Supplementary Material (ESI) for Chemical Communications

Electronic Supporting Information

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Experimental.

Materials

2,2'-azobisisobutyronitrile (AIBN), purchased from Wako Chemicals, was recrystallized twice from methanol prior to use. Carbon disulfide (CS_2, 99%+, Aldrich), diethyl ether (99%, Univar), *n*-hexane (95%, Univar), dichloromethane (DCM, 99%, Univar), *N*,*N*-Dimethylformamide (DMF, 99%, Univar), tetrahydrofurane (THF, Univar), triethylamine (TEA, 99%, Aldrich), acetone (99%, Univar), *N*,*N*-dimethylacetamide (DMAc, 99%, Aldrich), 3-mercaptopropionic acid (99+, Aldrich), *N*-Biotinoyl-N′-(6-maleimidohexanoyl)hydrazide (biotin maleimide, Aldrich, 95%), Ellman's reactant (5,5'-dithiobis(2-nitrobenzoic acid), Aldrich, 99%) streptadivin (Aldrich, 99%), D-glucosamine hydrochloride (Aldrich, 99%), D-galactose amine hydrochloride (Aldrich, 98%), acryloyl chloride (Aldrich, 98%), pentafluorophenol (Aldrrich, 99%), concanavalin A (Con A) (Fluka), 4'-hydroxyazobenzene-2-carboxylic acid (HABA, Aldrich, 99%) and silica gel (150-200 nm) were used as received. Membranes for dialysis (MWCO 2000 and 3500 Da) were purchased from Fisher Biotec (Cellu SepT1, regenerated cellulose-Tubular membrane).

Characterizations

NMR Spectroscopy. ^{1}H, ^{19}F and ^{13}C NMR spectra were recorded using Bruker ACF300 (300 MHz) or ACF500 (500 MHz) spectrometers. D_2O, DMSO-D_6 or $CDCl_3$ were used as solvents.

Monomer conversions were determined via ^{1}H NMR spectroscopy, comparing the signal area from the vinyl protons (δ ~ 6.70-6.16 ppm, 3H/mol to the signal area from the backbone protons (δ ~2.0-3.5, 3H/mol).

Gel Permeation Chromatography (GPC). Gel permeation chromatography (GPC) was conducted using *N*,*N*-dimethylacetamide [DMAc; 0.03% w/v LiBr, 0.05% 2, 6–di-Butyl-4-methylphenol (BHT)], THF or aqueous solutions (deionized water containing sodium azide) as the mobile phases. Aqueous

GPC was performed using a Shimadzu modular system comprised of a DGU-12A solvent degasser, a LC-10AT pump, a CTO-10A column oven, and a RID-10A refractive index detector and a SPD-10A Shimadzu UV/Vis detector (flow rate: 1 ml/min). The column system was made up of a Polymer Laboratories 5.0 mm bead-size guard column (50 × 7.8 mm^2) followed by two PL aquagel MIXED-OH columns (8 μm). Calibration was performed with PEO standards ranging from 10^6 to 909,500 g/mol.

DMAc GPC analyses were performed using a Shimadzu modular system comprised of a SIL-10AD auto-injector, a Polymer Laboratories 5.0-mm bead-size guard column (50 × 7.8 mm) followed by four linear PL (Styragel) columns (10^5, 10^4, 10^3, and 500Å) at 50 °C (flow rate = 1 mL/min) and an RID-10A differential refractive-index detector. The calibration was performed with polystyrene standards with narrow polydispersity ranging from 500 to 10^6 g/mol.

UV-vis Spectroscopy. UV-vis spectra were recorded using a CARY 300 spectrophotometer (Varian) equipped with a temperature controller.

Infrared Spectroscopy. ATR-FTIR spectra were obtained using a Bruker Spectrum BX FTIR system using diffuse reflectance sampling accessories.

Dynamic light scattering (DLS). Dynamic light scattering studieswere were conducted using a Malvern Instruments Zetasizer NaNo ZS instrument equipped with a 4 mV He-Ne laser operating at λ = 633 nm, together with an avalanche photodiode detector with high quantum efficiency, and an ALV/LSE-5003 multiple tau digital correlator electronics system.

X-ray Photoelectron Spectrometer (XPS). A Kratos Axis ULTRA XPS incorporating a 165 mm hemispherical electron energy analyzer was used. The incident radiation was Monochromatic A1 X-rays (1486.6 eV) at 225 W (15 kV, 15 ma). Survey (wide) scans were taken at an analyzer pass energy of

160 eV and multiplex (narrow) higher resolution scans at 20 eV. Survey scans were carried out over 1200–0 eV binding energy range with 1.0 eV steps and a dwell time of 100 ms. Narrow higher resolution scans were run with 0.2 eV steps and 250 ms dwell time. Base pressure in the analysis chamber was 1.0×10^{-9} Torr and during sample analysis 1.0×10^{-8} Torr. The data were analyzed by XPS peak 4.1 software for deconvulation.

