Supporting Information for

Gated Multivalent GlycoNanoparticles for Triggered Carbohydrate-Carbohydrate Interactions

*Sangho Won[(a)], Steven Hindmarsh[(c)] and Matthew I. Gibson [(a),(b)]**

(a) Department of Chemistry, University of Warwick, UK, CV4 7AL

(b) Warwick Medical School, University of Warwick, UK, CV4 7AL

(c) Department of Physics, University of Warwick, UK, CV4 7AL

CORRESPONDING AUTHOR INFORMATION

Email. M.i.gibson@warwick.ac.uk

Experimental Section

Materials

All chemicals were used as supplied unless otherwise stated. Methanol, hexane, hydrochloric acid, dichloromethane, toluene, acetone, tetrahydrofuran, chloroform, ethyl acetate, petroleum ether and diethyl ether were purchased from Fisher Scientific at laboratory reagent grade. Deuterochloroform (99.9 atom % D), deuteromethanol (99.5 atom % D), deuterium oxide (99.9 atom % D), 4,4'-azobis(4-cyanovaleric acid) (> 97.0 %), dodecane thiol (≥ 98.0 %), potassium phosphate tribasic (reagent grade, ≥ 98.0 %), carbon disulfide (≥ 99.9 %), 2-bromo-2-methylpropionic acid (98.0 %), *N*-isopropylacrylamide (97.0 %), hydroxyethylacrylamide (97.0 %), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (98.0 %), *N*,*N*-dimethylformamide (99.8%), 3-bromo-1-propanol (97.0 %), boron trifluoride diethyl etherate (46.5 % BF_3 basis), sodium bicarbonate (99.5 %), sodium chloride (99.5 %), sodium azide (99.5 %), calcium chloride (≥ 96.0 %), manganese(II) chloride (99.0 %) mesitylene (analytical standard) and magnesium sulfate (≥ 99.5 %) were all purchased from Sigma-Aldrich. 4-(Dimethylamino)pyridine (99.0 %), pentafluorophenol (99.0 %), trimethylamine (99.0 %) and sodium methoxide solution (30 wt. % in methanol) were purchased from Acros. Clear, polystyrene, flat-bottom, half-area 96-well microtiter plates and 96-well high binding microtitre plates were purchased from Greiner Bio-one. 10 mmol HEPES buffer containing 0.15 M NaCl, 0.1 mM $CaCl_2$ and 0.01 mM $MnCl_2$ (pH 7.5, HEPES) was prepared in 200 mL of milli-Q water (with a resistance of 18.2 MΩ cm). Pre formulated phosphate buffered saline tablets dissolved in 200 mL of milli-Q water (with a resistance of 18.2 MΩ cm) to give 0.137 NaCl, 0.0027 M KCl, 0.01 M Na_2HPO_4, 0.0018 M KH_2PO_4 and pH = 7.4. Gold nanoparticle solutions for 60 nm (0.288 mmol L^{-1}) were purchased from BBI Solutions. Amberlite IR-120 hydrogen form, ion exchange resin was purchased from Alfa Aesar. Soybean Agglutinin (SBA)

was purchased from Vector Labs. D-Galactosamine hydrochloride, β-D-Lactose octaacetate and GM3-Ganglioside were purchased from Carbosynth Ltd.

Physical and analytical methods

^{1}H, ^{13}C and ^{19}F NMR spectra were recorded for analysis of monomer conversions and polymer compositions on Bruker HD-400 and HD-500 spectrometer using deuterated solvents obtained from Sigma-Aldrich. All chemical shifts are reported in ppm (δ) relative to tetramethylsilane (TMS). FTIR spectra were acquired using a Bruker Vector 22 FTIR spectrometer with a Golden Gate diamond attenuated total reflection cell. A total 64 (or 128) scans with resolution of 4 cm^{-1} were collected. Samples were pre-dried as a thin film for FTIR analysis. SEC analysis was conducted on Varian 390-LC MDS system equipped with a column, two PL-AS RT/MT auto sampler, a PL-gel 3 mm (50 × 7.5 mm) guard column, two PL-gel 5 mm (300 × 7.5 mm) mixed-D columns using dimethylformamide (DMF) with 1 mg mL^{-1} LiBr at 50 °C as the eluent at a flow rate of 1.0 mL min^{-1}. The GPC system was equipped with ultraviolet (UV) (set at 280 nm) and differential refractive index (DRI) detections. Narrow molecular weight poly(methyl methacrylate) (PMMA) standards (200 - 1.0 × 10^{6} g mol^{-1}) were used for calibration using a second order polynomial fit. Polymer solutions at 1 mg mL^{-1} were prepared in the eluent and filtered through 0.45 mm filters prior to injection. UV-vis spectra were recorded in a disposable cuvette using a Cary 60 UV-vis spectrometer from Agilent at 25 °C. Lower critical solution temperatures of free PNIPAM and PNIPAM nanoparticles were also analyzed using an Agilent Cary 60 UV-vis spectrometer equipped with a temperature controller at 700 nm with a heating/cooling rate of 1 °C min^{-1}. The cloud point of PNIPAM and PNIPAM nanoparticles were determined by normalising the turbidimetry curve such that the values were in the range of 0 to 1, and the transition temperature was defined as being the temperature corresponding to a normalised absorbance of 0.5. A polymer concentration of 1.0 mg mL^{-1} was used in all

experiments. DLS and Zeta potential measurements were performed using a Nano-Zs from Malvern Instruments, UK running DTS software (4 mW, He-Ne laser, λ = 633 nm) and an avalanche photodiode (APD) detector. The scattered light was measured at an angle of 173° for DLS measurement. The temperature was stabilized to ± 0.1 °C of the set temperature. All samples were prepared at the concentration of 0.051 mg mL^{-1} gold nanoparticles. Hydrodynamic radii were determined using the manufacturer's software. Absorbance measurements of the nanoparticles incubated with lectin were recorded on a BioTek SynergyTM HT multi-detection microplate reader obtained using Gen5 1.11 multiple data collection and analysis software. The size and morphology of the synthesized gold nanoparticles and polymer coated gold nanoparticles were estimated by JEOL 2100FX transmission electron microscopy (TEM) at an accelerating voltage 200 kV. A drop of sample solution was deposited onto a copper grid and the water was evaporated under air. No staining was applied. Scanning Electron Microscopy (SEM): The samples were contacted to the stub using silver paint for SEM analysis. They were then carbon coated to provide a thin conductive covering in the region of a few nm. They were imaged using a Zeiss Gemini 500 SEM, using a 5 keV beam and the in-Lens detector.

