Facile *in Situ* Preparation of Biologically Active Multivalent Glyconanoparticles: Supplementary Information

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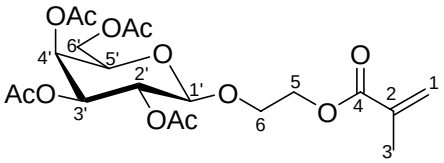
General

β-D-Galactose pentaacetate (98 %), 2-hydroxyethyl methacrylate (HEMA, >98 %), cation exchange resin DOWEX x 50W x 2-200 (H^+), sodium (99 %), methanol (99.8 %) and sodium borohydride (98 %) were purchased from Aldrich. Boron trifluoride diethyl etherate (purum, dist.) and 4,4'-azobis(4-cyanopentanoic acid) (>98 %) were purchased from Fluka. Peanut agglutinin agarose conjugate, methyl β-D-galactopyranoside and methyl β-D-glucopyranoside were purchased from Sigma. Hydrogen tetrachloroaurate (99.9 %) was purchased from Alfa Aesar. (4-Cyanopentanoic acid)-4-dithiobenzoate was synthesized according to a literature procedure.[1] Before use, boron trifluoride diethyl etherate was distilled in vacuo; all other chemicals were used without further purification.

NMR spectra were recorded using a Varian Inova 500 spectrometer at 499.87 (^{1}H) and 125.67 MHz (^{13}C) (^{1}H decoupled at 500 MHz) or using a Bruker Avance 400 at 400.13 MHz (^{1}H). NMR spectra were analyzed using the Varian VNMR 6.1C software. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer by casting a film on NaCl plates from either DCM or MeOH. Mass spectra were acquired using a

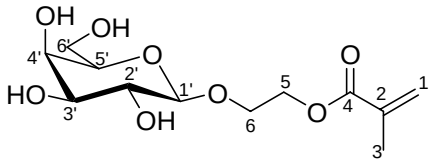
Micromass LCT spectrometer using ES+ and ES- modes of ionization. Elemental analyses were obtained using an Exeter Analytical Inc. CE-440 Elemental Analyzer. Aqueous SEC was performed using a triple detection method (with angular correction) and measurements were performed on a Viscotek TDA 301 triple detection SEC fitted with two (300 x 7.5 mm) GMPWxl methacrylate-based mixed bed columns with an exclusion limit of $5x10^7$ $gmol^{-1}$, having refractive index, viscometer and RALLS detectors. The eluent was 0.05 M $NaNO_3$ solution (80/20 v/v water/methanol) containing 2.5ml/litre 1.0 M NaOH, at a flow rate of 1.0 ml/minute and at a constant temperature of 30 °C. Calibration (for detector response) was achieved using a single narrow PEO standard (Polymer Labs) of 82,500 $gmol^{-1}$ and a d*n*/d*c* value of 0.133 mlg^{-1}. Molecular weights were determined using the Omnisec 4.0 for Windows software with a d*n*/d*c* value of 0.140 mlg^{-1} for poly[2-(β-D-galactosyloxy)ethyl methacrylate]. Dynamic light-scattering measurements were acquired using a Brookhaven Instruments 90 Zeta-Plus particle size analyser; samples were passed through a 0.22 μm syringe filter prior to analysis. Samples for TEM analysis were prepared by deposition of a drop of the particle solution on to a carbon-coated copper grid and the excess solution removed using filter paper, leaving a thin film of the particles. The samples were imaged using a Hitachi H7600 microscope.

Synthesis of 2-(2',3',4',6'-tetra-*O*-acetyl-β-D-galactosyloxy)ethyl methacrylate[2]