Synthesis of the 3-(benzylsulfanylthiocarbonylsulfanyl)-propionic acid (BSPA).

BSPA was synthesized as described in the literature.[1]

^{1}H NMR (300.17 MHz, $CDCl_3$, 298K): δ/ppm = 2.85 (2H, t, J = 6.8 Hz, C*H*$_2$C(O)OH), 3.62 (2H, t, C*H*$_2$CH$_2$), 4.61 (2H, s, C*H*$_2$-Ph), 7.31 (5H, m, CH(C$_6$*H*$_5$).

^{13}C NMR (75.48 MHz, $CDCl_3$, 298K): δ/ppm = 224.1, 179.2, 136.2, 130.7, 130.1, 129.2, 42.9, 34.4, 32.3.

Synthesis of pentafluorophenyl acrylate (FP-A). Pentafluorophenol (6.2 g, 33.8 mmol), triethylamine (3.5 g, 35.0 mmol) and dichloromethane (30 mL) were mixed and placed in an ice bath. Acryloyl chloride (3.0 mL, 36.8 mmol) was then added slowly. The solution was stirred for 4 hours at room temperature. The triethylammonium chloride salt issue of the esterification reaction was removed by filtration, and the solid was washed with DCM (10 mL). The solution was extracted twice with acidic water (10 mL, pH = 2.0), then twice with basic solution (10 mL, Na_2CO_3), and finally, twice with of distilled water (10 mL). The organic phase was dried over anhydrous sodium sulfate (Na_2SO_4), and then, DCM was removed using a rotary evaporator to yield a yellow liquid. This liquid was distilled under reduced pressure to give a colorless liquid (yield: 60%). Note: 100 mg of 6–di-Butyl-4-methylphenol (BHT) was added before the distillation as an inhibitor.

^{1}H NMR (300.17 MHz, $CDCl_3$, 298K): δ/ppm = 6.70 (C*H*$_2$, 1H, d), 6.36 (C*H*$_2$, 1H, dd) and 6.16 (C*H*, 1H, dd),

^{19}F NMR (282.41 MHz, $CDCl_3$, 298K): δ/ppm = -163.6 (2F, dd), -158.4 (1F, t), -153.5 (2f, d).

^{13}C NMR (75.48 MHz, $CDCl_3$, 298K): δ/ppm = 125.5, 127.9, 133.5, 133.7, 134.2, 140.0, 165.2.

RAFT polymerization of pentafluorophenyl acrylate (FP-A) in the presence of 3-(benzylsulfanylthiocarbonylsulfanyl)-propionic acid. An example of polymerization of FP-A is given as follows: FP-A(0.238 g, 1 mmol), 3-(benzylsulfanylthiocarbonylsulfanyl)-propionic acid (5.5 mg 2.02 × 10^{-5} mol), AIBN solution (0.1 mL, 6.6 mg diluted in 1 mL of benzene, 0.04 M) and benzene (1.9 mL) were added to a small vial (volume = 3mL). The solution was purged with nitrogen in an ice bath for 30 min. The solution was then heated to 70 ^{o}C for 6 hrs. Conversion was determined by ^{1}H NMR. The solution was partially evaporated under vacuum, and the polymer was precipitated in methanol (at 0 ^{o}C). The operation was repeated three times to remove any unreacted RAFT agent and any trace monomers. The polymer was dried under vacuum to yield a yellow powder. The sample was characterized by DMAc and THF GPC and by ^{1}H NMR ($CDCl_3$).

Theoretical M_n was calculated using the following equation $([M]_0/[RAFT]_0) \times \alpha_M \times MW^{monomer} + MW^{RAFT\ agent}$, where $[M]_0$, $[RAFT]_0$, $MW_{monomer}$ and $MW_{RAFT\ agent}$ represent monomer and RAFT agent concentrations, molar mass of monomer and RAFT agent, respectively;

Experimental M_n was measured by by ^{1}H NMR and calculated by the following equation: $(I^{CH\ at\ 3.0\ ppm} / (I^{CH2C(O)OH\ group\ at\ 3.5\ ppm})/2) \times MW^{FP\text{-}A} + MW^{RAFT}$, with $I^{CH\ at\ 3.0\ ppm}$, $I^{CH2C(O)OH\ group}$, $MW^{FP\text{-}A}$ and MW^{RAFT} corresponding to the intensity of signals at 3.0 ppm and 3.5 ppm, the molecular weight of FP-A and the molecular weight of RAFT respectively.

Chemical modification of P(FP-A) in the presence of sugar moieties.