Experimental procedures

Synthesis of 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (DMP)

Dodecane thiol (4.00 g, 4.73 mL, 19.76 mmol) was added dropwise to a stirred suspension of K_3PO_4 (4.20 g, 19.76 mmol) in acetone (60 mL) over 25 minutes. CS_2 (4.10 g, 3.24 mL, 53.85 mmol) was added and the solution turned bright yellow. After stirring for ten minutes 2-bromo-2-methylpropionic acid (3.00 g, 17.96 mmol) was added and a precipitation of KBr was noted. After stirring for 16 hour, the solvent was removed under reduced pressure and the residue was extracted into CH_2Cl_2 (2 × 200 mL) from 1M HCl (200 mL). The organic extracts were washed with water (200 mL) and brine (200 mL) and further dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by recrystallization in hexane to yield a bright yellow solid (4.00 g, 56 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm}: 3.31 (2H, t, $J_{12\text{-}11}$= 7.32 Hz, H^{12}); 1.76 (6H, s, H^{13}); 1.70 (2H, m, H^{11}); 1.41 (2H, m, H^{10}); 1.28 (16H, br. s, $H^{2\text{-}9}$); 0.90 (3H, t, $J_{1\text{-}2}$= 6.79 Hz, H^1).

^{13}C NMR (500 MHz, $CDCl_3$) δ_{ppm}: 220.97 (C^{13}); 176.62 (C^{16}); 55.43 (C^{14}); 37.09 (C^{12}); 31.92, 29.64, 29.56, 29.45, 29.35, 29.24, 29.11, 22.70 ($C^{2\text{-}9}$); 28.96 (C^{10}); 27.81 (C^{11}); 25.27 (C^{15}); 14.13 (C^1).

IR cm^{-1}: 2916 (alkyl-H stretch); 1710 (C=O stretch); 1068 (S-(C=S)-S stretch).

Polymerisation of N-isopropylacrylamide using 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (DMP*)*

N-isopropylacrylamide (1 g, 8.84 mmol), 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (64.45 mg, 177 μmol), and 4,4'-azobis(4-cyanovaleric acid) (ACVA) (9.9 mg, 35.3 μmol) were dissolved in methanol/toluene (1 : 1; 4mL) in a glass vial containing a stir bar giving [monomer] : [chain transfer agent] : [initiator] = 50 : 1 : 0.2. Mesitylene (150 μL) was added as an internal reference and the mixture was stirred (5 mins). An aliquot of this

starting mixture was removed for ^{1}H NMR analysis. The vial was fitted with a rubber septum and degassed by bubbling with nitrogen gas (30 mins). The vial was then placed in an oil bath thermostated at 70 °C. After 35 minutes, the reaction mixture was opened to air and quenched in liquid nitrogen. An aliquot was removed and conversion determined by ^{1}H NMR. The remainder was precipitated into diethyl ether (45 mL). The polymer was re-precipitated and purified from THF to diethyl ether three times. The product was purified three times by precipitation from toluene into diethyl ether, isolated centrifugation, and dried under vacuum overnight to give a yellow solid. The overall monomer conversion was determined from the ^{1}H NMR spectrum by measuring the decrease in intensity of the vinyl peaks associated with the monomer relative to mesitylene. Conversion (NMR): 86 %; M_n (theoretical), 5200 g mol^{-1}; M_n (SEC), 7100 g mol^{-1}; M_w/M_n (SEC), 1.10.

Synthesis of pentafluorophenyl 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (PFP-DMP)

2-(dodecylthiocarbonothiolythio)-2-methylpropanoic acid (0.50 g, 1.37 mmol), *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC) (0.39 g, 2.05 mmol) and 4-(dimethylamino)pyridine (DMAP) (0.25 g, 2.05 mmol) in 40 mL dichloromethane (DCM) was stirred for 20 minutes under N_2. Pentafluorophenol (0.78 g, 4.24 mmol) in 5 mL DCM was added. The reaction was stirred overnight at room temperature. The reaction was washed successively with 3 M HCl (50 mL), 1 M $NaHCO_3$ (50 mL) and 0.5 M NaCl (50 mL). The reaction was then dried over $MgSO_4$, filtered and then concentrated in vacuum to yield a yellow oil (0.35 g, 48 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm}: 3.24 (2H, t, $J_{12\text{-}11}$= 7.48 Hz, H^{12}); 1.79 (6H, s, H^{13}); 1.67 (2H, m, H^{11}); 1.31 (2H, m, H^{10}); 1.19 (16H, br. s, $H^{2\text{-}9}$); 0.81 (3H, t, $J_{1\text{-}2}$= 6.56 Hz, H^{1}).

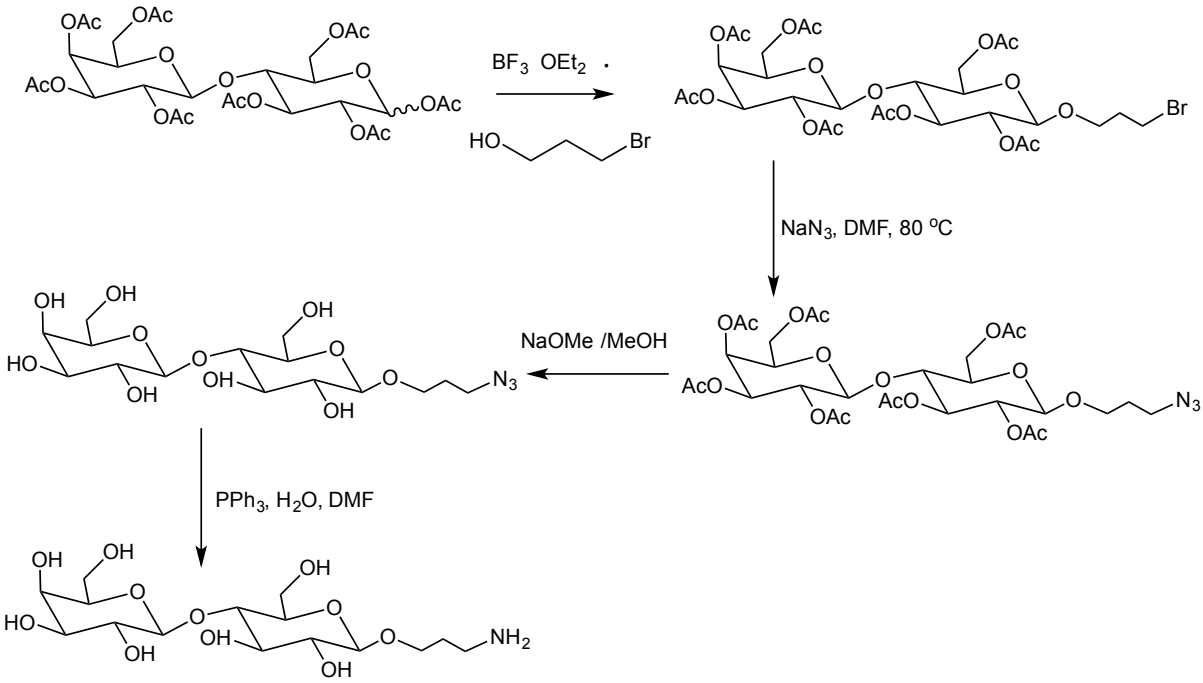
^{13}C NMR (500 MHz, $CDCl_3$) δ_{ppm}: 219.94 (C^{13}); 169.62 (C^{16}); 55.42 (C^{14}); 37.17 (C^{12}); 31.92, 29.63, 29.55, 29.43, 29.35, 29.12, 29.09, 22.70 (C^{2-9}); 28.92 (C^{10}); 27.82 (C^{11}); 25.43 (C^{15}); 14.13 (C^{1}).