β-D-Galactose pentaacetate (5.00 g, 12.8 mmol), 2-hydroxyethyl methacrylate (HEMA) (1.27 ml, 1.35 g, 10.4 mmol) and 3Å molecular sieves were stirred under N_2 in anhydrous dichloromethane (50 ml). Boron trifluoride diethyl etherate (4.54 g, 3.95 ml, 32.0 mmol) was added by syringe over 15 minutes, and the solution stirred for 36 hours. The resulting suspension was filtered to remove the molecular sieves, washed with a saturated brine solution (100ml) and the organic phase dried over $MgSO_4$. The solvent was removed under reduced pressure and the crude oil purified by flash column chromatography to yield pure 2-(2',3',4',6'-tetra-O-acetyl-β-D-galactosyloxy)ethyl methacrylate as a light yellow oil (2.46 g, 52 %). Found C, 51.88; H, 6.18 $C_{20}H_{28}O_{12}$ requires C, 52.17; H, 6.18. FT-IR ν/cm^{-1}: 1751 (C=O of acetate groups) 1719 (C=O of methacrylate ester) 1637 1320 1298 (C=C). δ_H: (400 MHz, $CDCl_3$) 1.98 (3H, s, 3 x H-3) 2.01, 2.04, 2.05, 2.15, (3H x 4, 4s, Ac x 4), 3.84 (1 H, ddd, $J_{6a,6b}$ 11.5 Hz, $J_{5a,6a}$ 7.5 Hz, $J_{5b,6a}$ 4.0 Hz, H-6a), 3.92 (1H, td $J_t = J_{5',6a'} = J_{5',6b'}$ = 6.6 Hz, $J_{4',5'}$ 1.1 Hz, H-5'), 4.06 (1H, ddd, $J_{6a,6b}$ 11.5 Hz, $J_{5a,6b} = J_{5b,6b}$ = 5.0 Hz, H-6b) 4.10 (1H dd, $J_{6'a,6'b}$ 10.3 Hz, $J_{5',6'a}$ 6.7 Hz, H-6'a), 4.18 (1H, dd, $J_{6'a,6'b}$ 10.9 Hz, $J_{5'6'b}$ 6.5 Hz, H-6'b), 4.26 (1H ddd, $J_{5a,5b}$ 12.0 Hz, $J_{5a,6a}$ 7.5 Hz, $J_{5a,6b}$ 4.5 Hz, H-5a), 4.31 (1H, ddd, $J_{5a,5b}$12.5 Hz, $J_{5b,6a} = J_{5b,6b}$ = 5.0 Hz, H-5b), 4.55 (1H, d, $J_{1',2'}$ 8.0 Hz, H-1'), 5.01 (1H, dd, $J_{2',3'}$ 10.4 Hz, $J_{3',4'}$ 3.6 Hz, H-3'), 5.23 (1H, dd, $J_{1',2'}$ 8.0 Hz, $J_{2',3'}$ 10.4 Hz, H-2'), 5.39 (1H, dd, $J_{3',4'}$ 4.5 Hz, $J_{4',5'}$ 1.2 Hz H-4'), 5.59-5.61 (1H, m, H-1 *Z* to Me-C=C), 6.13-6.14 (1H, m, H-1 *E* to Me-C=C). δ_C: (100.26 MHz, decoupled, ^{1}H 400 MHz; $CDCl_3$) 18.25 (C-3), 20.6, 20.8, 21.0 (4 x $H_3\boldsymbol{C}CO_2$), 61.3 (C-6'), 63.5 (C-5), 66.8 (C-4'), 67.4 (C-6), 68.7 (C-2'), 70.7 (C-3'), 70.9 (C-5'), 101.3 (C-1'), 125.9 (C-1), 136.1 (C-2), 167.1 (C-4), 169.4, 170.2, 170.3 (Me$\boldsymbol{C}CO_2$). LR-MS (ES+) *m/z* requires 483.4, found 483.2 ($M + Na^+$).

Synthesis of 2-(β-D-galactosyloxy)ethyl methacrylate[2]