An example is given for the chemical modification of P(FP-A) as follows:. P(FP-A) (0.1 g, 4.10 × 10^{-4} mol of pentafluorophenyl groups) was dissolved in of DMF (2 mL). Gluosamine solution was prepared by dissolution of glucosamine (0.123 g, 4.9 × 10^{-4} mol) in water (1 mL). Triethylamine (85 μL, 5.9 × 10^{-4} mol) was then added to the glucosamine solution. The glucosamine solution was subsequently added slowly to the FP-A solution using a syringe. The solution was stirred for 3 h at 30 ^{o}C. The

polymer was dialyzed (MWCO = 3 500 Da) against water for 3 days to remove any excess of glucosamine. The polymer was freeze dried and analyzed by ^{1}H NMR in deuterium oxide. (N.B. the polymer became totally water-soluble).

Chemical modification of P(FP-A) in the presence of sugar moieties.

The thiol-terminated glycopolymers obtained previously (after purification) were also reacted in the presence of biotin modified maleimide. Glycopolymer (**G1** of Table 2, 50 mg, M_n DMAc GPC = 5600 g/mol, PDI: 1.18) was dissolved in a water/DMAc mixture (50/50 V-%) at room temperature. The solution was purged with nitrogen. A solution of biotin-modified maleimide (1 mL, 20 mg) was dissolved in DMF solution in the presence of triethylamine (5 mg) that was then added to the glycopolymer solution. The reaction was carried out at room temperature for 3 hours. The polymer was dialyzed (MWCO = 2 000 Da) against a DMF/water mixture (90/10 V-%) for 2 days (the DMF solution was changed every 3 hours), followed by dialysis against pure water for 1 day to remove sugar traces and DMF. The polymer was freeze dried and analyzed by ^{1}H NMR in deuterium oxide (yield: 70%). A similar procedure was used for all the polymers. Ellman's assay was used to assess the absence of free thiol after this reaction.

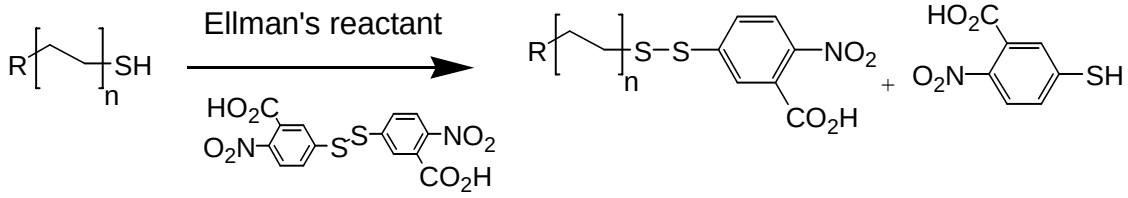
One-pot synthesis method for the attachment of sugar moieties and simultaneous end-group polymer modification using a thiol-ene reaction.

P(FP-A) (0.1 g, 4.10×10^{-4} mol of pentafluorophenyl groups, polymer (2.70×10^{-5} mol $M_{n,\ ^1H\ NMR}$ = 3 700 g/mol (**P1** of Table 1), PDI 1.20) and biotin modified maleimide (60 mg, 1.35×10^{-4} mol) were dissolved in DMF (3 mL). The solution was purged with nitrogen for 30 min. A solution of glucosamine was prepared (as before). This solution was purged with nitrogen, and added slowly to the P(FP-A) solution. The solution was shaken for 3 h at 30 ^{o}C. The polymer was dialyzed (MWCO = 2 000 Da) against a DMF/water mixture (90/10 V-%) for 2 days (the DMF solution was changed every 3

hours), followed by dialysis against pure water for 1 day to remove sugar traces and DMF. The polymer was freeze dried and analyzed by ^{1}H NMR in deuterium oxide. The biotin functionality was assessed by ^{1}H NMR using the following equation $F^{biotin} = [I^{4.3ppm}/(I^{7.2ppm]/}5)] \times 100$).

Titrations:

Ellman's assay. The thiol content of the polymers was determined by Ellman's assay.[2,3] The Ellman reagent was prepared by dissolving 5,5'-dithiobis(2-nitrobenzoic acid) in 0.1 M phosphate buffer (pH = 8.0) at a defined concentration (0.4 mg/mL ,1.0 mmol/mL). Biotin terminated P(glucose acrylamide) (4 mg, M_n = 5 500 g/mol, PDI = 1.15, 7.27 × 10^{-4} mol) was dissolved in phosphate buffer (2 mL) and Ellman's solution (1 mL) was added. The reactional mixture was stirred for 15 min and kept in the dark at room temperature. The reaction mixture (1 mL) was analyzed by U.V.-visible spectrophotometry and the solution absorbance, at 412 nm, was recorded. Phosphate buffer and Ellman's solution were used as blanks. The thiol concentration was calculated by $[thiol]_0 = Ab^{412nm}/ \varepsilon^{412\ nm}$, with Ab and ε as the absorbance at 412 nm and extension coefficient of Ellman's reactant at 412 nm (ε =14 250 L/mol/cm) respectively. All the experiments were carried out in triplicate.



