Glycosylated Polyplex Micelles from Oppositely Charged Block Copolymers

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Supplementary Information

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Materials

2-Cyanopropan-2-yl propyl trithiocarbonate (CPP-TTC) was synthesized according to literature[1]. 2-(Dimethylamino)ethyl methacrylate (DMAEMA, 98%), *tert*-butyl methacrylate (*t*-BMA, 98%), D-(+)-glucose, methacryloyl chloride (MCC, 97%), 2,2'-azobis(2-methylpopionitrile) (AIBN), trifluoroacetic acid (TFA, ≥99.0%), 3-sulfopropyl methacrylate potassium salt (K-SPMA, 98%), isobutanol (*i*-BuOH, ≥99%), oxalyl chloride ($(COCl)_2$, ≥99%), aluminum oxide (Al_2O_3, activated, basic), sodium iodide (NaI, ≥99.5%), potassium nitrate (KNO_3, ≥99.0%) and methyl iodide (MeI, ≥99.0%) were purchased from Sigma-Aldrich. Triethylamine (TEA, >99.0%) was obtained from TCI. Sodium hydroxide (NaOH, 99.0%), hydrochloric acid solution (HCl, 37%), sulfuric acid (H_2SO_4, 95-97%), and magnesium sulfate ($MgSO_4$, anhydrous, ≥99.5%) were purchased from Boom. Sodium chloride (NaCl, ≥99.0%) was sourced from Merck. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP, AR grade) was sourced from Biosolve. Diethyl ether (Et_2O, HPLC grade), ethyl acetate (EtOAc, HPLC grade), *n*-hexane (HPLC grade), *N,N*-dimethylformamide (DMF, ≥99%), dichloromethane (DCM, HPLC grade), 1,4-dioxane and dimethyl sulfoxide (DMSO) were obtained from Marcon Fine Chemicals. Silica gel for flash chromatography (40–63 μm) was obtained from Silicycle. Dialysis tubing (SERVAPOR® 3, Membra-Cel, cellulose, MWCO = 3500 Da) was purchased from Serva.

AIBN was recrystallized from THF/hexane before use. Commercially available liquid monomers were passed through a short basic Al_2O_3 column to remove the inhibitor before polymerization. Anhydrous dichloromethane was obtained through a MB-SPS 800 purification machine from MBraun, equipped with HPLC grade DCM Ossum chemicals. All other chemicals were used as received.

Instruments

^{1}H and ^{13}C nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on a Bruker 400 MHz spectrometer at 298 K. Deuterated chloroform ($CDCl_3$, 99.8%) and deuterium oxide (D_2O, 99.9%) were purchased from Sigma-Aldrich. The samples were dissolved in an appropriate solvent (≈ 4 g L^{-1}) and shimmed with a pulse width of 45 µs, spectral width of 12/-2 ppm, a recycle delay of 1 s and measurements via either 32 or 128 scans. The spectra were analyzed with MestreNova software version 14.1.

High-resolution mass spectrometry (HRMS) was performed on an LTQ Orbitrap XL spectrometer (Thermo Fisher Scientific) with an electrospray ionization (ESI) source. The samples were dissolved in DCM at a concentration of ≈ 1 g/L and passed through a 0.45 µm nylon filter prior to analysis.

Size exclusion chromatography (SEC) was performed on a GPCMax system from Viscotek equipped with a 302 TDA detector array and two columns in series (PolarGel L and M, both 8 µm 30 cm) from Agilent Technologies. The columns and detectors were maintained at a temperature of 50 °C. DMF containing 0.01 M LiBr was used an eluent at a flow rate of 1 mL min^{-1}. Nearly monodisperse poly(methyl methacrylate) standards ($Đ$ = 1.03) from the Polymer Standard Service were used for the construction of a calibration curve. The samples were prepared with the eluent at a concentration of 3 g L^{-1} and passed through a 0.45 µm nylon filter before injection. Data acquisition and calculations were performed via Viscotek Omnise software version 5.0.

Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectra were collected via a Bruker VERTEX 70 spectrometer equipped with an ATR diamond single reflection module. Spectra were recorded from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1} with 64 scans for each sample. Atmospheric compensation and baseline correction were applied to the collected spectra via Bruker's OPUS spectroscopy software version 7.0.

Thermogravimetric analysis (TGA) measurements were conducted on a TA Instruments TGA5500 analyzer. The samples (~ 5 mg) were heated from 30 °C to 700 °C at a rate of 10 °C min^{-1} under a continuous nitrogen flow. The data acquisition and analysis was performed via TA Instruments TRIOS software.

Differential scanning calorimetry (DSC) measurements were conducted on a TA Instruments DSCQ1000 analyzer. The samples (~ 5 mg) were subjected to the following methods: (i) equilibration at -60 °C, (ii) 5 min isotherm, (iii) ramp to 180 °C at 10 °C min^{-1}, (iv) 5 min isotherm, (v) ramp to -60 °C at 10 °C min^{-1}, (vi) 5 min isotherm, and (vii) ramp to 180 °C at 10 °C min^{-1}. Data analysis was performed via the heat second cycle TA Instruments TRIOS software.

Dynamic light scattering (DLS) measurements were conducted on a Malvern Panalytical Zetasizer Ultra system, equipped with a helium-neon laser (λ = 633 nm) and an avalanche photodiode detector. Samples were prepare in a 10 mM KNO_3 solution. The nanoparticle solutions were measured at 25 °C in backscattering mode (173°) and multi-angle scattering mode (17°, 90°, 173°) after 120 s equilibration time using 30 cumulative recordings and each sample was recorded five times. The measurements were carried out with BRAND® Macro cuvettes, and the results were analyzed with ZS Xplorer software.

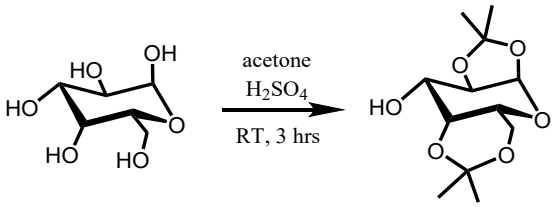
ζ–Potential measurements were performed on a Malvern Panalytical Zetasizer Ultra system, equipped with a helium neon laser (λ = 633 nm) and an avalanche photodiode detector. The measurements were taken at 25 °C while the acquisition times were automatically determined, and each sample was recorded in triplicate. The measurements were conducted with a folded capillary zeta cell and results were analyzed with ZS Xplorer software.

Transmission electron microscopy (TEM) imaging was conducted on a Philips CM120 transmission electron microscope equipped with a LaB_6 filament and operated at an accelerating voltage of 120 kV. Images were acquired via a Gatan slow-scan CCD camera. Negatively stained samples were prepared by depositing 5 µL of the nanoparticle dispersion onto a glow-discharged (15 s at 40 mA and 300 V) 400-mesh copper grid with a carbon support film and adsorbing for 1 min before blotting. Before the sample was fully dried, 5 µL of 2 wt. % uranyl acetate staining solution was deposited onto the grid and immediately blotted and a new 5 µL drop of staining solution was deposited and left to adsorb for 1 min before blotting. The TEM images were analyzed via ImageJ software, which uses brightness and contrast correction tools to enhance the general quality of the snapshots. A software embedded measurement tool was used to determine the dimensions of the nanoparticles.

Methods

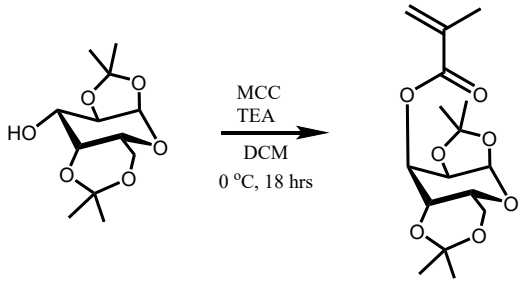
Synthesis of the monomers

Synthesis of acetonide-protected glucose (PrGlc-OH)



PrGlc-OH was synthesized according to previously reported procedures[2], with adaptations. A round bottom flask equipped with a stirring egg containing acetone (200 mL) was cooled over an ice bath. Sulfuric acid (10 mL) was added dropwise to the flask, followed by the addition of D(+)-glucose (10 g). The suspension was stirred at room temperature for 3 h. Afterwards, the flask was cooled in an ice bath, and an ice-cold NaOH solution (30 g NaOH in 120 mL deionized water) was added dropwise to neutralize the acid. The residual acetone was removed under vacuum, and 100 mL of deionized water was added. The resulting aqueous solution was extracted with dichloromethane (DCM) (3 × 150 mL), and the combined organic phases were washed with deionized water (3 × 150 mL) and dried with anhydrous $MgSO_4$. The crude product was obtained by removing DCM via rotary evaporation, and the final product was isolated by recrystallization from cold *n*-hexane. Yield: 5.76 g. ^{1}H NMR ($CDCl_3$): (ppm) = 5.94 (d, CH), 4.54 (d, CH), 4.33 (m, CH), 4.16 (m, CH_2), 4.07 (dd, CH), 3.98 (m, CH), 1.50 (s, CH_3), 1.44 (s, CH_3), 1.36 (s, CH_3), 1.32 (s, CH_3).

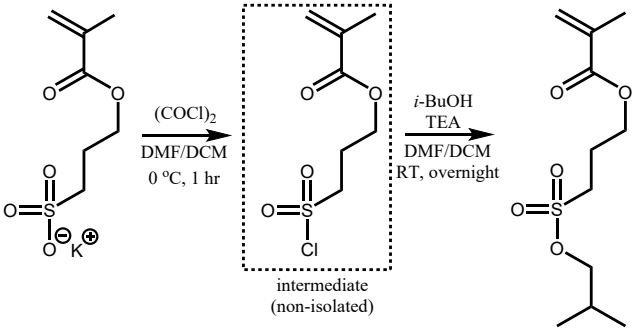
Synthesis of acetonide-protected glucose methacrylate (PrGlcMA).



