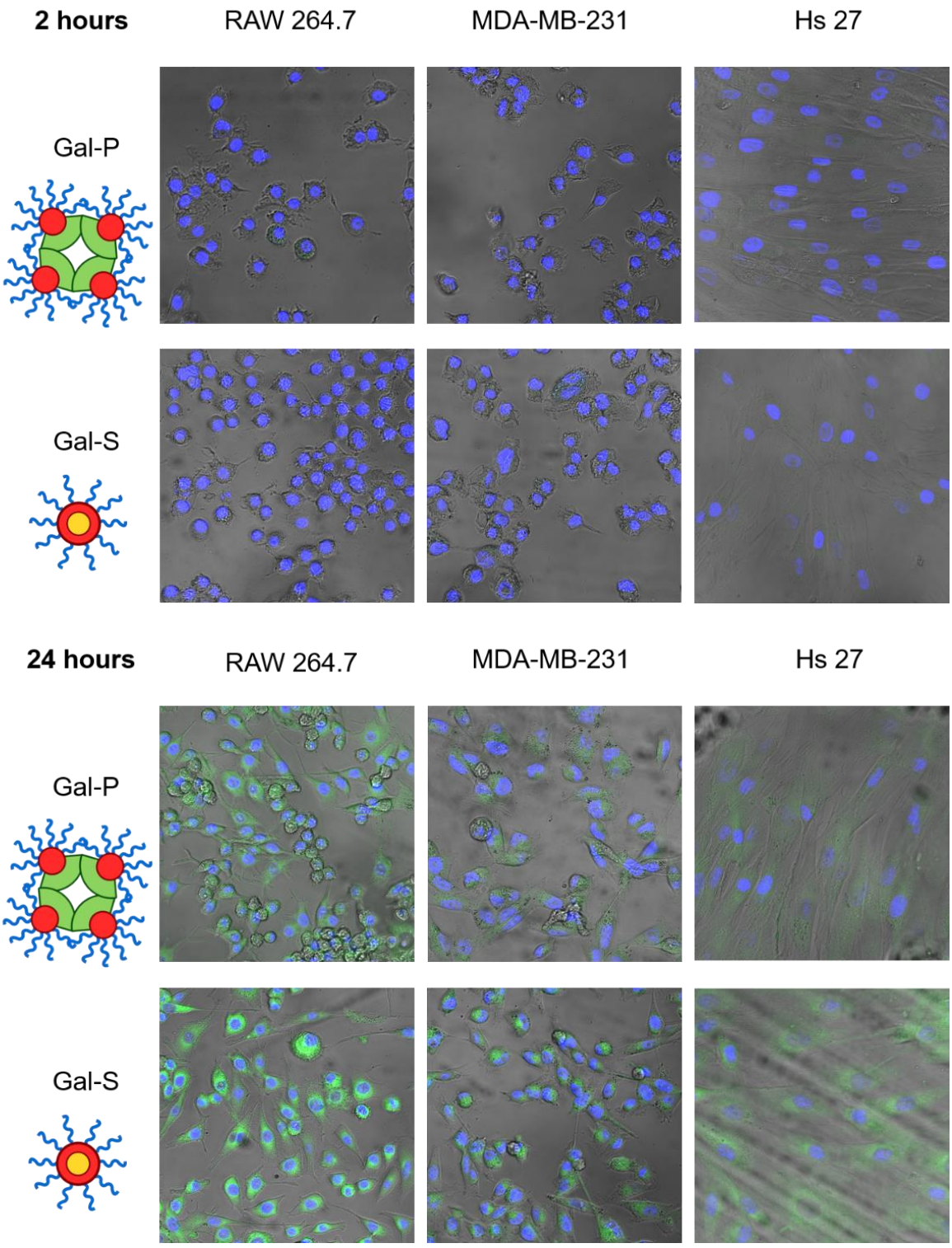

Fig. S10. Confocal microscopy images of patchy and smooth galactose-functionalised nanoparticles (labelled with FITC, green) internalised by macrophages (RAW 264.7), breast cancer cells (MDA-MB-231) and healthy fibroblasts (Hs 27) after incubation for 2 hours and 24 hours. Nuclei are stained with Hoechst solution (blue). Fluorescence images were merged with DIC images.

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