Scheme S1. Titration by Ellman's assay.

Streptavidin/HABA assay.[4,5] HABA solution (7 mM) was prepared by dissolving HABA (8.4 mg) in NaOH solution (5 ml, 10 mM). SAv (1 mg) was dissolved in PBS buffer (1 ml, pH 6.0). To prepare the SAv/HABA solution, firstly SAv solution (0.25 ml) was diluted with PBS buffer (pH 6.0) to 0.5 ml. HABA solution (17.8 µl) was then added to the SAv solution and the solution was vigorously stirred.

Biotin (1.22 mg) was dissolved in PBS buffer (50 ml, pH 6.0) to yield a 0.1 mM solution. Biotin solution (5 μl) was added to SAv/HABA solution (0.3 ml) repeatedly. After every addition of biotin solution, the solution was stirred and was analyzed by UV-vis spectroscopy.

The biotin functionality was calculated using the following equation $F^{biotin} = [(\Delta Ab/n^{polymer})/\Delta^{calibration}] \times 100$, ΔAb, $n^{polymer}$ and $\Delta^{calibration}$ correspond to difference between the absorbance before and after addition of polymer, amount of polymer added to the solution and slope of calibration curve, respectively.

Turbidimetric Assay.[6] Concanavalin A (Con A) was diluted fresh for all experiments. The lectin was dissolved (~ 1 mg/mL) in HBS buffer (pH =7.4), and the resulting solution was mixed. The concentration of Con A stock solution was determined by using UV absorbance at 280 nm ([mg/mL Con A] = A^{280nm} /1.37). The solution was then diluted to 1 μM (based on Con A tetramer at 104 000 Da). Turbidity measurements were performed by adding the diluted Con A solution (300 μL) to a dry quartz cuvette (500 μL volume, 1 cm path length). A solution of the ligand of interest in HBS buffer was then added (30 μL at 500 μM, final concentration was 50 μM per sugar moieties). Upon addition, the solution was mixed vigorously for 5s using a micropipette before placement in the spectrometer. Absorbance data were recorded at 420 nm for 10 min.

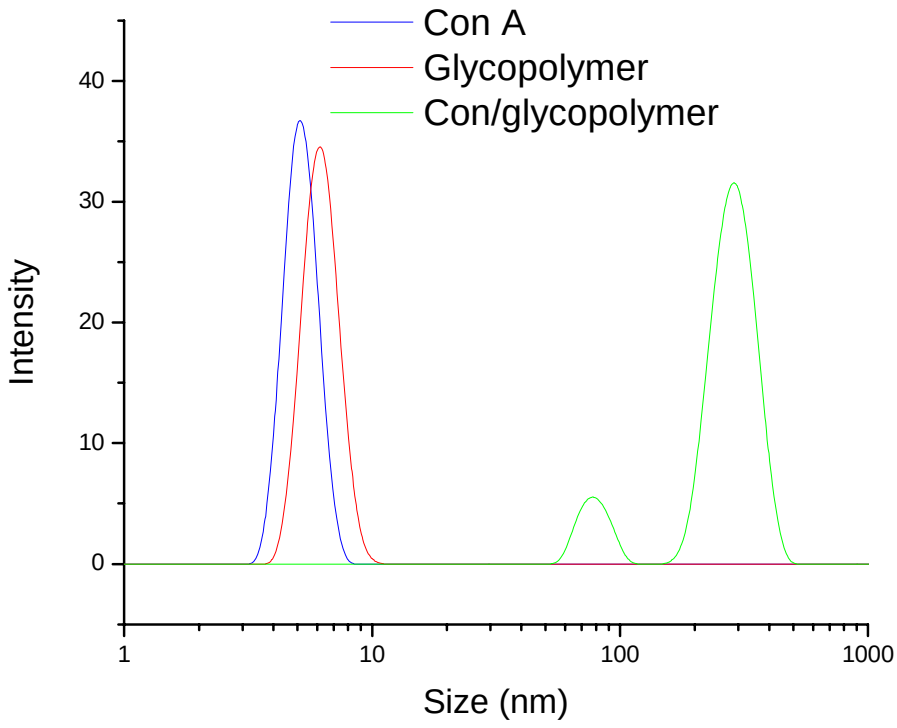


Figure S1. DLS distributions of concanavalin A (con A), typical glycopolymer (glycopolymer **G2**) of Table 2) and con A/glycopolymer (glycopolymer **G2**) complex.