PrGlcMA was synthesized according to previously reported procedures[3], with adaptations. PrGlc-OH (5.60 g, 21.5 mmol, 1 eq), trimethylamine (TEA) (3.26 g, 32.3 mmol, 1.5 eq), and anhydrous dichloromethane (150 mL) were added to a round-bottom flask equipped with a stirring egg and cooled in an ice bath. The mixture was

purged with argon for 5 minutes. Methacryloyl chloride (MCC) (3.37 g, 32.3 mmol, 1.5 eq) was then added dropwise under argon flow into the cold mixture. The reaction mixture was subsequently allowed to warm to room temperature and stirred overnight. The reaction was quenched with 3 mL of cold deionized water, and the mixture was washed sequentially with 0.1 M HCl (3 × 100 ml), 0.1 M NaOH (3 × 100 mL) and deionized water (3 × 100 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated under vacuum, yielding a light brown crude product. PrGlcMA was obtained via a flash column using *n*-hexane/ethyl acetate (4/1) as the eluent ($R_f \approx 0.5$), resulting in a transparent viscous oil. Yield: 3.70 g. ^{1}H NMR ($CDCl_3$): (ppm) = 6.11 (s, CH), 5.89 (d, CH), 5.61 (s, CH), 5.29 (s, CH), 4.53 (d, CH), 4.25 (m, CH_2), 4.06 (m, CH), 4.01 (m, CH), 1.95 (s, CH_3), 1.52 (s, CH_3), 1.40 (s, CH_3), 1.30 (s, CH_3).

Synthesis of 3-isobutoxysulfopropyl methacrylate (BSPMA)

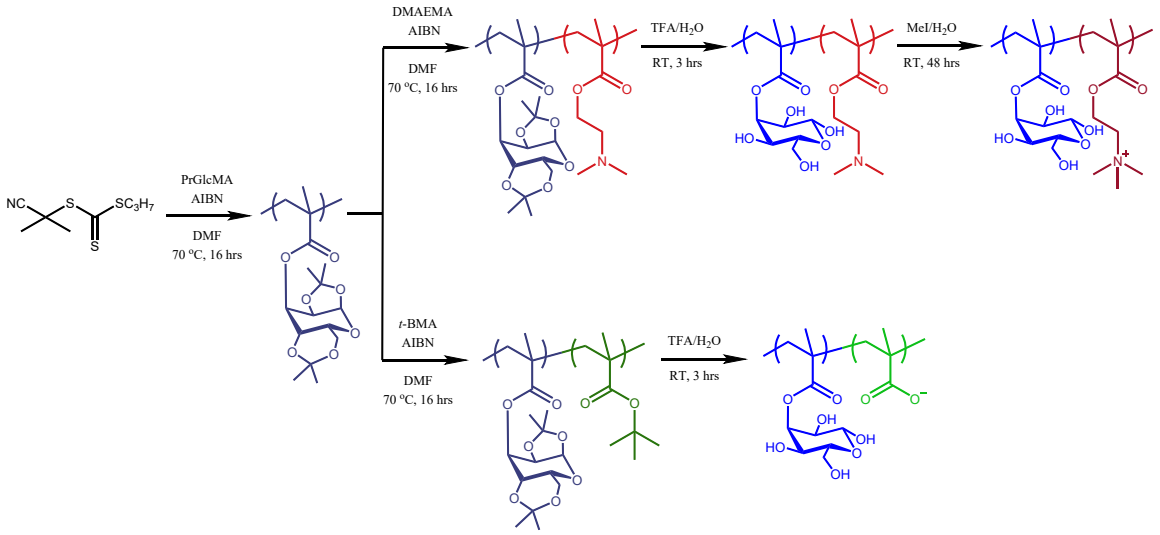


BSPMA was synthesized according to a previously reported method[1]. K-SPMA (10.2 g, 41.4 mmol, 1 eq) and a stir bar were placed in a three-neck round-bottom flask and subjected to three vacuum/argon cycles to remove moisture. Anhydrous *N,N*-dimethylformamide (DMF) (30 mL) was then added. The suspension was cooled in an ice bath, followed by the dropwise addition of oxalyl chloride $(COCl)_2$ (5.78 g, 45.5 mmol, 1.1 eq) in anhydrous dichloromethane (15 mL) under an argon atmosphere. In a separate three-neck round-bottom flask, isobutanol (3.70 g, 49.9 mmol, 1.2 eq) and triethylamine (8.41 g, 83.1 mmol, 2 eq) were dissolved in anhydrous dichloromethane (30 mL) under an argon flow and cooled in an ice bath. After 1 h, the intermediate suspension from the first flask was slowly added to the second flask via a dropping funnel under argon overpressure. Once the addition was complete, the mixture was allowed to warm to room temperature and stirred overnight. Deionized water (200 mL) was then added, and the desired product was extracted with diethyl ether (3×200 ml). The combined organic phases were concentrated under vacuum, washed with

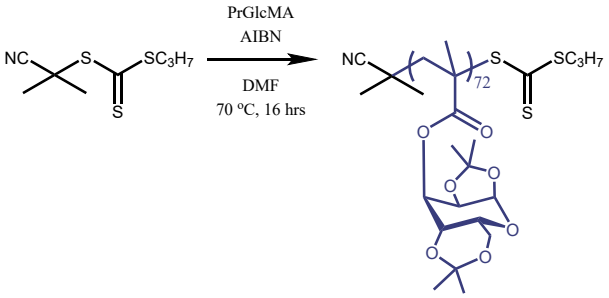
deionized water (200 mL) and dried over anhydrous $MgSO_4$. The crude product was purified via flash column chromatography using *n*-hexane/ethyl acetate (3/1) as the eluent ($R_f \approx 0.6$), yielding a light yellow oil. Yield: 7.62 g. ^{1}H NMR ($CDCl_3$): (ppm) = 6.09 (s, CH), 5.58 (s, CH), 4.26 (t, CH_2), 3.98 (d, CH_2), 3.19 (t, CH_2), 2.23 (m, CH_2), 2.01 (m, CH), 1.92 (m, CH_3), 0.96 (d, CH_3).

Synthesis of the homopolymers and block copolymers

Scheme S1: Synthesis strategy for the fabrication of positively and negatively charged glycosylated block copolymers



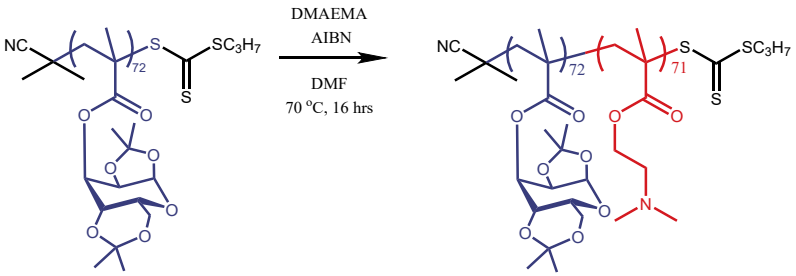
Synthesis of poly(acetonide-protected glucose methacrylate) (PPrGlcMA)



CPP-TTC (10.4 mg, 47.5 µmol, 1 eq), PrGlcMA (1.18 g, 3.59 mmol, 76 eq), AIBN (1.5 mg, 9.28 µmol, 0.2 eq; prepared from 105.7 mg of a stock solution containing 14.6 mg AIBN in 997.8 mg DMF) and 1.43 g DMF were added to a round-bottom flask with a stir bar. The reaction mixture was deoxygenated by nitrogen bubbling for 10 min before the flask was immersed in a preheated oil bath to initiate polymerization. After 16 h at 70 °C, the flask was removed from the oil bath, cooled, and exposed to air to terminate the reaction. The viscous solution was precipitated into cold *n*-hexane/ethyl acetate

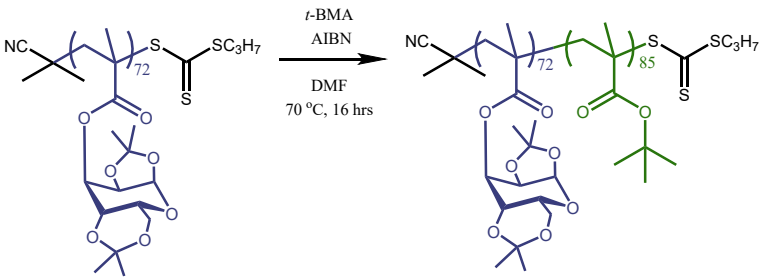
(6/1), redissolved in a minimal amount of acetone and precipitated in cold *n*-hexane twice. The precipitate was dried under high vacuum overnight, yielding a yellow solid. Yield: 957.1 mg. ^{1}H NMR ($CDCl_3$): conversion = 94.5%, DP_{NMR} = 72, $M_{n\ NMR}$ = 23 800 Da. SEC (DMF): $M_{n\ SEC}$ = 24 500 Da, Đ = 1.56.

Synthesis of poly(acetonide-protected glucose methacrylate)-*block*-poly[2-(dimethylamino)ethyl methacrylate] (PPrGlcMA-*b*-PDMAEMA)



$PPrGlcMA_{72}$-CTA (151.2 mg, 6.3 µmol, 1 eq), DMAEMA (100.1 mg, 0.63 mmol, 100 eq), AIBN (207 µg, 1.26 µmol, 0.2 eq; prepared from 14.6 mg of a stock solution containing 14.6 mg AIBN in 997.8 mg DMF) and 0.62 g DMF were added to a round-bottom flask with a stir bar. The reaction mixture was deoxygenated by bubbling with nitrogen for 10 minutes before the flask was immersed in a preheated oil bath to initiate polymerization. After 16 h at 70 °C, the flask was removed from the oil bath, cooled, and exposed to air to terminate the reaction. The viscous solution was precipitated into cold *n*-hexane/ethyl acetate (6/1), redissolved in a minimal amount of acetone and reprecipitated in cold *n*-hexane twice. The precipitate was dried under high vacuum overnight, yielding a light yellow solid. Yield: 185.3 mg. ^{1}H NMR ($CDCl_3$): conversion = 71.1%, DP_{NMR} = 71, $M_{n\ NMR}$ = 35 000 Da. SEC (DMF): $M_{n\ SEC}$ = 32 300 Da, Đ = 1.66.