^{19}F NMR (300 MHz, $CDCl_3$) δ_{ppm}: -151.54 (d, 2F, ortho F); -157.74 (t, F, para F); -162.34 (t, 2F, meta F).

IR cm^{-1}: 2923 (CH_2); 1779 ($C_6F_5C=O$); 1073 (S-(C=S)-S).

Polymerisation of hydroxyehtylacrylamide usting pentafluorophenyl 2-(dodecylthiocarbonothioylthio)-2-methylpropanoic acid (PFP-DMP)

In a typical reacation, hydroxyehtylacrylamide (HEA) (0.50 g, 4.34 mmol), pentafluorophenyl 2-(dodecylthiocarbonothiolythio)-2-methylpropanoic acid (PFP-DMP) (0.154 g, 0.289 mmol), 4,4'-azobis(4-cyanovaleric acid) (ACVA) (0.0162 g, 0.058 mmol) were dissolved in 50 : 50 toluene : methanol (4 mL). Mesitylene (150 μL) was added as an internal reference. An aliquot was taken for NMR analysis in MeOD. The solution was degassed under N_2 for 30 mins. The reaction was stirred at 70 °C for 90 mins. An aliquot was taken for NMR analysis for NMR analysis in MeOD. The reaction was rapidly cooled in liquid nitrogen and precipitated into diethyl ether. The polymer was re-precipitated into diethyl ether from methanol twice to yield a yellow polymer product which was dried under vacuum. Conversion (NMR): 93 %; M_n (theoretical), 2100 g mol^{-1}; M_n (SEC), 4800 g mol^{-1}; M_w/M_n (SEC), 1.10.



Scheme S1. Synthesis of amino-propyl modified lactose.

Synthesis of 1-(3-bromopropoxy) 2,2',3,3',4',6,6'-hepta-*O*-acetyl-β-lactoside

β-D-Lactose octaacetate (5.0 g, 7.37 mmol) was dissolved in dry CH_2Cl_2 (40 mL) with stirring. 3-bromo-1-propanol (1.54 g, 11.07 mmol) was added to a solution at rt for 1 h under an N_2 atmosphere. The reaction mixture was cooled at 0 °C, and $BF_3{\cdot}OEt_2$ (3.64 mL, 29.45 mmol) was added. The reaction mixture was stirred at 0 °C for 24 h, and filtered over a filter paper. The filtrate was diluted with EtOAc (100 mL), and the organic layer was washed with satd $NaHCO_3$ and satd NaCl, dried over $MgSO_4$, then filtered and concentrated in *vacuuo*. The residue was purified by chromatography (silica gel, 1:2 pet ether-EtOAc) to give a compound 1 (1.72 g, 31%) as a colorless syrup.

^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm}: 5.37 (1H, d, *J* = 3.20 Hz, Gal-4); 5.23 (1H, t, *J* = 9.46 Hz, Glc-3); 5.13 (1H, dd, *J* = 10.38, 8.09 Hz, Gal-2); 4.98 (1H, dd, *J* = 10.53, 3.51 Hz, Gal-3); 4.91 (1H, dd, *J* = 9.31, 8.09 Hz, Glc-2); 4.54 (1H, d, *J* = 1.68, Glc-1); 4.51 (1H, d, *J* = 2.29, Glc-6); 4.49 (1H, d, *J* = 2.59, Gal-1); 4.18-4.08 (3H, m, Glc-6, Gal-6, Gal-6); 3.99-3.95 (1H, m, -CH_2-);

3.89 (1H, t, J = 7.02 Gal-5); 3.81 (1H, t, J = 9.31 Glc-4); 3.71-3.48 (5H, m, Glc-5, -CH_2-); 2.18 (3H, s, Ac); 2.15 (3H, s, Ac); 2.08 (3H, s, Ac); 2.07 (3H, s, Ac); 1.99 (3H, s, Ac).

^{13}C NMR (500 MHz, $CDCl_3$) δ_{ppm}: 170.40, 170.37, 170.17, 170.09, 169.77, 169.74, 169.08 (7 s, 7 $COCH_3$); 101.09 (Gal-1); 100.80 (Glc-1); 76.27 (Glc-4); 72.70 (Glc-5); 71.65 (Gal-3); 71.00 (Glc-2); 70.70 (Gal-5); 69.11 (Glc-3); 67.34 (Gal-2); 66.60 (Glc-4); 61.97 (Glc-6); 60.81 (Gal-6); 21.07, 20.90, 20.82, 20.76, 20.65, 20.53 (6 q, 6 $COCH_3$).

HRMS (ESI +) m/z: 779.1367 $[M+Na]^+$; expected 779.1368 ($C_{29}H_{41}BrO_{18}Na$).

Synthesis of 1-(3-azidopropoxy) 2,2',3,3',4',6,6'-hepta-*O*-acetyl-β-lactoside

Compound 1 (1.0 g, 1.3 mmol) was added to a suspension of NaN_3 (0.429 g, 6.60 mmol) in dry DMF (15 mL) at 80 °C for 16 h with stirring. The reaction mixture was concentrated in *vacuuo*, and the residue was diluted with $CHCl_3$ (50 mL). The organic layer was washed with H_2O and brine, dried over MgSO4, filtered, and concentrated in *vacuuo* to give a compound 2 (0.75 g, 79 %) as a colorless syrup.