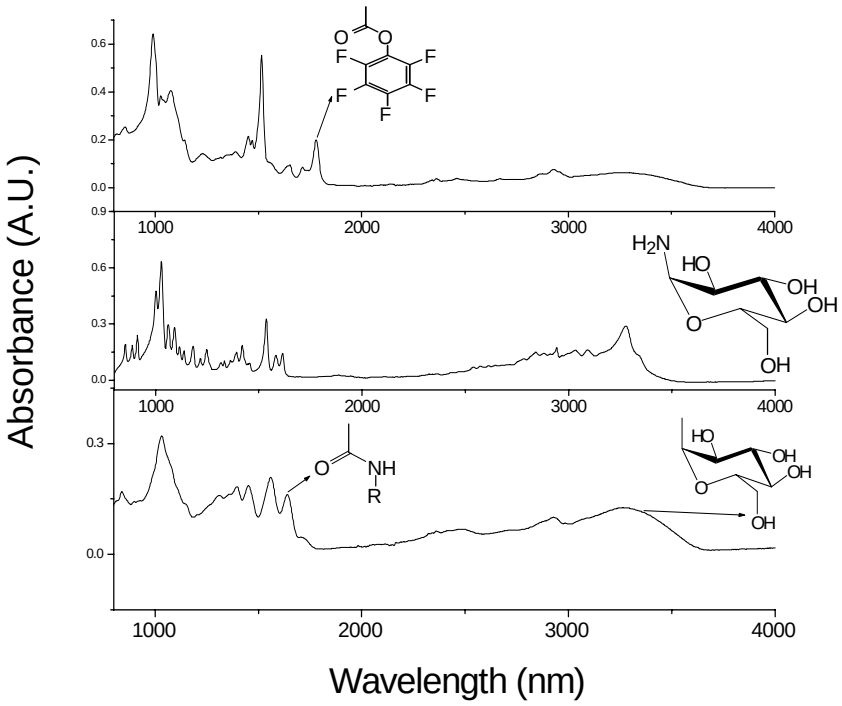


Figure S2. ATR-FTIR spectra of A- purified P(FP-A), B- glucosamine **(5)** and C- C- P(glucose acrylamide) obtained after reaction.

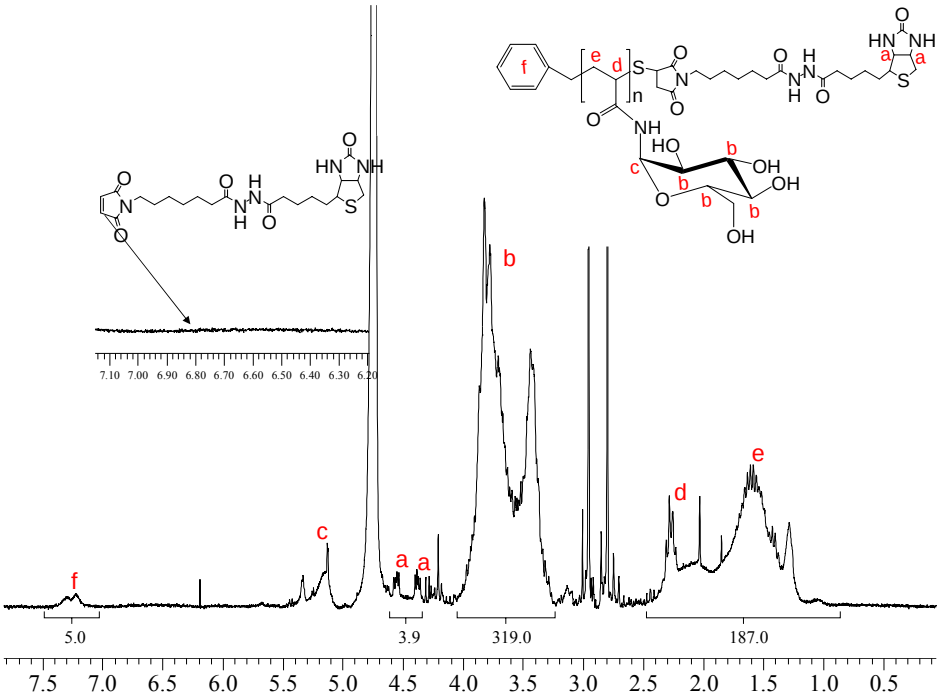


Figure S3. ^{1}H NMR of biotin functionalized poly(glucose acrylamide) (sample # **GB1** of Table 2) in D_2O. Inset: zoom of the region from 6.20 to 7.10 ppm (showing the absence of any signal at 6.8 ppm attributed to the maleimide group). The biotin functionality assessed by ^{1}H NMR using the following equation: $F^{biotin} = [I^{4.3ppm}/(I^{7.2ppm}/5)] \times 100)$.