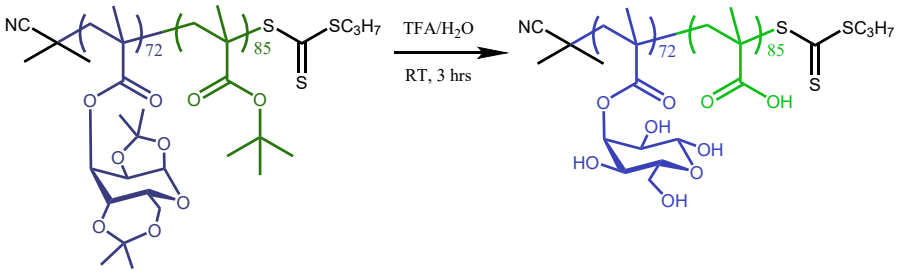
Synthesis of poly(acetonide-protected glucose methacrylate)-*block*-poly(2-(*tert*-butyl methacrylate) (PPrGlcMA-*b*-P*t*BMA)



$PPrGlcMA_{72}$-CTA (151.7 mg, 6.3 µmol, 1 eq), *t*-BMA (94.7 mg, 0.66 mmol, 105 eq), AIBN (207 µg, 1.26 µmol, 0.2 eq; prepared from 14.8 mg of a stock solution containing

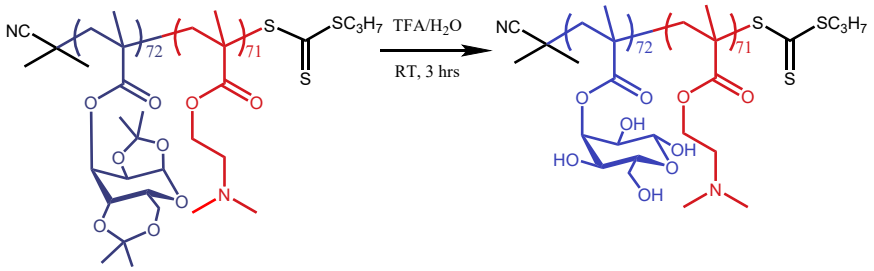
14.6 mg AIBN in 997.8 mg DMF) and 0.617 g DMF were added to a round-bottom flask with a stir bar. The reaction mixture was deoxygenated by bubbling with nitrogen for 10 minutes before the flask was immersed in a preheated oil bath to initiate polymerization. After 16 h at 70 °C, the flask was removed from the oil bath, cooled and exposed to air to terminate the reaction. The viscous solution was precipitated into cold *n*-hexane/ethyl acetate (6/1), redissolved in a minimal amount of acetone and reprecipitated in cold *n*-hexane twice. The precipitate was dried under high vacuum overnight, yielding a light yellow solid. Yield: 185.6 mg. ^{1}H NMR ($CDCl_3$): conversion = 81.5%, DP_{NMR} = 85, $M_{n\ NMR}$ = 35 900 Da. SEC (DMF): $M_{n\ SEC}$ = 34 300 Da, Đ = 1.55.

Synthesis of poly(glucose methacrylate)-*block*-poly(methacrylic acid) (PGlcMA-*b*-PMAA)



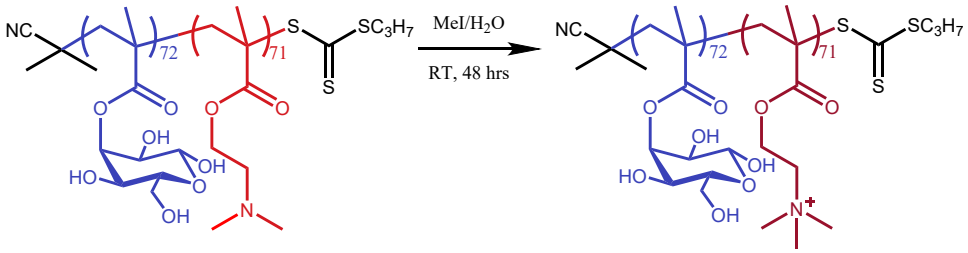
PPrGlcMA$_{72}$-P*t*BMA$_{85}$ (85.0 mg) was placed into a glass vial equipped with a stir bar, and a premixed solution of trifluoroacetic acid/water (9 mL trifluoroacetic acid, 1 mL deionized) was added to the vial upon stirring. After 3 h at room temperature, the clear solution was transferred to a dialysis membrane (MWCO = 3500 Da) and extensively dialyzed against deionized water until the pH became constant. The water was removed by freeze-drying, yielding a white fluffy solid. Yield: 58.4 mg. ^{1}H NMR (D_2O): *tert*-butyl removal ≈ 100%.

Synthesis of poly(glucose methacrylate)-*block*-poly[2-(dimethylamino)ethyl methacrylate] (PPrGlcMA-*b*-PDMAEMA)



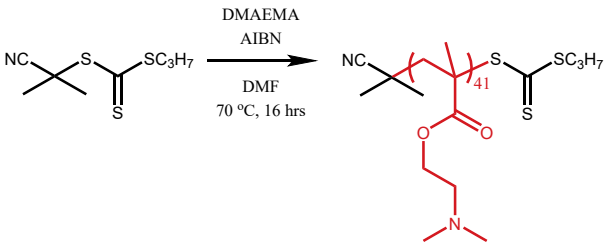
PPrGlcMA$_{72}$-PDMAEMA$_{71}$ (110.2 mg) was placed into a glass vial equipped with a stir bar, and a premixed solution of trifluoroacetic acid/water (9 mL trifluoroacetic acid, 1 mL deionized) was added to the vial upon stirring. After 3 h at room temperature, the clear solution was transferred to a dialysis membrane (MWCO = 3500 Da) and extensively dialyzed against DI water until the pH remained constant. The water was removed by freeze-drying, yielding a white fluffy solid. Yield: 85.3 mg. ^{1}H NMR (D_2O): acetonide removal ≈ 100%.

Synthesis of poly(glucose methacrylate)-*block*-poly[2-(methacryloyloxy)ethyl trimethylammonium iodide] (PGlcMA-*b*-PMETAI)



PGlcMA$_{72}$-*b*-PDMEMA$_{71}$ (60.3 mg, 17.1 mg DMEMA, 109.9 µmol, 1 eq), methyl iodide (156 mg, 1.1 mmol, 10 eq) and deionized water (10 mL) were added to a glass vial equipped with a stir bar. The reaction mixture was stirred at room temperature for 48 h. To remove residual methyl iodide, the polymer solution was bubbled with nitrogen for 4 h, followed by freeze-drying to obtain a light brown, fluffy solid. Yield: 69.6 mg. ^{1}H NMR (D_2O): quaternization ≈ 70%

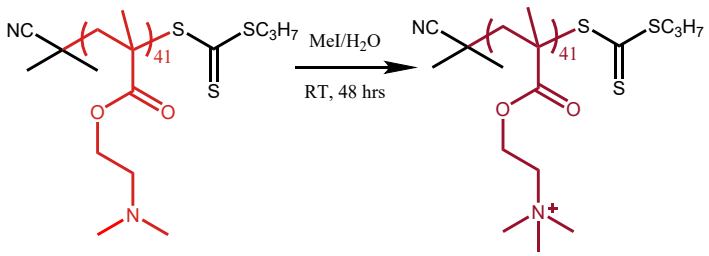
Synthesis of poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA)



CPP-TTC (31.5 mg, 0.144 mmol, 1 eq), DMAEMA (1089.6 mg, 6.93 mmol, 48 eq), AIBN (2.3 mg, 14.0 µmol, 0.1 eq; 244.2 mg of a stock solution of 8.5 mg AIBN in 996.2 mg DMF) and 5.70 g DMF were added to a round-bottom flask with a stir bar. The reaction mixture was deoxygenated by bubbling with nitrogen for 10 minutes, after which the flask was immersed in a preheated oil bath to initiate the polymerization. After 16 h at 70 °C, the flask was removed from the oil bath, cooled and exposed to air

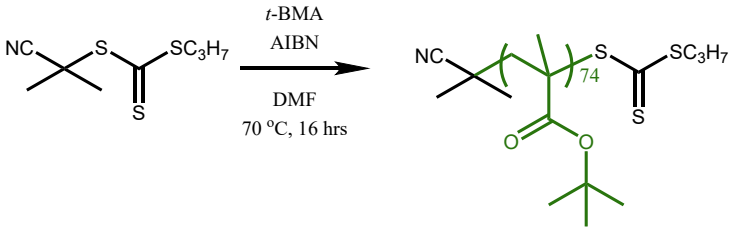
to terminate the reaction. The viscous solution was precipitated into cold *n*-hexane/ethyl acetate (6/1), redissolved in a minimal amount of acetone and reprecipitated in cold *n*-hexane twice. The precipitate was dried under high vacuum overnight, yielding a viscous yellow liquid. Yield: 853.3 mg. ^{1}H NMR ($CDCl_3$): conversion = 84.2%, DP_{NMR} = 41, $M_{n\ NMR}$ = 6700 Da. SEC (DMF): $M_{n\ SEC}$ = 7300 Da, Đ = 1.29.