^{1}H NMR (500 MHz, $CDCl_3$) δ_{ppm}: 5.37 (1H, d, J = 3.05 Hz, Gal-4); 5.22 (1H, t, J = 9.31 Hz, Glc-3); 5.11 (1H, dd, J = 10.38, 8.09 Hz, Gal-2); 4.96 (1H, dd, J = 10.38 3.36 Hz, Gal-3); 4.93 (1H, dd, J = 9.46, 8.09 Hz, Glc-2); 4.54 (1H, d, J = 1.68, Glc-1); 4.51 (1H, d, J = 2.29, Glc-6); 4.50 (1H, d, J = 2.59, Gal-1); 4.18-4.08 (3H, m, Glc-6, Gal-6, Gal-6); 3.95-3.91 (1H, m, CH); 3.89 (1H, t, J = 6.87 Gal-5); 3.82 (1H, t, J = 9.61 Glc-4); 3.65-3.33 (5H, m, Glc-5, -CH_2-); 2.18 (3H, s, Ac); 2.15 (3H, s, Ac); 2.09 (3H, s, Ac); 2.07 (3H, s, Ac); 1.99 (3H, s, Ac).

^{13}C NMR (500 MHz, $CDCl_3$) δ_{ppm}: 170.40, 170.37, 170,16, 170.09, 169.77, 169.66, 169.09 (7 s, 7 $COCH_3$); 101.10 (Gal-1); 100.59 (Glc-1); 76.25 (Glc-4); 72.76 (Glc-3); 72.68 (Glc-5); 71.65 (Glc-2); 70.99 (Gal-3); 70.70 (Gal-5); 69.11 (Gal-2); 66.60 (Gal-4); 61.93 (Glc-6); 60.80 (Gal-6); 47.95 ($CH_2CH_2N_3$); 20.87, 20.82, 20.72, 20.65, 20.53 (5 q, 5 $COCH_3$).

HRMS (ESI +) m/z: 742.2283 $[M+Na]^+$; expected 742.2277 ($C_{29}H_{41}N_3O_{18}Na$).

Synthesis of 1-(3-azidopropoxy) β-lactoside

Compound 2 (700 mg, 0.97 mmol) was deacetylated with large excess of NaOMe in MeOH (30 % soln, 20 mL) in THF/MeOH (3:1, 80 mL) for overnight with stirring (not exceeding 10 °C). The reaction mixture was neutralized by acidic amberlite ion exchange resin. The residue was filtered and condensed in *vacuuo* to give a compound 3 (240 mg, 58 %) as a colorless solid.

^{1}H NMR (500 MHz, CD_3OD) δ_{ppm}: 4.37 (1H, d, *J* = 7.63 Hz, Gal-1); 4.30 (1H, d, *J* = 7.93 Hz, Glc-1); 4.00 (1H, m, CH); 3.90 (1H, dd, *J* = 12.36, 2.14 Hz, Glc-6); 3.83 (1H, dd, *J* = 10.53, 4.12 Hz, Glc-6); 3.80 (1H, d, *J* = 3.97, Gal-4); 3.73-3.64 (4H, m, Gal-6, Gal-6, -CH_2-); 3.61-3.41 (9H, m, Gal-5, Gal-3, Gal-2, Glc-4, Glc-3, Gal-2, Glc-5, -CH_2-); 3.26 (1H, t, *J* = 8.39, Glc-2).

^{13}C NMR (500 MHz, CD_3OD) δ_{ppm}: 103.69 (Gal-1); 102.91 (Glc-1); 79.18 (Glc-4); 75.69 (Gal-5); 75.05 (Glc-5); 75.01 (Glc-3); 73.43 (Gal-3); 73.33 (Glc-2); 71.16 (Gal-2); 68.90 (Gal-4); 66.23 ($CH_2CH_2N_3$); 61.08 (Gal-6); 60.49 (Glc-6); 48.10 (CH_2N_3).

HRMS (ESI +) m/z: 448.1541 $[M+Na]^+$; expected 448.1538 ($C_{15}H_{27}N_3O_{11}Na$).

Synthesis of 1-(3-aminopropoxy) β-lactoside

To a mixture of compound 3 (200 mg, 0.47 mmol) in DMF (10 mL), PPh_3 (247 mg, 0.940 mmol) was added at 0 °C. The mixture was stirred at rt for 2 h. H_2O (1.0 mL) was added and the reaction mixture was stirred at rt for 22 h until showing white precipitation (Ph_3PO). The reaction mixture filtered and concentrated in *vacuuo* to give a final product.

^{1}H NMR (500 MHz, D_2O) δ_{ppm}: 4.40 (1H, d, *J* = 8.09 Hz, Gal-1); 4.34 (1H, d, *J* = 7.78 Hz, Glc-1); 3.99-3.95 (1H, m, -CH_2-); 3.84 (1H, d, *J* = 3.20 Hz, Gal-6); 3.78 (1H, m, Gal-4); 3.80 (1H,

d, J = 3.97, Gal-4); 3.76-3.61 (6H, m, Gal-6, Gal-5, Glc-6, Glc-6, Glc-4, -CH_2-); 3.59-3.43 (6H, m, Gal-3, Glc-4, Glc-3, Glc-5, Glc-2, Gal-2); 3.07 (2H, t, J = 6.87, -CH_2-).

^{13}C NMR (500 MHz, D_2O) δ_{ppm}: 102.91 (Gal-1); 102.03 (Glc-1); 78.38, 75.35, 74.77, 74.34, 72.80, 72.10, 70.99, 69.64, 68.50, 67.90, 61.00, 60.46; 36.87 ($CH_2CH_2NH_2$).

HRMS (ESI +) m/z: 400.1815 $[M+H]^+$; expected 400.1813 ($C_{15}H_{30}NO_{11}$).

End group modification of PFP-polyhydroxyethylacrylamide using galactosamine/lactosamine

In a typical reaction, PFP-pHEA (50 mg, 0.024 mmol), galactosamine (25.2 mg, 0.117 mmol) were dissolved in 5 mL DMF with 0.05 M triethylamine (TEA). The reaction was stirred at 50 °C for 16 hrs. The polymer was precipitated into diethyl ether from methanol three times and dried under vacuum. IR indicated loss of C=O stretch corresponding to the PFP ester.