A solution of 2-(2',3',4',6'-tetra-*O*-acetyl-β-D-galactosyloxy)ethyl methacrylate (2.0 g, 4.35 mmol) in anhydrous MeOH (30 ml) was stirred at room temperature under an N_2 atmosphere. Freshly prepared NaOMe in MeOH (0.03M, 10 ml) was added and the reaction monitored by TLC (acetonitrile : water, 9 : 1) until the product of methacrylate ester cleavage was evident (R_f = 0.2). DOWEX 50W x 2-200 proton ion exchange resin was added and the suspension stirred for a further 15 minutes. The resin was removed by filtration and the solvent removed under reduced pressure. The resulting oil was purified by flash chromatography (chloroform : methanol 8 : 2) to yield 2-(β-D-galactosyloxy)ethyl methacrylate as a colourless oil that upon lyophilizing gave a hygroscopic white solid (670 mg, 53 %). Found C, 49.12; H, 6.94 ; $C_{12}H_{20}O_8$ requires C, 49.31; H, 6.90. FT-IR ν/cm^{-1}: 3360 (br, OH), 1708 (C=O of methacrylate ester) 1636, 1320, 1298 (C=C); δ_H: (400 MHz; D_2O) 1.83 (3H, m, 3 x H-3), 3.45 (1H, dd, $J_{2',3'}$ 9.7 Hz, $J_{3',4'}$ 3.3 Hz, H-3'), 3.50 (1H, td, $J_t = J_{5',6'a} = J_{5'6'b}$ = 5.5 Hz, $J_d = J_{4',5'}$ = 1.0 Hz, H-5'), 3.52 (1H, dd, $J_{1',2'}$ 7.5 Hz, $J_{2',3'}$ 10.0 Hz, H-2'), 3.71 (1H, dd, $J_{6'a,6'b}$ 11.0 Hz, $J_{5',6'a}$ 5.5 Hz, H-6'a), 3.74 (1H, dd, $J_{6'a,6'b}$ 11.0 Hz, $J_{5',6'b}$ 7.0 Hz, H-6'b), 3.82 (1H, dd, $J_{3',4'}$ 3.3 Hz, $J_{4',5'}$ 0.7 Hz, H-4'), 3.84 (1H, ddd, $J_{6a,6b}$ 12.0 Hz, $J_{5a,6a}$ 6.0 Hz, $J_{5b,6a}$ 4.0 Hz, H-6a), 4.10 (1H, ddd, $J_{6a,6b}$ 12.0 Hz, $J_{5a,6b}$ 3.7 Hz, $J_{5b,6b}$ 6.0 Hz, H-6b), 4.26 (1H, d, $J_{1',2'}$ 7.5 Hz, H-1'), 4.31 (1H, ddd, $J_{5a,5b}$ 12.0 Hz, $J_{5a,6a}$ 6.0 Hz, $J_{5a,6b}$ 3.5 Hz, H-5a), 4.35 (1H, ddd, $J_{5a,5b}$ 12.0 Hz, $J_{5b,6a}$ 3.5 Hz, $J_{5b,6b}$ 6.0 Hz, H-5b), 5.61–5.62 (1H, m, H-1 *Z* to CH_3–C=C), 6.12

(1H, m, H-1 *E* to CH_3–C=C); δ_C (100.62 MHz; decoupled ^{1}H 400 MHz; D_2O) 18.4 (C-3), 62.5 (C-6'), 65.3 (C-5), 68.5 (C-6), 70.3 (C-4'), 72.4 (C-2'), 74.9 (C-3'), 76.7 (C-5'), 105.3 (C-1'), 126.4 (C-1), 137.7 (C-2), 168.8 (C-4). LR MS (ES+) *m/z* requires 315.3, found 315.1 ($M + Na^+$).

Synthesis of poly[2-(β-D-galactosyloxy)ethyl methacrylate] (pGalEMA)[2]

To a solution of GalEMA (100 mg, 0.348 mmol) in UHQ water was added (4-cyanopentanoic acid)-4-dithiobenzoate (**2**, 3.5 μmol) and 4,4'-azobis(4-cyanopentanoic acid) (**3**, 1.75 μmol) as 8 $mgml^{-1}$ solutions in absolute ethanol. The solution was degassed by three freeze-pump-thaw cycles and purged with nitrogen before sealing. The solution was stirred at 70°C for 3 h then dialyzed and lyophilized to yield pGalEMA as a hygroscopic pink solid (80 mg, 80 %). IR (NaCl plates)ν/cm^{-1} 3446 (OH), 1718 (C=O of methacrylate ester), 1052 (C=S); δ_H: (500 MHz; D_2O) 0.93-1.09 (3H, br, CH_3), 1.89-2.13 (2H, br, CH_2), 3.58-4.5 (10H, br, carbohydrate and methylene side chain protons), 4.39 (1H, br, anomeric H). M_n (SEC) 24,100; M_w/M_n (SEC) 1.09; $M_{n,th}$ 23,751 at 80% conversion (theoretical number average molecular weight, $M_{n,th} = (x * ([M]_0/[RAFT]_0) * M_m) + M_{In} + M_{RAFT}$, where x = fractional conversion, M_m = monomer molecular weight, M_{In} is the initiating radical molecular weight and M_{RAFT} = RAFT agent molecular weight).

Kinetics of polymerization were investigated using ^{1}H NMR spectroscopy in D_2O:EtOH (9:1). ^{1}H NMR spectra were recorded every 5 minutes over 3 hours, and the ratio of integrals from the vinyl protons and the carbohydrate protons was used to determine conversion. Data from a typical polymerization are shown in Figure S1.