Synthesis of poly[2-(methacryloyloxy)ethyl trimethylammonium iodide] (PMETAI)



$PDMEMA_{41}$ (60.0 mg, 381.7 µmol, 1 eq), methyl iodide (542.2 mg, 3.82 mmol, 10 eq) and deionized water (10 mL) were added to a glass vial equipped with a stir bar. The reaction mixture was stirred at room temperature for 48 h. To remove residual methyl iodide, the polymer solution was bubbled with nitrogen for 4 h, followed by freeze-drying to obtain a light brown, fluffy solid. Yield: 92.1 mg. ^{1}H NMR (D_2O): quaternization ≈ 100%

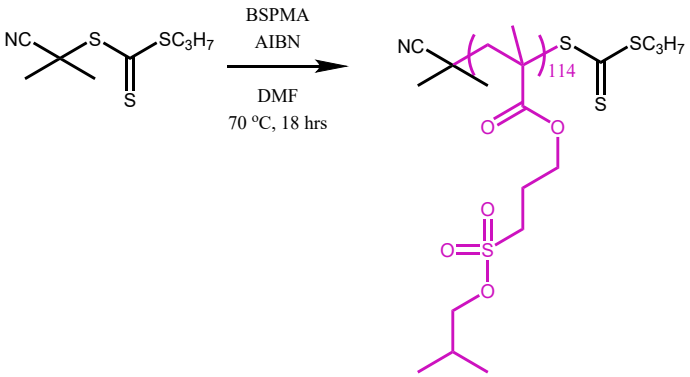
Synthesis of poly(*tert*-butyl methacrylate) (P*t*BMA)



CPP-TTC (15.0 mg, 68.5 µmol, 1 eq), *t*-BMA (977.3 mg, 6.85 mmol, 100 eq), AIBN (1.08 mg, 6.9 µmol, 0.1 eq; prepared from 186.8 mg of a stock solution containing 11.7 mg AIBN in 2012.4 mg DMF) and 3.01 g DMF were added to a round-bottom flask with a stir bar. The reaction mixture was deoxygenated by bubbling with nitrogen for 10 minutes, after which the flask was immersed in a preheated oil bath to initiate the polymerization. After 16 h at 70 °C, the flask was removed from the oil bath, cooled and exposed to air to terminate the reaction. The viscous solution was precipitated into

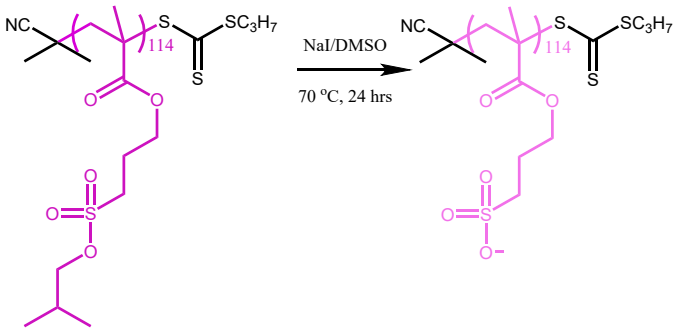
cold *n*-hexane/ethyl acetate, redissolved in a minimal amount of acetone and reprecipitated in cold *n*-hexane twice. The precipitate was dried under high vacuum overnight, yielding a yellow solid. Yield: 853 mg. ^{1}H NMR ($CDCl_3$): conversion = 74.1%, DP_{NMR} = 74, $M_{n\ NMR}$ = 10 700 Da.

Synthesis of poly[3-(isobutoxysulfonyl)propyl methacrylate] (PBSPMA)



CPP-TTC (30.2 mg, 108 µmol, 1 eq), BSPMA (3.41 g, 12.9 mmol, 119 eq), AIBN (2.0 mg, 12.2 µmol, 0.1 eq; prepared from 183.1 mg of a stock solution containing 20.6 mg AIBN in 1888.0 mg DMF) and 4.23 g DMF were added to a round-bottom flask with a stir bar. The reaction mixture was deoxygenated by bubbling with nitrogen for 10 minutes, after which the flask was immersed in a preheated oil bath to initiate polymerization. After 18 h at 70 °C, the flask was removed from the oil bath, cooled and exposed to air to terminate the reaction. The viscous solution was precipitated into cold *n*-hexane/ethyl acetate (6/1), followed by freeze-drying from 1,4-dioxane to obtain a yellow solid. Yield: 3.13 g. ^{1}H NMR ($CDCl_3$): conversion = 95.8%, DP_{NMR} = 114, $M_{n\ NMR}$ = 33 800 Da. SEC (DMF): $M_{n\ SEC}$ = 43 000 Da, Đ = 1.10.

Synthesis of poly(3-sulfopropyl methacrylate) (PSPMA-Na)

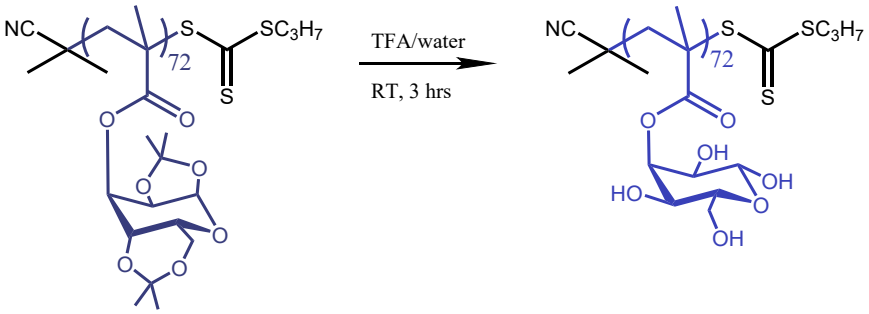


Deprotection was conducted according to a previously reported protocol[1], with adaptations. $PBSPMA_{114}$ (200.5 mg, 0.758 mmol BSPMA), sodium iodide (NaI) (390.0

mg, 2.60 mmol, 3 eq) and 3 mL dimethyl sulfoxide (DMSO) were added to a glass vial equipped with a stir bar. After 24 h at 70 °C, the resulting yellow fluffy polymer was isolated via precipitation in *n*-hexane/ethyl acetate (1/1) and freeze-dried from water. Yield: 166.0 mg. ^{1}H NMR (D_2O): isobutyl removal ≈ 100%.

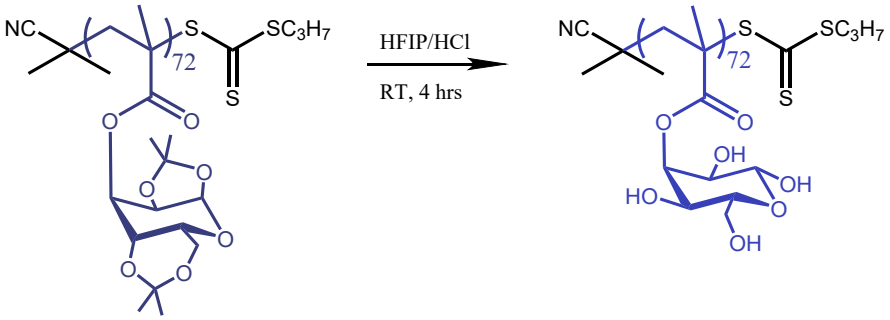
Deprotection tests on homopolymers

TFA/water deprotection of PPrGlcMA



Deprotection was conducted according to a previously reported protocol[2], with adaptations. $PPrGlcMA_{72}$ (60.2 mg, 0.183 mmol PrGlcMA, 0.367 mmol acetonide group) was added to a glass vial equipped with a stir bar, and a premixed solution of trifluoroacetic acid/water (5.4 mL trifluoroacetic acid, 0.6 mL deionized water) was added into the vial under stirring. After 3 h at room temperature, the clear solution was transferred to a dialysis membrane (MWCO = 3500 Da) and extensively dialyzed against deionized water until the pH remained consistent. The water was removed by freeze-drying, yielding a white fluffy solid. Yield: 45.2 mg. ^{1}H NMR (D_2O): acetonide removal ≈ 100%.

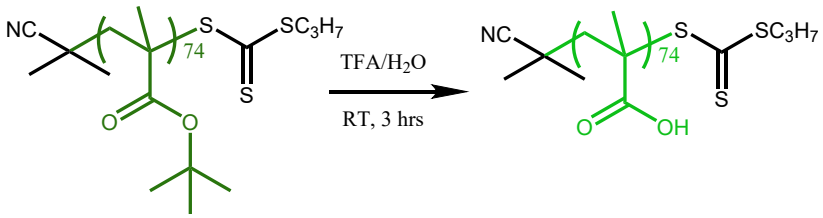
HFIP/HCl deprotection of PPrGlcMA



$PPrGlcMA_{72}$ (50.1 mg, 0.153 mmol PrGlcMA, 0.305 mmol acetionide group) was added to a glass vial equipped with a stirring bar and dispersed in 5.5 mL of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). HCl solution (46 µL, 0.457 mmol) was then added to the

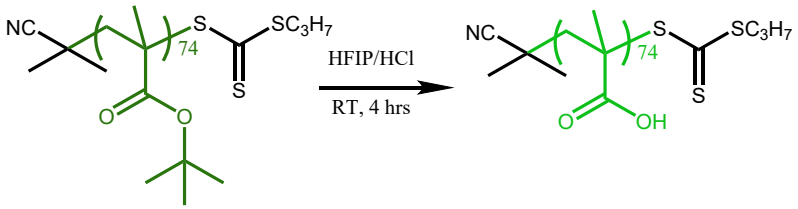
mixture. After 4 h at room temperature, the lightly cloudy solution was transferred to a dialysis membrane (MWCO = 3500 Da) and extensively dialyzed against deionized water until the pH remained consistent. The water was removed by freeze-drying, yielding a white fluffy solid. Yield: 32.2 mg. ^{1}H NMR (D_2O): acetonide removal ≈ 100%.

TFA/water deprotection of P*t*BMA



P*t*BMA$_{74}$ (100.1 mg, 0.704 mmol *tert*-BMA, 0.704 mmol *tert*-butyl) was added into a glass vial equipped with a stir bar, and a premixed trifluoroacetic acid/water (9 mL trifluoroacetic acid, 1 mL deionized water) was added to the polymer under stirring. After 3 h at room temperature, the clear solution was transferred to a dialysis membrane (MWCO = 3500 Da) and extensively dialyzed against deionized water until the pH remained constant. The water was removed by freeze-drying, yielding a white fluffy solid. Yield: 53.3 mg. ^{1}H NMR (D_2O): *tert*-butyl removal ≈ 100%.