General procedure for the synthesis of polymer-coated gold nanoparticles

Approximately 1 mg of the desired thiol-terminated polymer (pNIPAM or GalNAc-pHEA / Lac-pHEA) was added to a microcentrifuge tube, and dissolved in 100 μL of high-purity water. 900 μL of the citrated-stabilized gold nanoparticle solution was added to this tube (60 nm: 0.288 mmol L^{-1}, 0.057 mg mL^{-1} total gold concentration), which was then agitated 30 mins in the absence of light. To remove excess polymer, the particles were centrifuged and following careful decantation of the supernatant, the particles were then re-dispersed in 1 mL of high-quality water and the centrifugation-resuspension process repeated for a total of 3 cycles. After the final cycle the particles were dispersed in 1 mL of high-quality water for future use. Assuming complete incorporation of the citrate coated gold particles into the final polymer coated particles the total concentration of gold in the final solution was 0.259 mmol L^{-1}, 0.051 mg mL^{-1}.

Gold nanoparticle functionalisation using a mixture of pNIPAM and Lac-pHEA (1 : 9 molar ratio)

100 μL of total polymer solution with different molar ratio between DP50 pNIPAM (0.25 mg, 4.76 mmol, 10 μL) and Lac-pHEA (0.90 mg, 4.76 mmol, 90 μL) was added to 900 μL of 60 nm gold nanoparticles. Left for 30 minutes at room temperature and centrifuged to remove any attached polymer and resuspended in water.

Calcium and lectin induced aggregation studies by absorbance

A stock solution of the Ca^{2+} or SBA was made up (0.1 mg mL^{-1} for SBA) in 10 mM HEPES buffer with 0.15 M NaCl, 0.1 mM $CaCl_2$ and 0.01 mM $MnCl_2$. 25 μL serial Ca^{2+} (0.1 to 100 mM) or SBA (0 to 10 μg mL^{-1}) dilution was made up in the same buffer in a 96-well micro-titre plate. 25 μL of the multi-polymer functionalised gold nanoparticle (0.051 mg mL^{-1} in water) were added to each well. Initial and after 30 minutes, both absorbance spectrum were recorded from 450 nm – 700 nm with 10 nm intervals at 20 °C and 40 °C, respectively.

Carbohydrate-carbohydrate interaction surface binding assays

Greiner high binding 96-well plates (half volume) were incubated for 17 h with solutions of GM-3 (180 μL of 0.1 mg mL^{-1} in PBS) per well. After incubation, unbound GM-3 was removed by washing vigorously with Milli-Q water, after which the plates were dried and stored at 4 °C. 50 μL of gold nanoparticle solution (0.255 mg mL^{-1} in water) and 5 μL of Ca^{2+} solution (5 M in HEPES) were then added to 96-well plates functionalized with GM-3 and incubated at room temperature and 40 °C for 1 h, respectively. After removing the unbound gold nanoparticles by extensively washing with Milli-Q water, the absorbance in each well was measured between 450 and 700 nm in 1 nm steps. Finally, each well was prepared for SEM analysis.

Additional Data

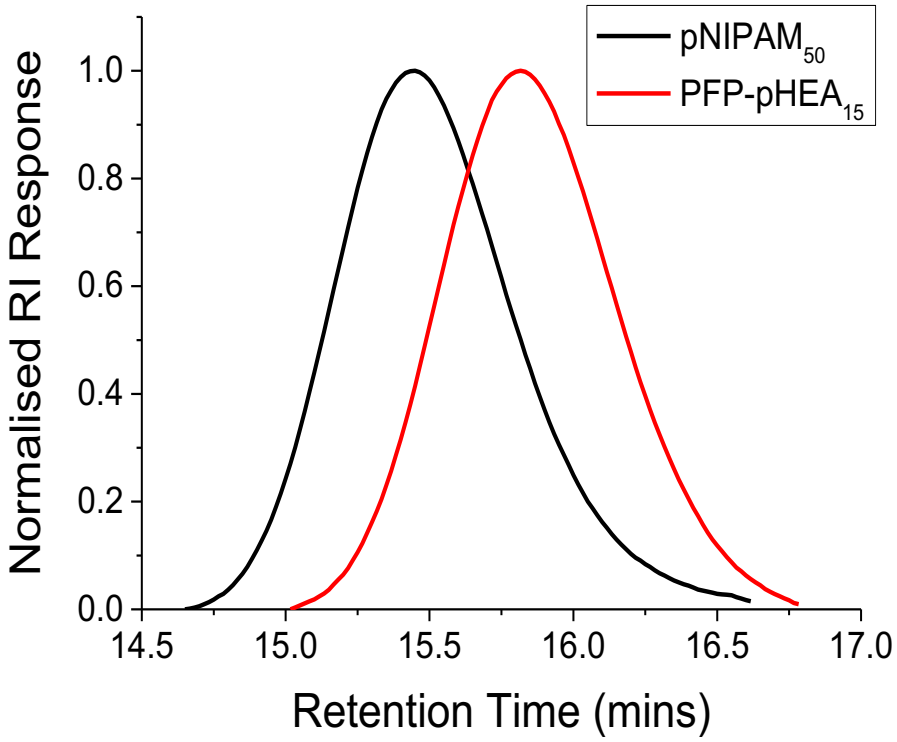


Figure S1. SEC chromatograms of polymers used in this work

Infrared analysis of PHEA before and after reaction of pentafluorophenol end-group with galactosamine/lactosamine. Disappearance of C=O at around 1750 cm^{-1} attributable to the carbonyl associated with the PFP end-group being removed.