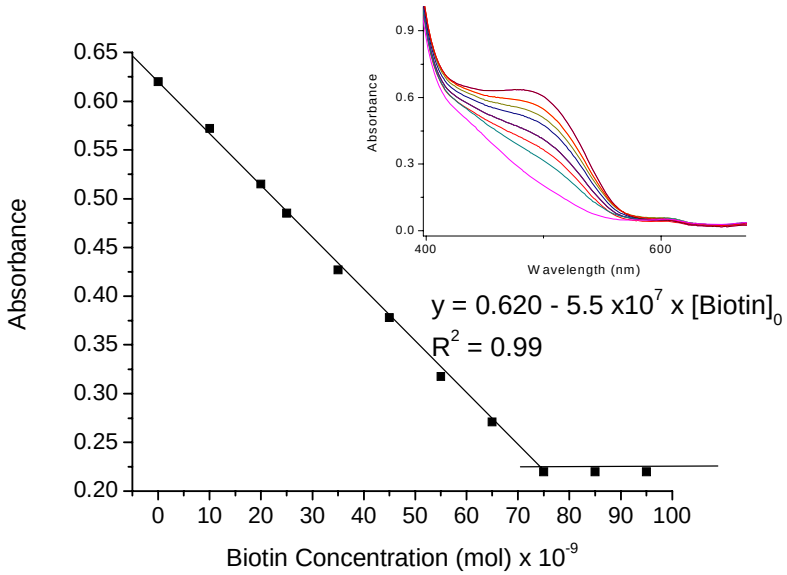


Figure S4. Calibration curve for HABA/streptadivin assay.

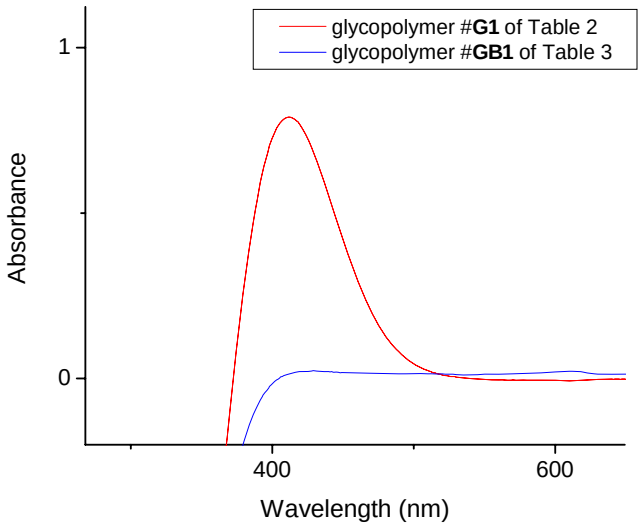


Figure S5. Ellman's assay of glycopolymer #**G1** (Table 2) obtained in the absence of biotin modified maleimide and (-) glycopolymer #**GB1** (Table 2) obtained in the presence of biotin modified maleimide. Note: The baseline was carried out using the Ellman's solution (before addition of polymer).