HFIP/HCl deprotection of P*t*BMA



Deprotection was conducted according to a previously reported protocol[4], with adaptations. P*t*BMA$_{74}$ (50.4 mg, 0.354 mmol *tert*-BMA, 0.354 mmol *tert*-butyl) was added to a glass vial equipped with a stir bar, and dispersed in 5.5 mL of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP). HCl solution (53 µL, 0.528 mmol) was added to the mixture. After 4 h at room temperature, the cloudy solution was transferred to a dialysis membrane (MWCO = 3500 Da) and extensively dialyzed against deionized water until the pH kept consistent. The water was removed by freeze-drying, yielding a white fluffy solid. Yield: 25.3 mg. ^{1}H NMR (D_2O): *tert*-butyl removal ≈ 100%.

Preparation of glycosylated polyplex micelles

Each polymer was dissolved in triple-filtered 10 mM KNO_3 buffer at a concentration of 1 g/L. The repeated units account for 100 wt.% of the charged homopolymers (neglecting end groups), the amino decorated units account for 46 wt.% of the $PGlcMA_{72}$-*b*-$PMETAI_{71}$ and the carboxyl decorated units account for 29 wt.% of the $PGlcMA_{72}$-*b*-$PMAA_{85}$. The volumes of the charged polymer solutions were calculated to determine the stoichiometric ratio between the amino-decorated units and the carboxyl/sulfo-decorated units.

Glycosylated polyplex micelles (GPM) were prepared as follows:

- GPM_1: 844 µL of $PGlcMA_{72}$-*b*-$PMETAI_{71}$ (1) and 538 µL of $PGlcMA_{72}$-*b*-$PMAA_{85}$ (1)
- GPM_2: 538 µL of $PGlcMA_{72}$-*b*-$PMAA_{85}$ (1) and 543 µL of $PMETAI_{41}$ (1)
- GPM_3: 844 µL of $PGlcMA_{72}$-*b*-$PMETAI_{71}$ (1) and 156 µL of $PMAA_{74}$ (1)
- GPM_4: 844 µL of $PGlcMA_{72}$-*b*-$PMETAI_{71}$ (1) and 418 µL of PSPMA-Na_{114} (1)
- GPM_5: 844 µL of $PGlcMA_{72}$-*b*-$PMETAI_{71}$ (1) and 209 µL of PSPMA-Na_{114} (0.5)

Each mixture was introduced into a dust-free vial containing stir bars, for subsequent preparation.

Supporting figures

S1: Nuclear magnetic resonance spectroscopy of the monomers

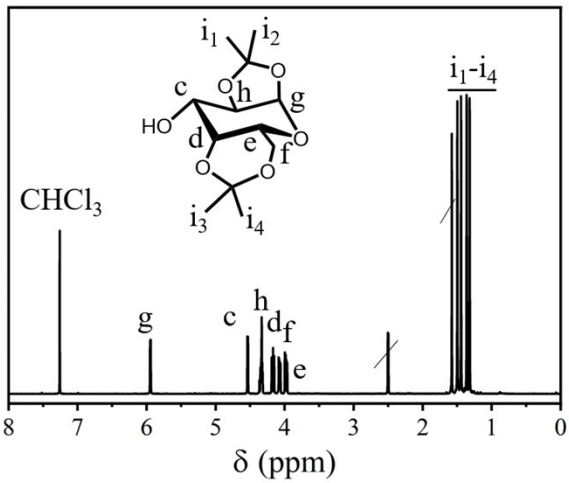


Figure S1-1. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of the protected glycomonomer precursor (PrGlc-OH).

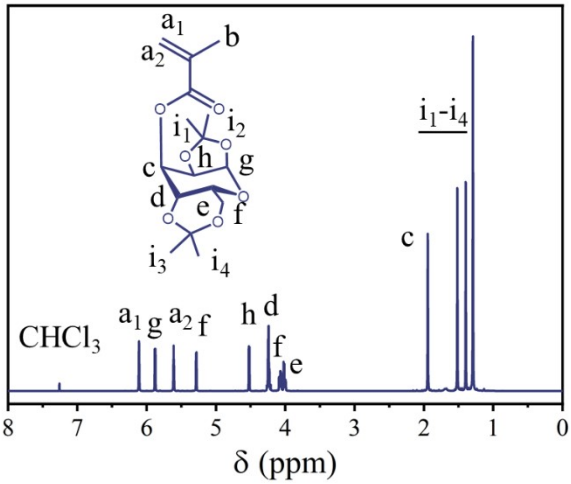


Figure S1-2. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of the protected glycomonomer (PrGlcMA).

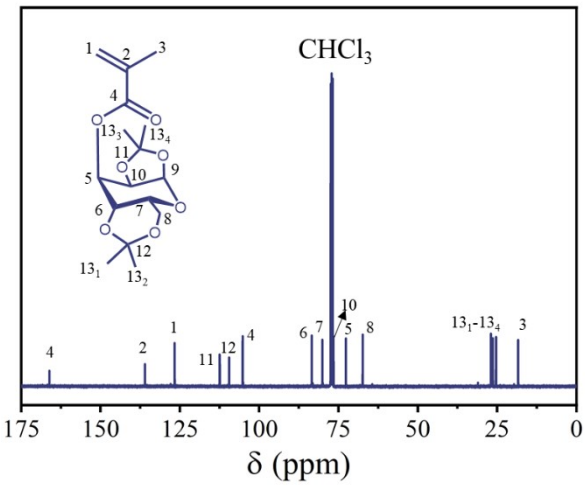


Figure S1-3. ^{13}C NMR spectrum ($CDCl_3$, 400 MHz) of the protected glycomonomer (PrGlcMA).

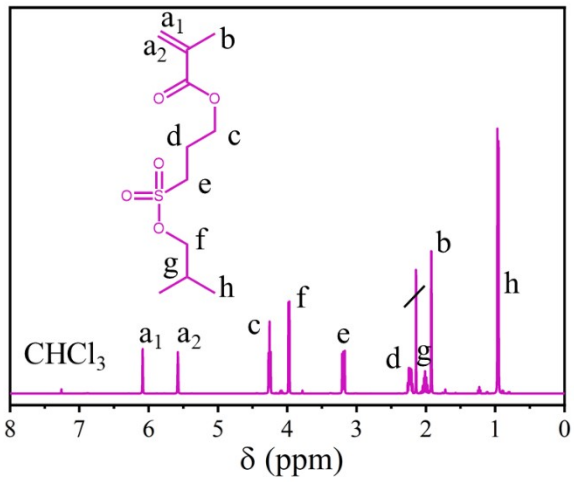


Figure S1-4. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of the 3-isobutoxysulfopropyl methacrylate (BSPMA).

S2: High resolution mass spectra of the protected glycomonomers

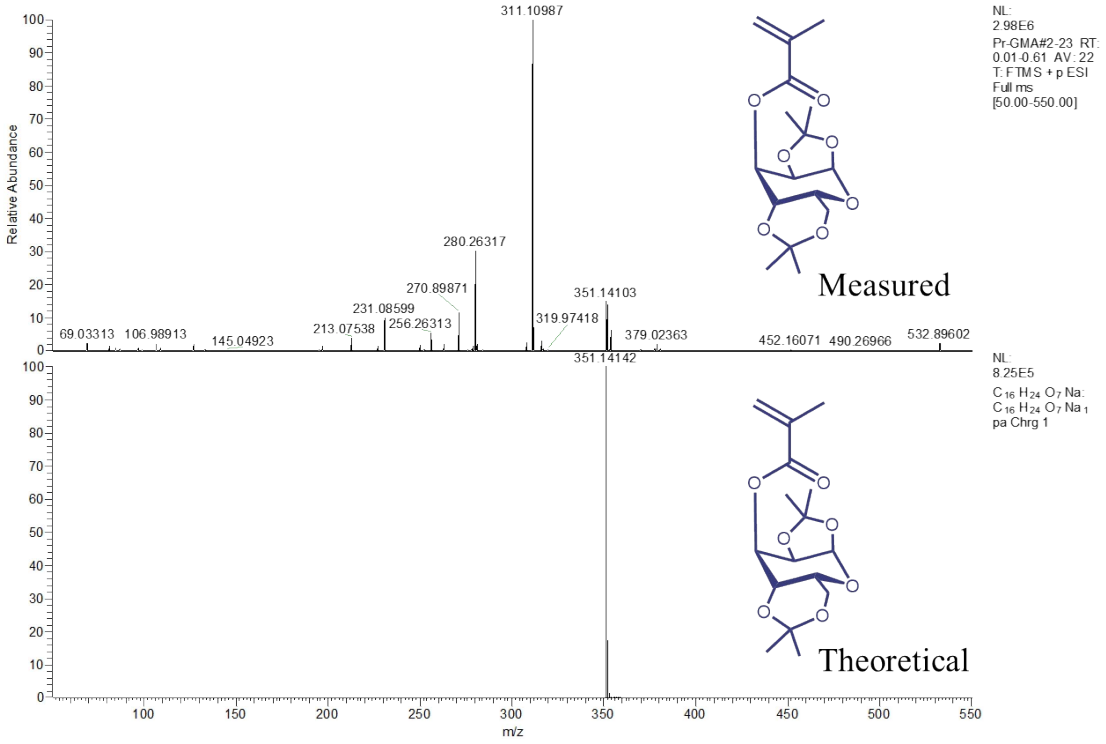


Figure S2. HRMS analysis of the acetonide-protected glycomonomer (PrGlcMA, c ≈ 1 g L^{-1} in DCM).