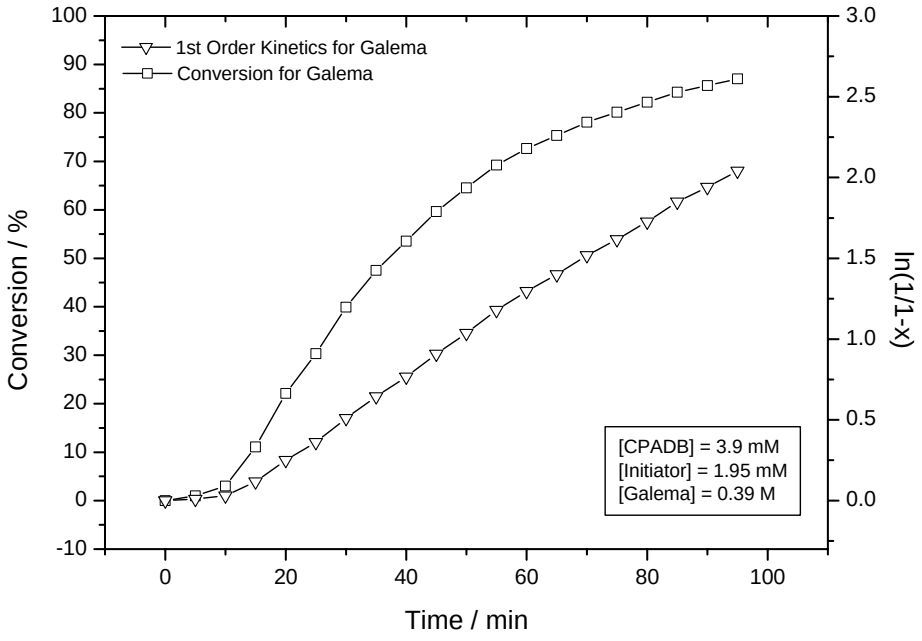
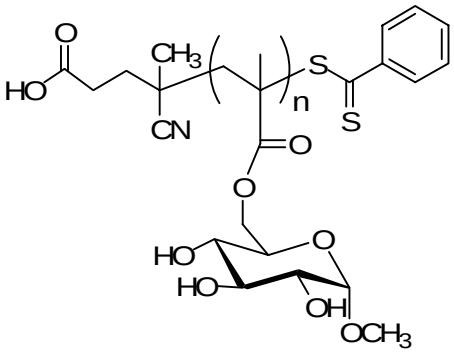


Figure S1. Polymerization data for GalEMA (**1**) (conversion vs. time and first order kinetic plot)

Synthesis of poly(methyl 6-*O*-methacryloyl-α-D-glucoside) (p6-*O*-MAMGlc)[3]



An aqueous solution of methyl 6-*O*-methacryloyl-α-D-glucoside (8.00 mL, 8.00 × 10^{-3} mol 6-*O*-MAMGlc, HPLC water) was introduced in a Schlenk tube and mixed with ethanol solutions of 4,4'-azobis-(4-cyanopentanoic acid) (6.22 × 10^{-2} M, 1.300 mL, 8.08 × 10^{-5} mol) and (4-cyanopentanoic acid)-4-dithiobenzoate (5.18 × 10^{-1} M, 0.345 mL, 1.79

$\times 10^{-4}$ mol). The tube was sealed with a greased glass stopper, degassed with 4 freeze-evacuate-thaw cycles and transferred to an oil bath pre-heated to 70 °C. After 50 min., the reaction was stopped by cooling in ice-water (5 min.) and two aliquots (50 and 100 μL) of solution were drawn for analysis. Both aliquots were freeze-dried overnight and re-dissolved in DMF eluant (2.00 mL) and D_2O (0.700 mL) for SEC and ^{1}H NMR analysis, respectively. The remaining macroRAFT agent was recovered by precipitation in excess ethanol followed by centrifugation and freeze-drying (overnight, dark). The product compound was recovered as a pink powder. Final conversion: 87% (NMR). Yield: 1.127 g, 54%. M_n (NMR) 8,400; M_n (SEC) 9,500; PDI (SEC) 1.09. ^{1}H-NMR (300 MHz, D_2O, 50 °C) δ (ppm): 0.97 and 1.13 (H-11), 1.91 (CH_2 chain), 3.40 (4-H), 3.46 (H-7), 3.60 (2-H), 3.70 (3-H), 3.82 (5-H), 4.10 and 4.37 (6-H), 4.82 (H-1), 7.56 ($H_{meta\ arom}$), 7.73 ($H_{para\ arom}$), 7.97 and 8.00 ($H_{ortho\ arom}$). ^{13}C-NMR (126 MHz, D_2O, 40 °C) δ (ppm): 17.38 (C-11), 45.5 (C-9), 54.5 (C-10), 55.75 (C-7), 65.22 (C-6), 69.78 (C-5), 70.62 (C-4), 71.89 (C-2), 73.82 (C-3), 99.86 (C-1), 179.5 (C-8).