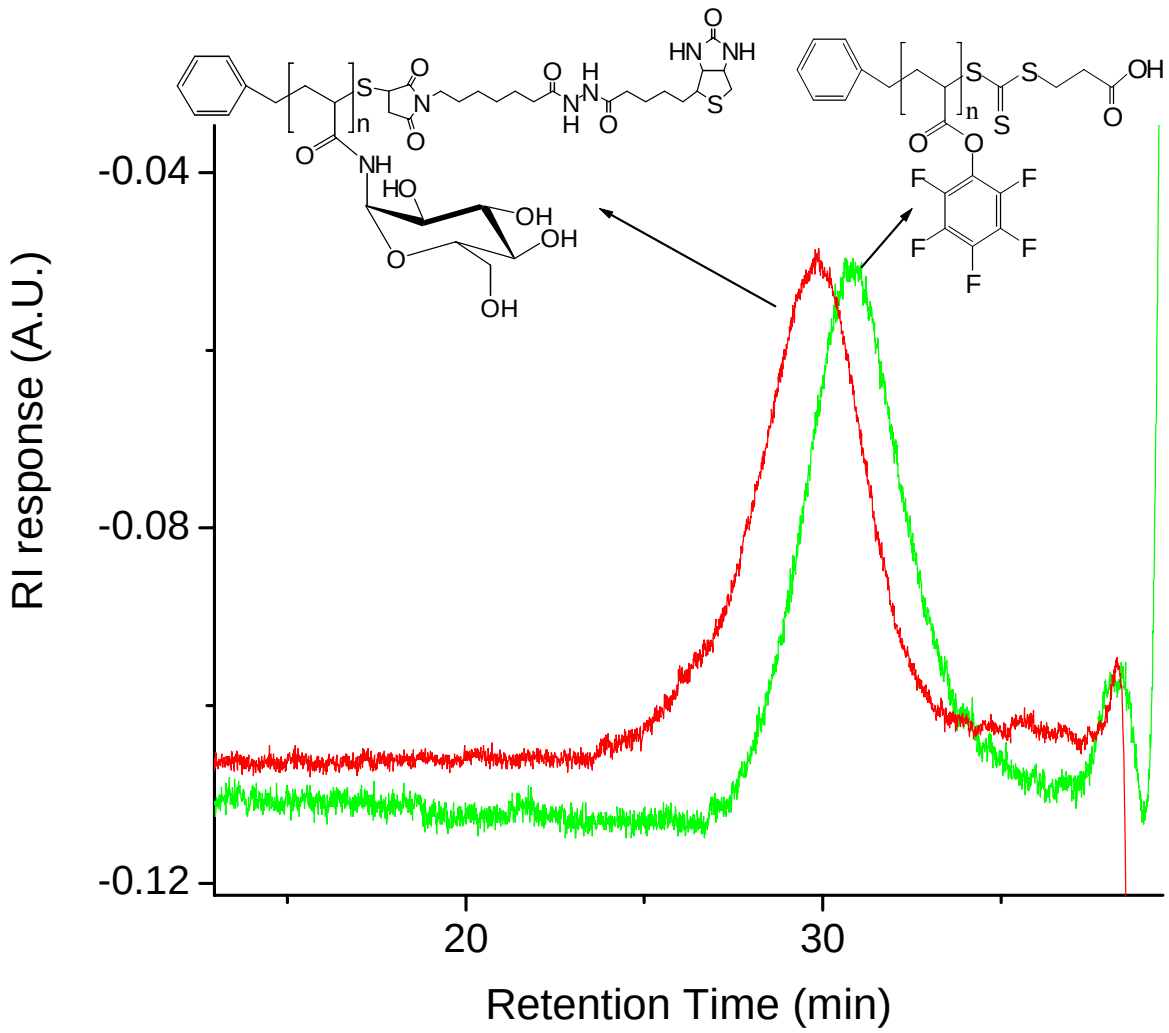


Figure S6. DMAc GPC traces of P(FP-A) polymer (# **P2** of Table 1) before and after modification in the presence of biotin and glucosamine (#**GB1** of Table 2).