S3: Nuclear magnetic resonance spectroscopy of the homopolymers

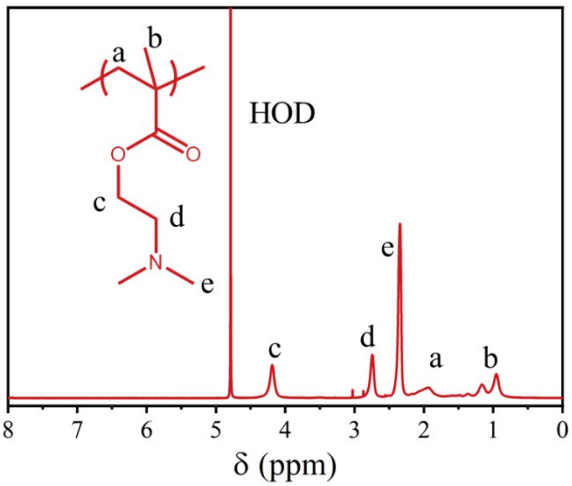


Figure S3-1. ^{1}H NMR spectrum (D_2O, 400 MHz) of the positively charged homopolymer precursor ($PDMAEMA_{41}$).

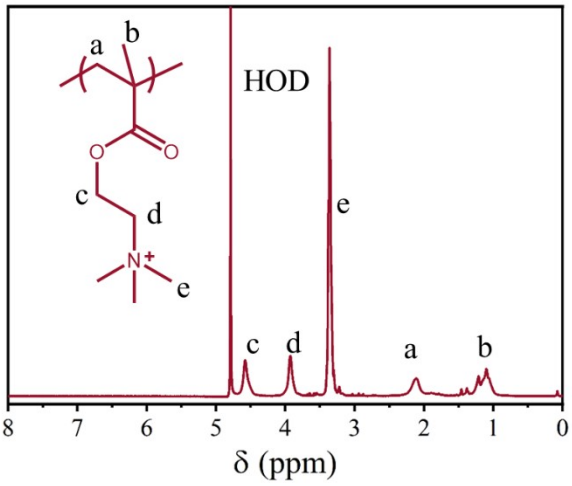


Figure S3-2. ^{1}H NMR spectrum (D_2O, 400 MHz) of the positively charged homopolymer ($PMETAI_{41}$).

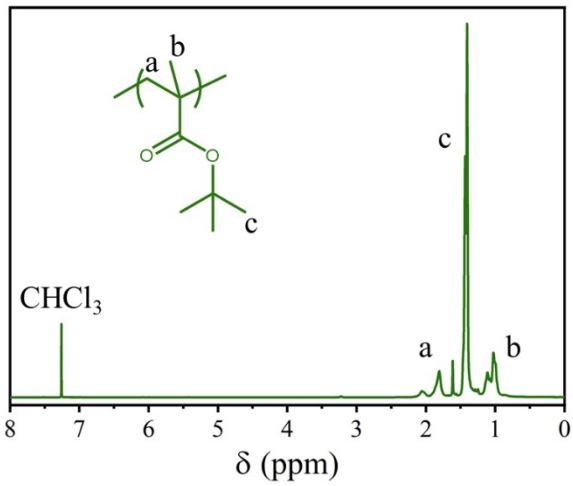


Figure S3-3. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of the negatively charged homopolymer precursor (P*t*BMA$_{74}$).

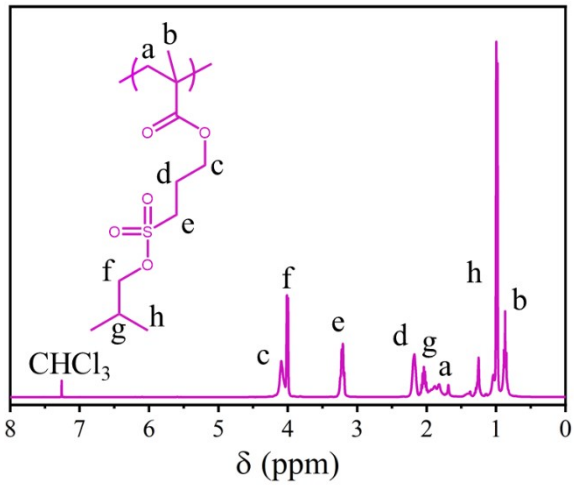


Figure S3-4. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of the negatively charged homopolymer precursor (PBSPMA$_{114}$).

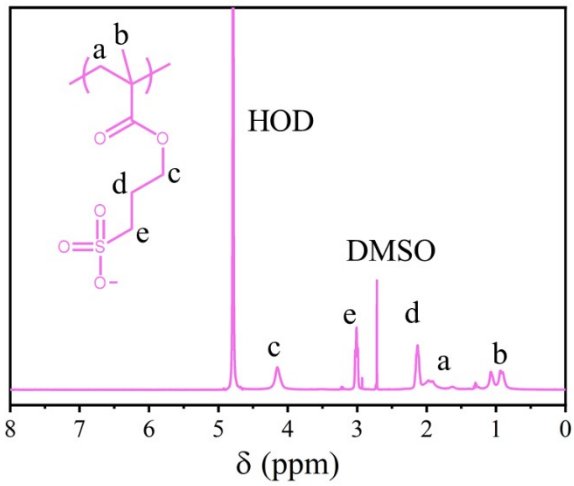


Figure S3-5. ^{1}H NMR spectrum (D_2O, 400 MHz) of the negatively charged homopolymer (PSPMA-Na_{114}).

S4: Nuclear magnetic resonance spectroscopy of deprotected homopolymers

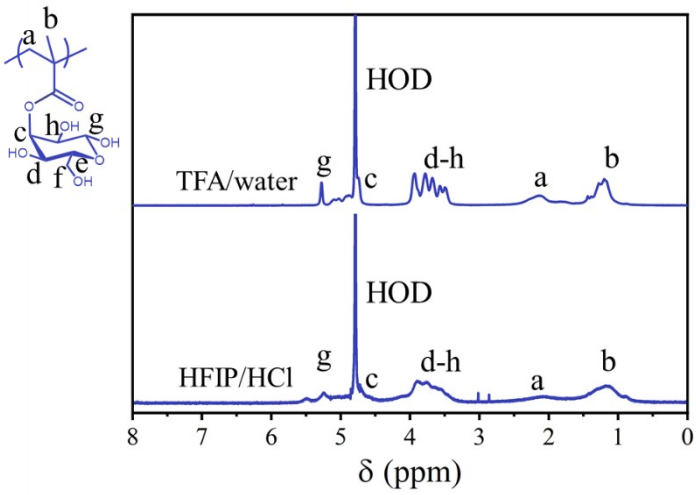


Figure S4-1. ^{1}H NMR spectrum (D_2O, 400 MHz) of $PGlcMA_{72}$ homonpolymers obtained through deprotection of $PPrGlcMA_{72}$ via either the TFA/water or HFIP/HCl route.

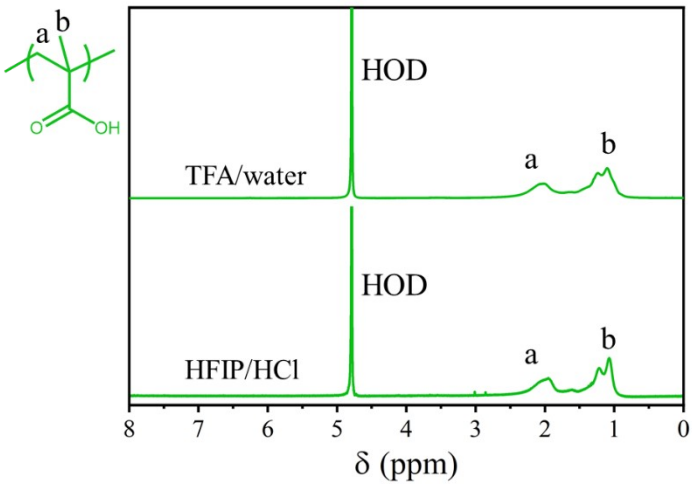


Figure S4-2. ^{1}H NMR spectrum (D_2O, 400 MHz) of $PMAA_{74}$ homonpolymers obtained through deprotection of P*t*BMA_{74} via either the TFA/water or HFIP/HCl route.

S5: Infrared spectroscopy of the homopolymers and block copolymers

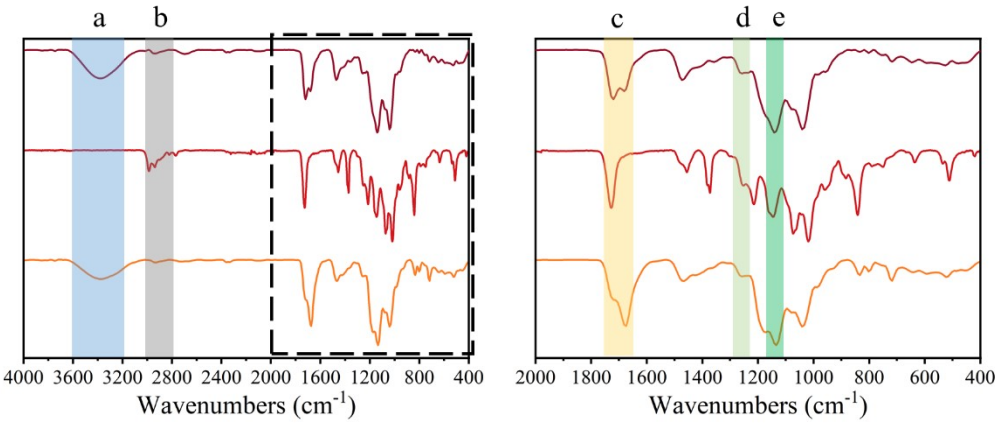


Figure S5-1. Infrared spectra of the positively charged glycosylated block copolymer PGlcMA$_{72}$-*b*-PMETAI$_{71}$ (dark red) and its precursors PPrGlcMA$_{72}$-*b*-PDMAEMA$_{71}$ (red) and PGlcMA$_{72}$-*b*-PDMAEMA$_{71}$ (orange). The assigned signals are (a) -OH stretching of sugar ring (3380 cm^{-1}), (b) -CH$_3$ stretching of backbone, amine and acetonide (2940, 2984 and 2930 cm^{-1}), (c) -C=O stretching of methacrylate (1720, 1728 and 1674 cm^{-1}), and (d) -C-N stretching of amine (1250 cm^{-1}). (e) -C-O stretching of methacrylate (1140 cm^{-1}).