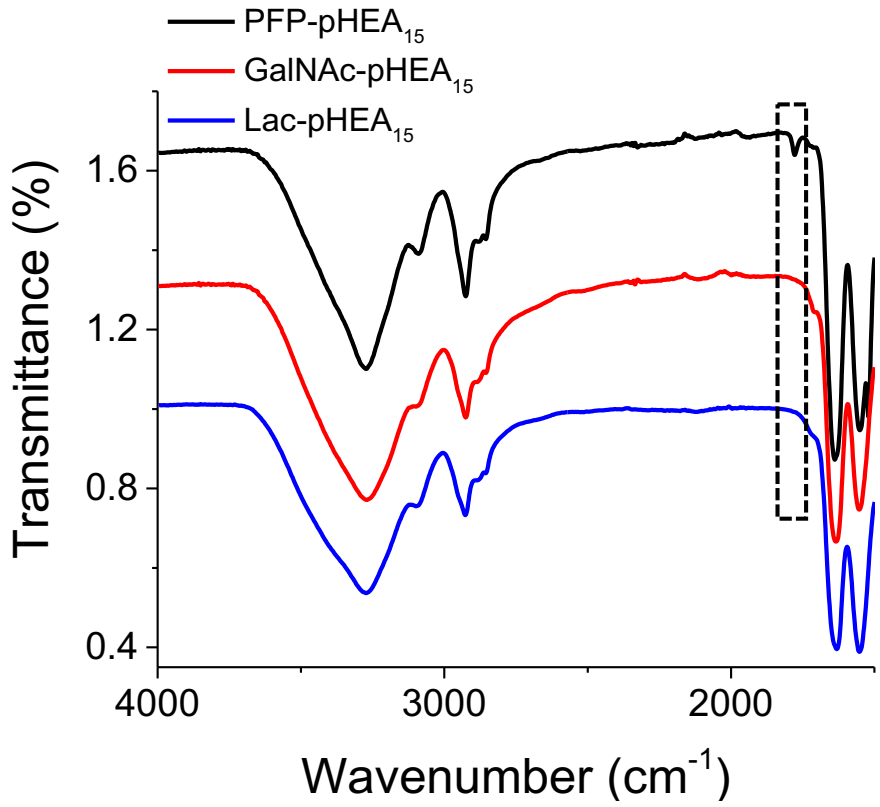


Figure S2. Infrared spectra of PFP-pHEA$_{15}$ (black), GalNAc-pHEA$_{15}$ (red) and Lac-pHEA$_{15}$ (blue). Box indicate region where PFP ester group would be found.

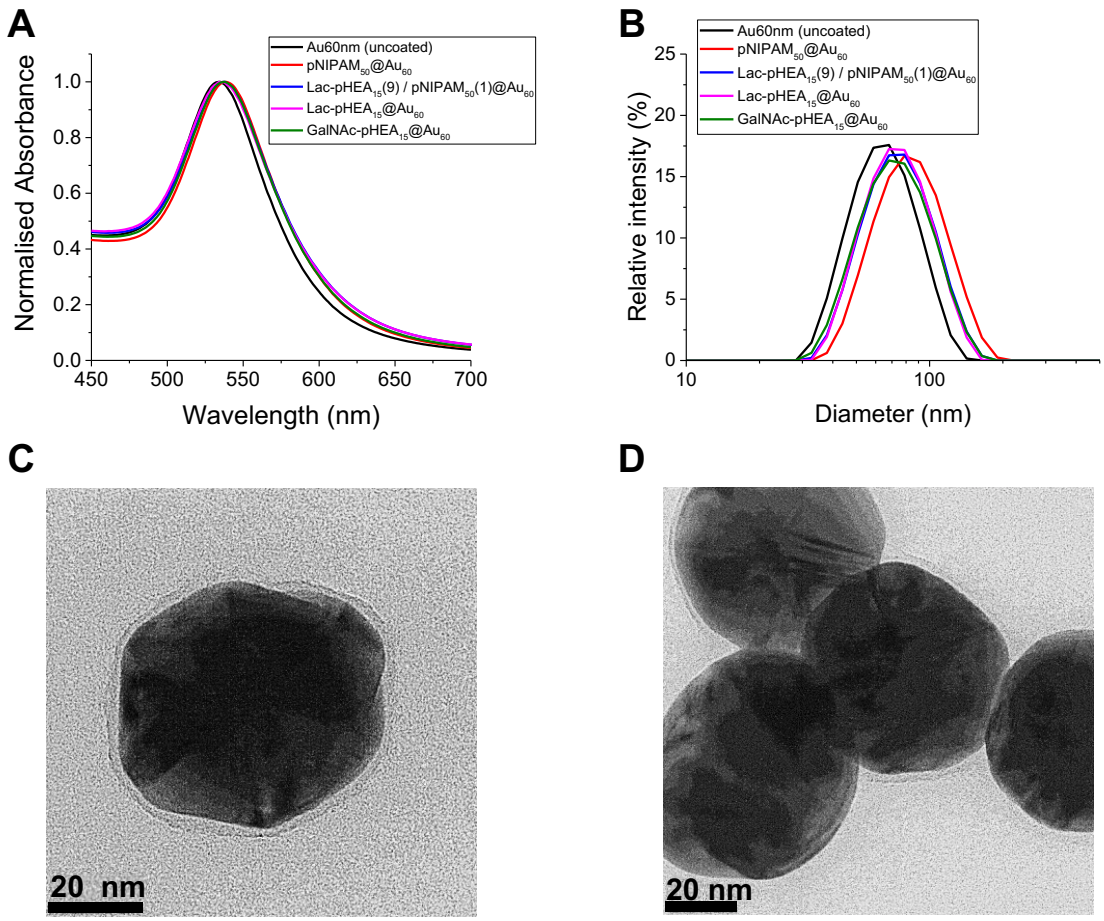


Figure S3. Characterization of polymer functionalized 60 nm gold nanoparticle A) UV-vis spectra; B) DLS analysis; C) Transmission electron microscope (TEM) image of Lac-pHEA$_{15}$ (9) /pNIPAM$_{50}$ (1) coated 60 nm gold nanoparticles at 20 ^{o}C showing polymer 'halo' and ; D) at 40 ^{o}C in presence of Ca^{2+} when aggregated.

Table S1. Solution properties of polymer coated gold nanoparticles.

Particle	Cloud point $_{Ca2+}$ [°C] [a]	Zeta-potential$_{water}$ [mV] [b]	Zeta-potential $_{Ca2+}$ [mV] [c]
Bare gold 60 nm	-	-41	-
pNIPAM@Au60	42	-38	-7
Lac-pHEA(9)/pNIPAM(1)@Au$_{60}$	45	-37	-5
Lac-pHEA@Au$_{60}$	-	-35	-5
GalNAc-pHEA@Au$_{60}$	-	-23	-3

[a]Cloud point was measured in HEPES buffer containing 100 mM Ca^{2+} upon heating from 25 °C to 80 °C, 0.0255 mg mL^{-1} total particle concentration. The CP (cloud point) of particles defined as being the point of 50% transmittance by UV-Vis spectroscopy; Zeta-potential of nanoparticles measured in [b]water and [c]HEPES buffer containing 100 mM Ca^{2+}.