Synthesis of glycopolymer-functionalized gold nanoparticles

AuNPs particles were prepared by a modified version of the synthesis of Brust *et al.*[4] 80 mg (1 equiv. by end groups) of pGalEMA (M_n (SEC) 24,100; M_w/M_n (SEC) 1.09) in 2.8 ml of distilled water was stirred with $HAuCl_4$ (0.03M, 114 μl, 1 equiv.) at room temperature. Freshly prepared $NaBH_4$ solution (0.4M, 214 μl, 25 equiv.) was added and the solution became golden brown. The resulting solution was analysed by TEM, dynamic light scattering and UV-vis spectroscopy. Particles stabilized with p6-*O*-

MAMGlc were prepared by an identical procedure, using 1 equiv. by end group of p6-*O*-MAMGlc (M_n (NMR) 8,400; M_n (SEC) 9,500; M_w/M_n (SEC) 1.09).

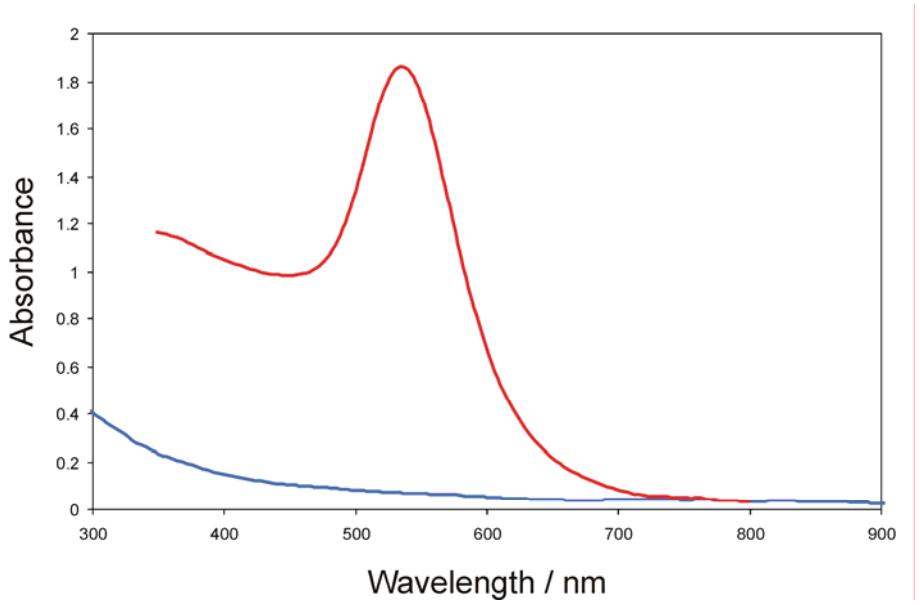


Figure S2. UV-vis spectra of dispersions of pGalEMA-stabilized (blue) and citrate-stabilized (red) gold nanoparticles (average diameter of latter particles 15 nm by TEM).[5]

In vitro tests of biological activity of glycopolymer-functionalized gold nanoparticles

Prior to these experiments, the nanoparticles were analysed by SEC to confirm the absence of free polymer. A suspension of agarose beads coated with peanut agglutinin (PNA) (20 µl, 2-4 mg PNA/ml solution) was pipetted onto a microscope slide. 40 µl of pGalEMA-coated nanoparticle solution was added to the beads and the aggregation monitored by optical microscopy; after 30 min. the beads appeared to have agglomerated completely. Redispersion experiments were attempted by addition of 10 µl of a 0.072 N solution methyl β-D-galactopyranoside or methyl β-D-glucopyranoside. A control experiment was also conducted in an identical manner using gold nanoparticles stabilized with p6-*O*-MAMGlc.

References

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