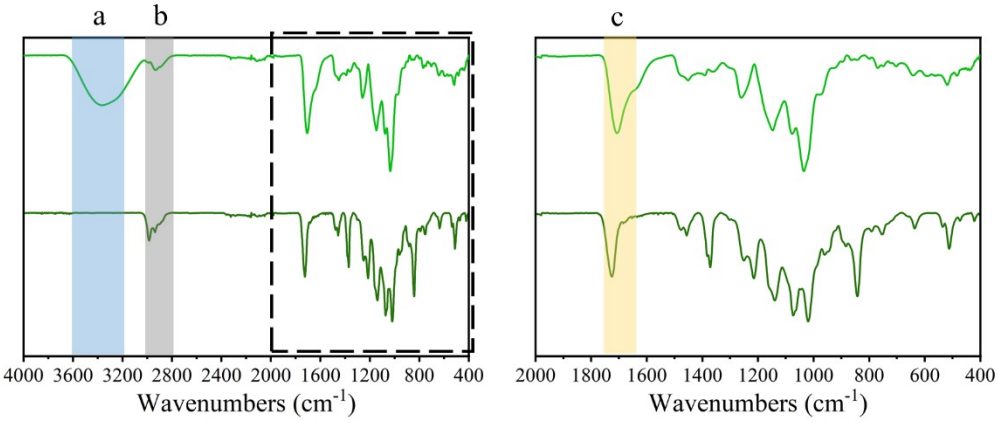


Figure S5-2. Infrared spectra of the negatively charged glycosylated block copolymer PGlcMA$_{72}$-*b*-PMAA$_{85}$ (light green) and its precursor PPrGlcMA$_{72}$-*b*-P*t*BMA$_{85}$ (dark green). The assigned signals are (a) -OH stretching of sugar ring and methacrylic acid (3376 cm^{-1}), (b) -CH$_3$ stretching of backbone and *tert*-butyl (2940 and 2980 cm^{-1}), and (c) -C=O stretching of methacrylate (1706 and 1726 cm^{-1}).

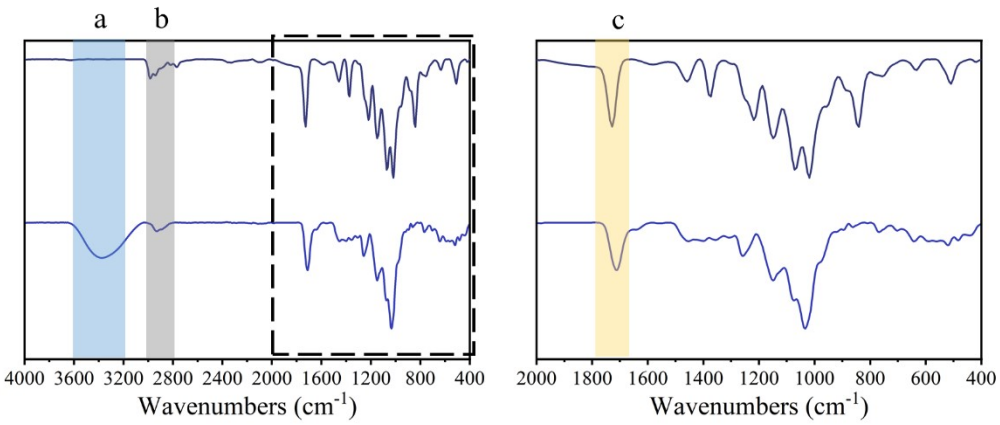


Figure S5-3. Infrared spectra of the acetonide-protected glycosylated homopolymer $PPrGlcMA_{72}$ (dark blue) and the deprotected glycosylated homopolymer $PGlcMA_{72}$ (blue). The assigned signals are (a) -OH stretching of sugar ring (3375 cm^{-1}), (b) $-CH_3$ stretching of backbone and acetonide (2980 and 2935 cm^{-1}), and (c) -C=O stretching of methacrylate (1728 and 1712 cm^{-1}).

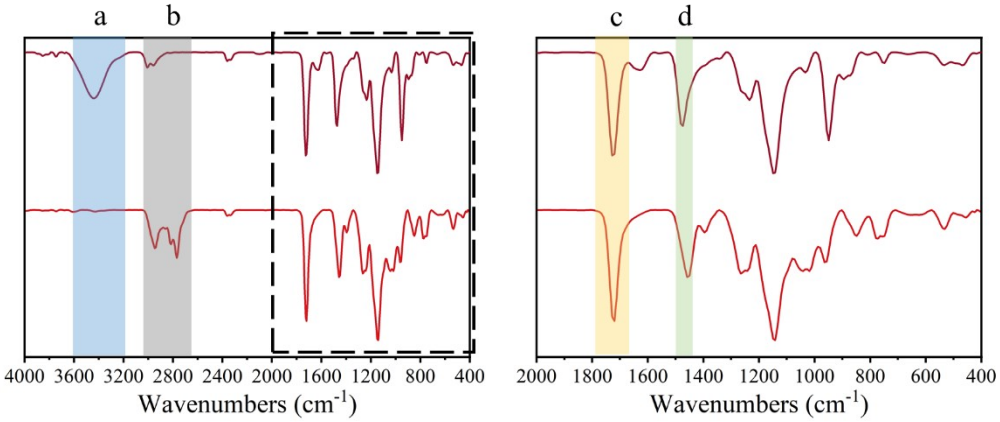


Figure S5-4. Infrared spectra of the positively charged homopolymer $PMETAI_{41}$ (dark red) and its precursor $PDMAEMA_{41}$ (red). The assigned signals are (a) -OH stretching (3440 cm^{-1}), (b) $-CH_3$ stretching of backbone and amine (3016, 2946 and 2770 cm^{-1}), (c) -C=O stretching of methacrylate (1728 cm^{-1}), (d) $-CH_3$ bending of amine (1455 cm^{-1}).

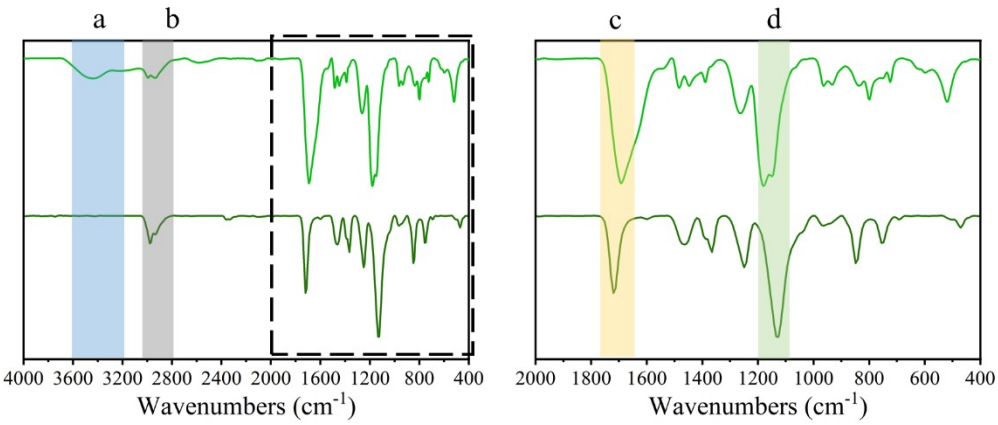


Figure S5-5. Infrared spectra of the negatively charged homopolymer $PMAA_{74}$ (light green) and its precursor P*t*BMA_{74} (dark green). The assigned signals are (a) -OH stretching of methacrylic acid (3440 cm^{-1}), (b) -CH_3 stretching of backbone and *tert*-butyl (2990 cm^{-1}), (c) -C=O stretching of methacrylate (1693 and 1720 cm^{-1}), and (d) -C-O stretching of ester (1184 and 1126 cm^{-1}).

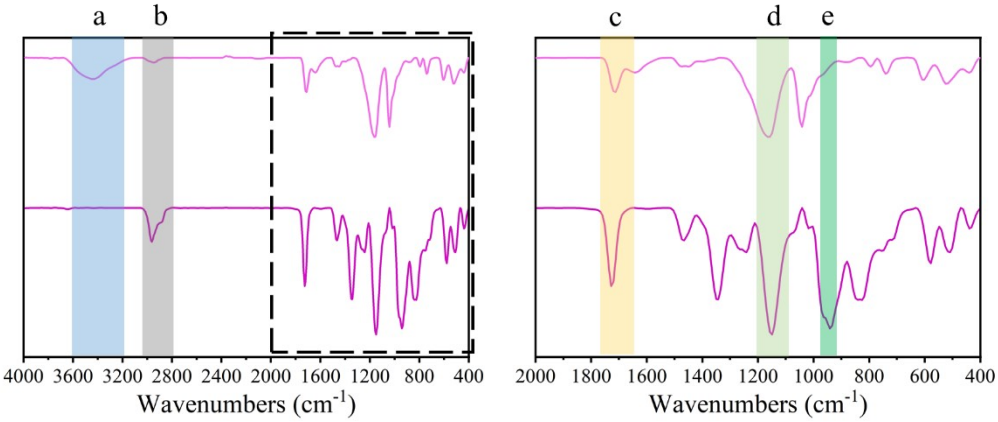


Figure S5-6. Infrared spectra of the negatively charged homopolymer PSPMA-Na_{114} (pink) and its precursor $PBSPMA_{114}$ (violet). The assigned signals are (a) -OH stretching (3330 cm^{-1}), (b) -CH_3 stretching of backbone and isobutyl (2954 and 2962 cm^{-1}), (c) -C=O stretching of methacrylate (1712 and 1728 cm^{-1}), (d) -C-O stretching of ester (1157 and 1149 cm^{-1}), and (e) S-O-R of sulfonic ester (941 cm^{-1}).

S6: Thermal analyses of block copolymers and homopolymers

Table S1: Degradation and glass transition temperatures of various block copolymers.