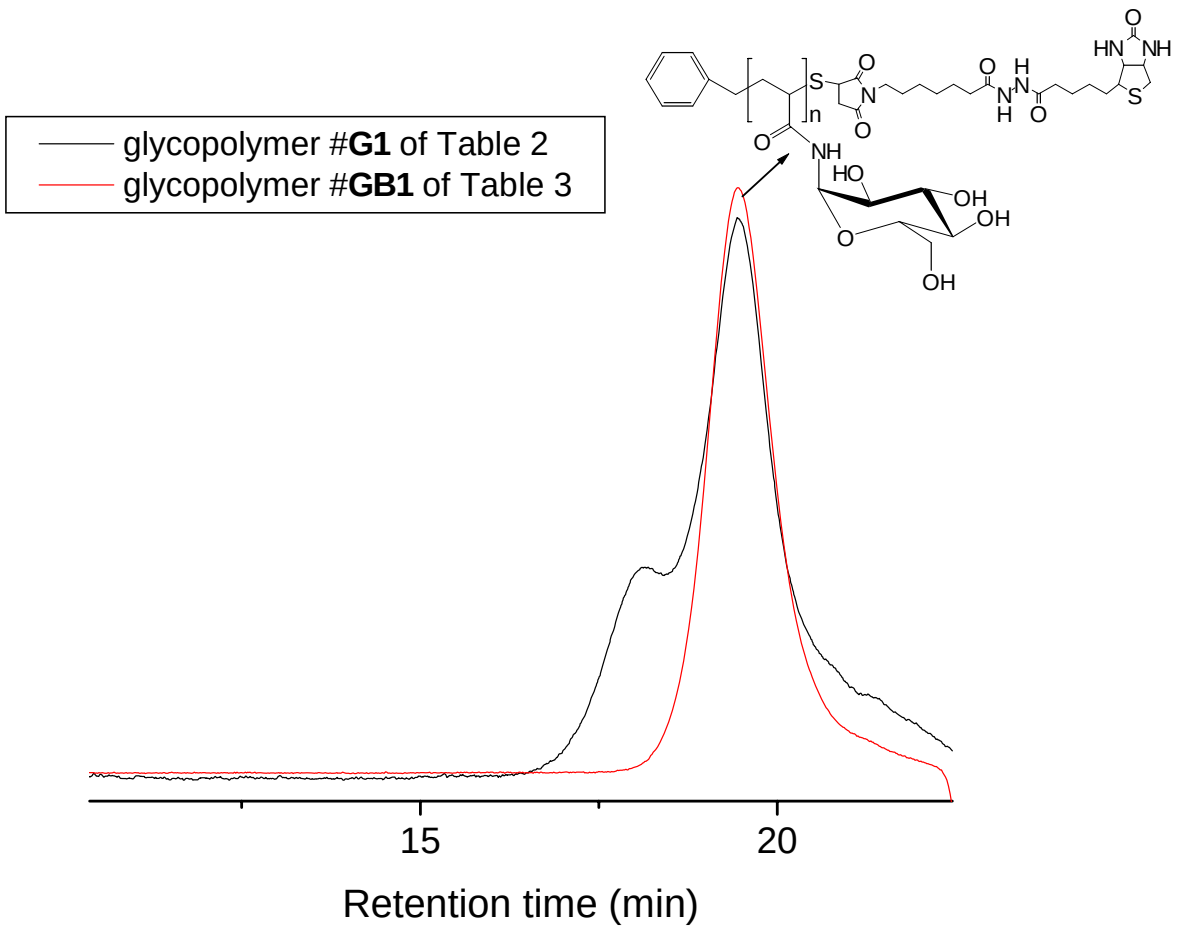


Figure S7. Aqueous GPC traces of (-) glycopolymer (#**G1** of Table 2) obtained in the absence of biotin modified maleimide and (-) glycopolymer (#**GB1** of Table 2) obtained in the presence of biotin modified maleimide.

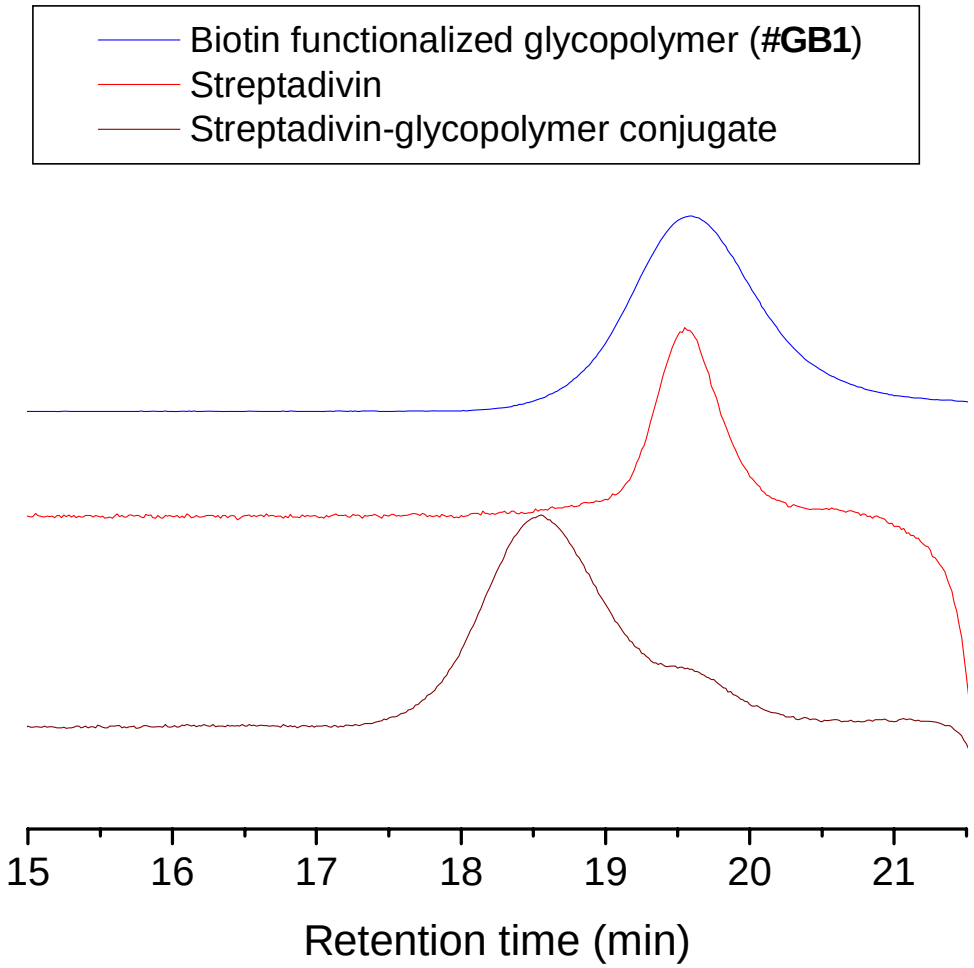


Figure S8. Aqueous GPC traces of (-) biotin modified glycopolymer (#**GB1** of Table 2), (-) streptadivin, and streptadivin/biotin-glycopolymer conjugates.

References

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