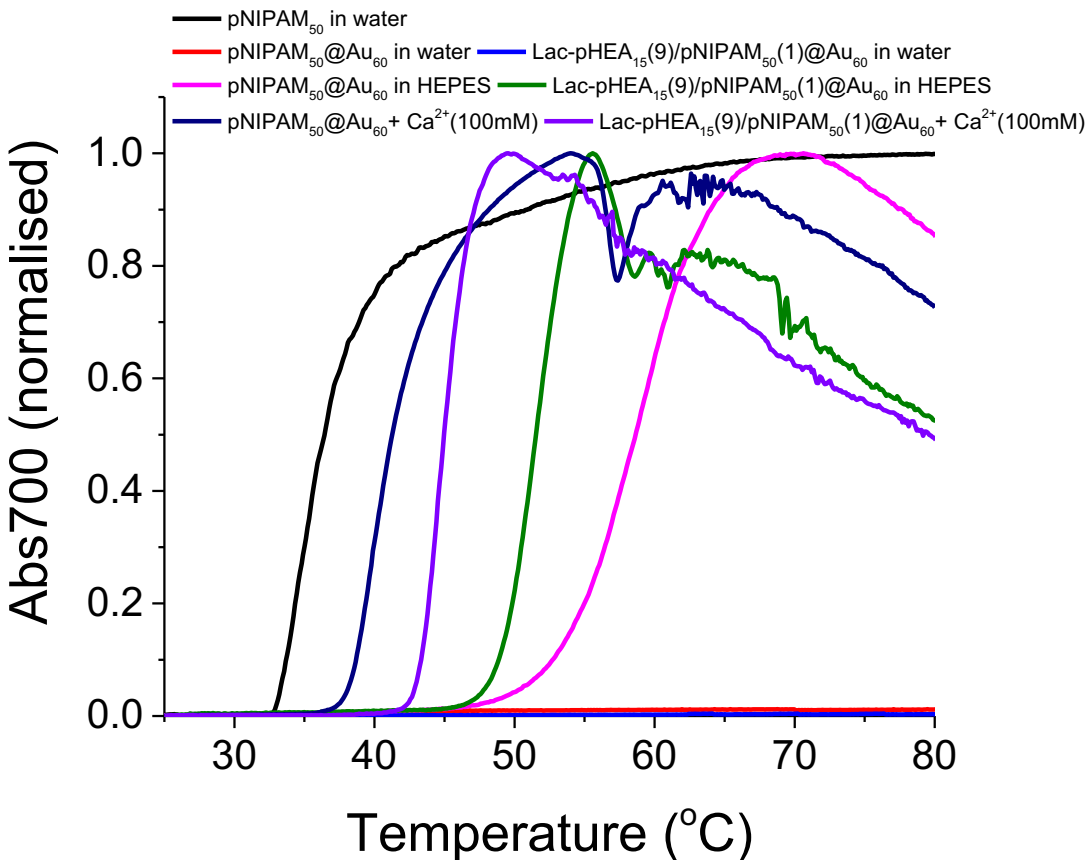


Figure S4. Turbidimetry scans (absorbance at 700 nm) of pure $pNIPAM_{50}$ (1.0 mg mL^{-1}) and polymer functionalised gold nanoparticles (0.0255 mg mL^{-1}) in different solution.

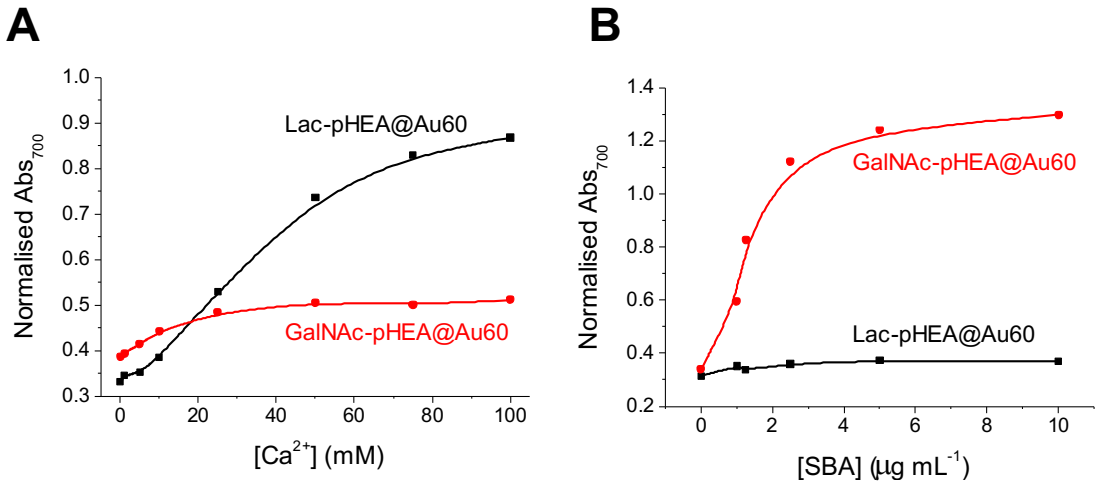


Figure S5. Binding isotherms of static particles with a gradient of A) Calcium and B) Soybean agglutinin.

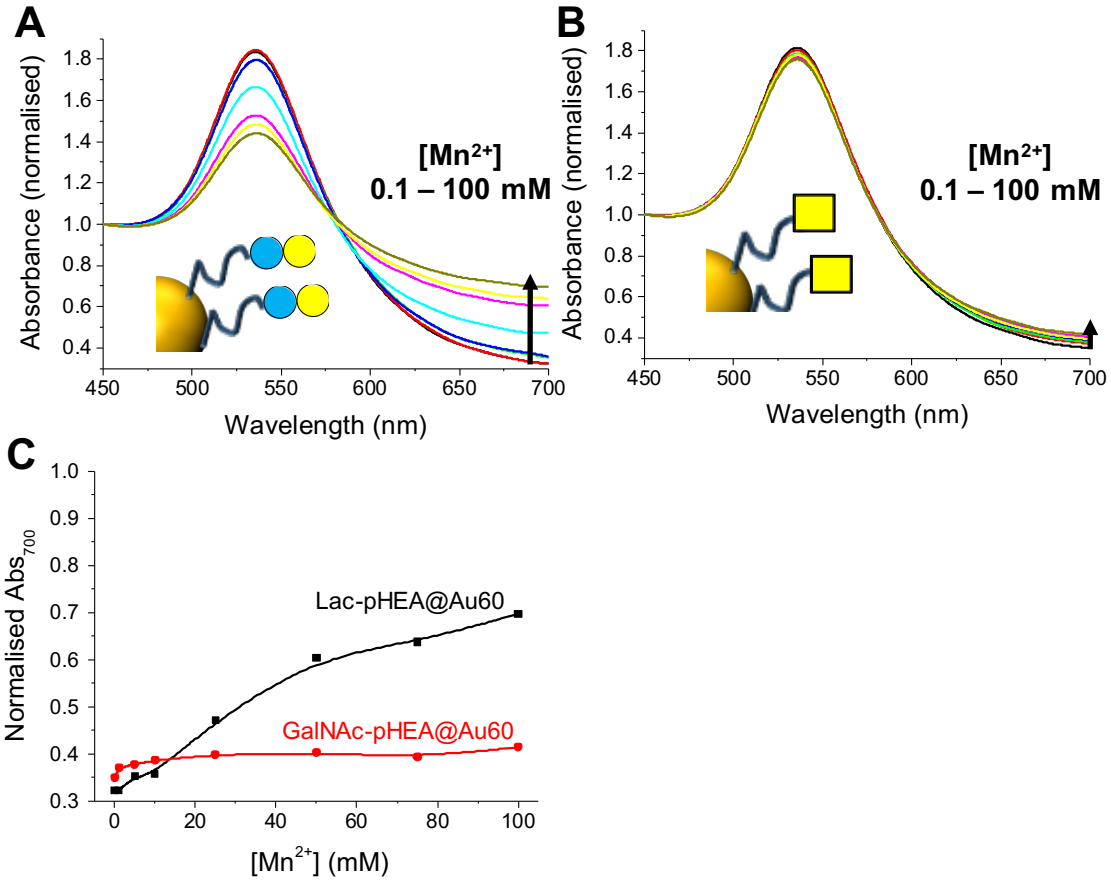


Figure S6. UV-Vis traces of different glyco-nanoparticle in presence of serial dilution of manganese (Mn^{2+}) after 30 minutes incubation at room temperature. A) Lac-pHEA$_{15}$@Au$_{60}$ with Mn^{2+}; B) GalNAc-pHEA$_{15}$@Au$_{60}$; C) Binding isotherm with Mn^{2+} gradient.

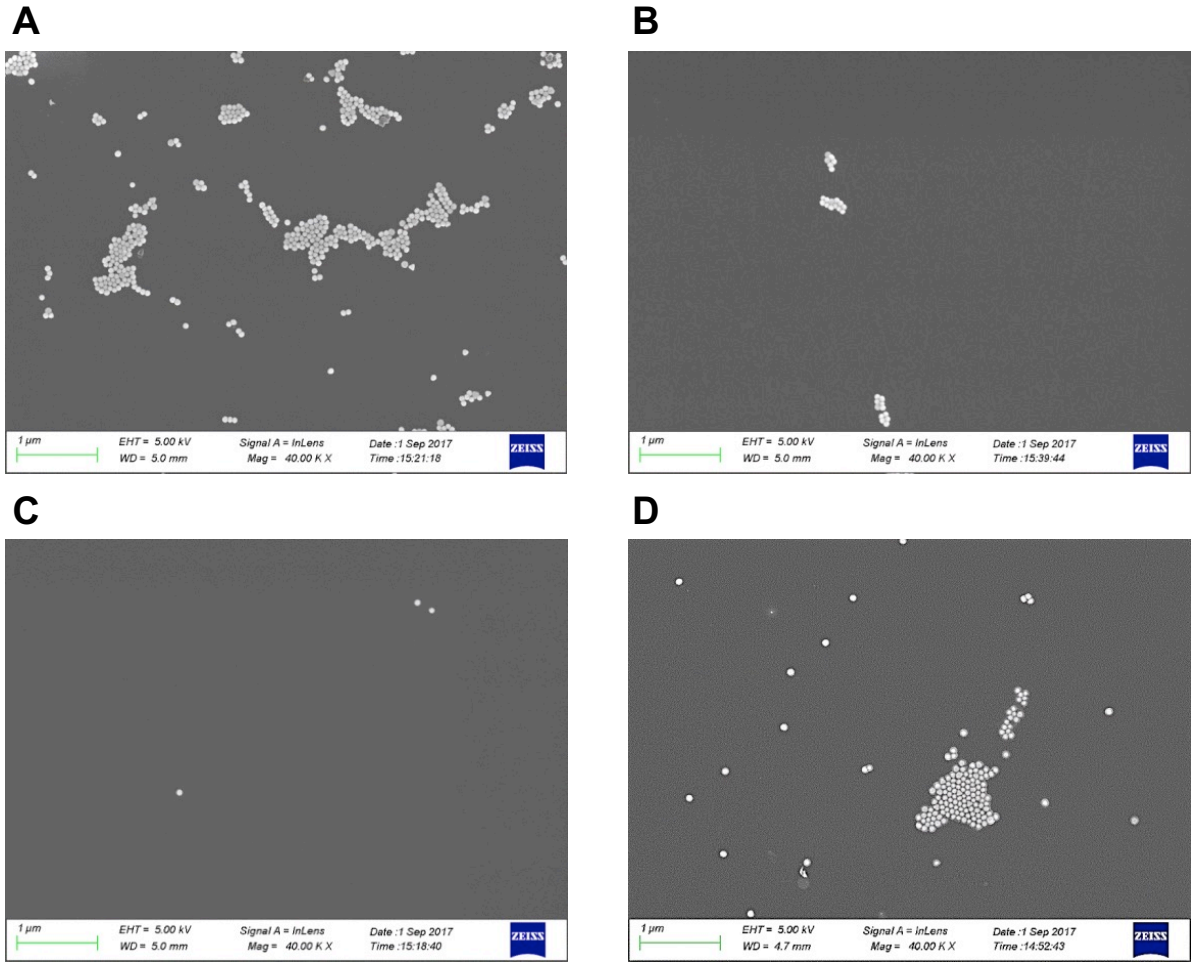


Figure S7. Non-colored SEM image of A) Lac-pHEA$_{15}$@Au$_{60}$ at 20 ºC; B) GalNAc-pHEA$_{15}$@Au$_{60}$ 20 ºC; C) Lac-pHEA$_{15}$(9)/pNIPAM$_{50}$(1)@Au$_{60}$ at 20 ºC; D) Lac-pHEA$_{15}$(9)/pNIPAM$_{50}$(1)@Au$_{60}$ at 40 ºC.