	$T_{deg\ 1}$ [a] (°C)	$W_{deg\ 1}$ [a] (%)	$T_{deg\ 2}$ [a] (°C)	W_{deg2} [a] (%)	T_g [b] (°C)
PPrGlcMA$_{72}$-*b*-PDMAEMA$_{71}$	263	8.1	325	29.2	5 + 119
PGlcMA$_{72}$-*b*-PDMAEMA$_{71}$	177+220	45.4	417	37.9	62
PGlcMA$_{72}$-*b*-PMETAI$_{71}$	179+211	36.1	257	13.8	45
PPrGlcMA$_{72}$-*b*-P*t*BMA$_{85}$	193	34.9	244	9.7	83 + 153
PGlcMA$_{72}$-*b*-PMAA$_{85}$	205	26.8	406	51.7	38

[a] determined by TGA at a rate of 10 °C min^{-1} and the temperature corresponding to the maximal rate at T_{deg} (*i.e.,* decomposition temperature), [b] determined by DSC on the second heating ramp at a rate of 10 °C min^{-1}.

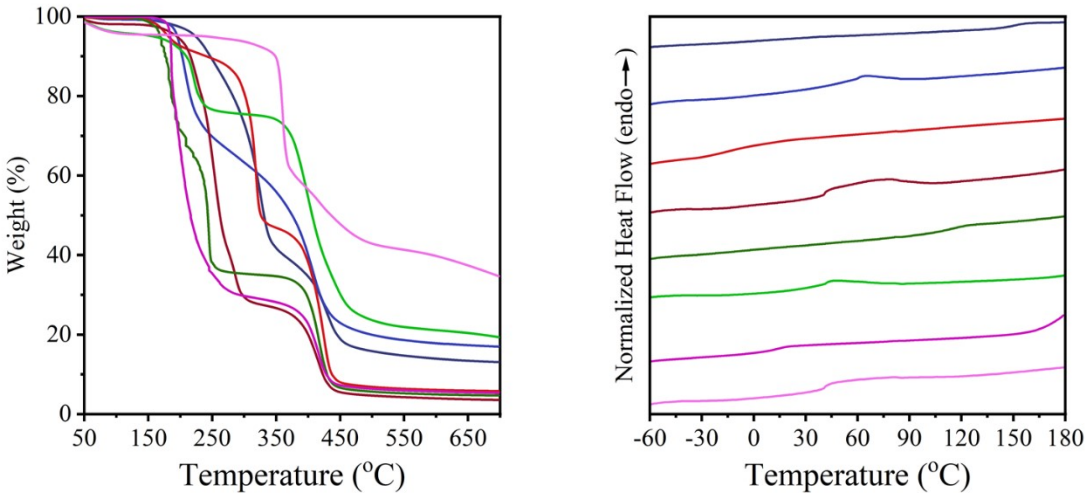


Figure S6. Thermogravimetric (left) and differential scanning calorimetry (right) analyses of various homopolymers: PPrGlcMA$_{72}$ (dark blue), PGlcMA$_{72}$ (blue), PDMAEMA$_{41}$ (red), PMETAI$_{41}$ (dark red), P*t*BMA$_{74}$ (dark green), PMAA$_{74}$ (light green), PBSPMA$_{114}$ (violet) and PSPMA-Na$_{114}$ (pink).

Table S2: Degradation and glass transition temperatures of various homopolymers.

	$T_{deg\ 1}$ [a] (°C)	$W_{deg\ 1}$ [a] (%)	$T_{deg\ 2}$ [a] (°C)	W_{deg2} [a] (%)	T_g [b] (°C)
$PPrGlcMA_{72}$	324	60.8	429	26.0	150
$PGlcMA_{72}$	207	33.1	406	45.7	61
$PDMAEMA_{41}$	176	8.4	316	43.1	6
$PMETAI_{41}$	252	58.0	285	12.8	48
P*t*BMA_{74}	185	35.3	242	29.6	124
$PMAA_{74}$	221	20.2	399	56.1	45
$PBSPMA_{114}$	191	60.1	235	5.6	15
$PSPMA\text{-}Na_{114}$	360	36.9	420	16.1	42

[a] determined by TGA at a rate of 10 °C min^{-1} and the temperature corresponding to the maximal rate at T_{deg} (*i.e.* decomposition temperature), [b] determined by DSC on the second heating ramp at a rate of 10 °C min^{-1}.

S7: Dynamic light scattering analysis of glycosylated polyplex micelles

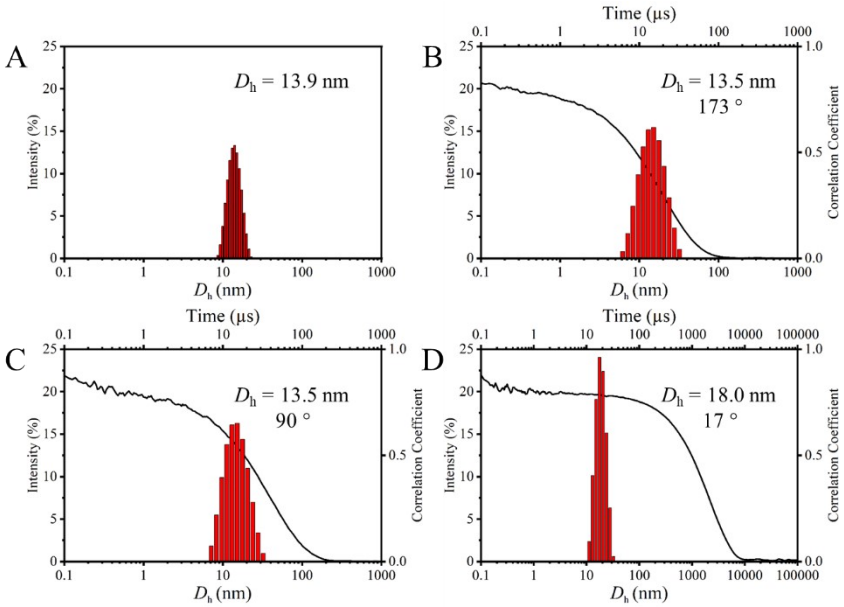


Figure S7-1. (A) Integrated DLS intensity plot (bars) of GPM_1. Individual DLS intensity plot (bars) correlation coefficient (lines) from three scattering angles (B) back angle (173°), (C) side angle (90°) and (D) front angle (17°).

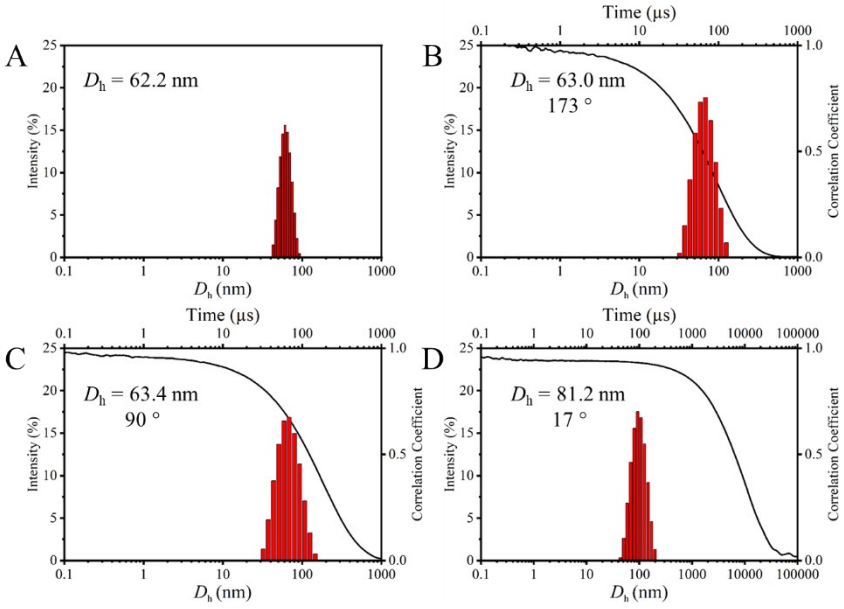


Figure S7-2. (A) Integrated DLS intensity plot (bars) of GPM_2. Individual DLS intensity plot (bars) correlation coefficient (lines) from three scattering angles (B) back angle (173°), (C) side angle (90°) and (D) front angle (17°).

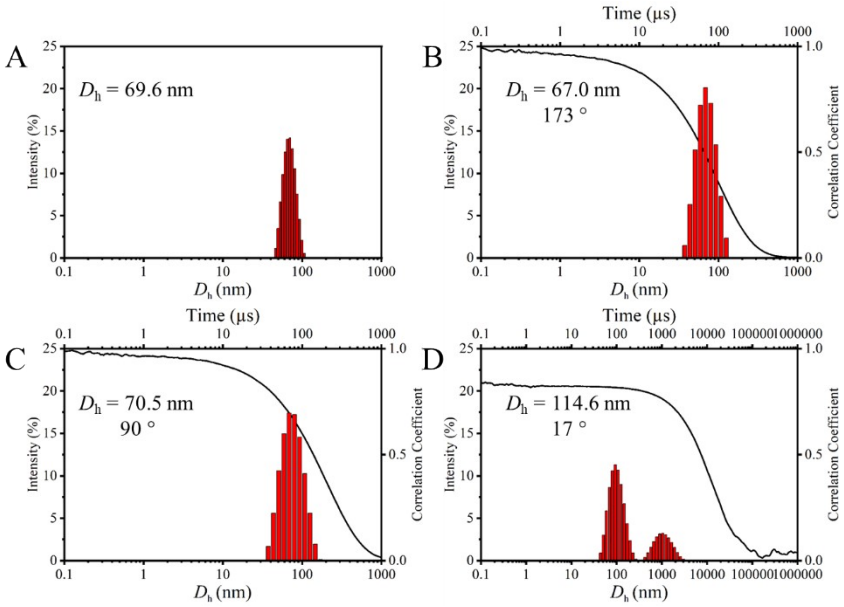


Figure S7-3. (A) Integrated DLS intensity plot (bars) of GPM_3. Individual DLS intensity plot (bars) correlation coefficient (lines) from three scattering angles (B) back angle (173°), (C) side angle (90°) and (D) front angle (17°).

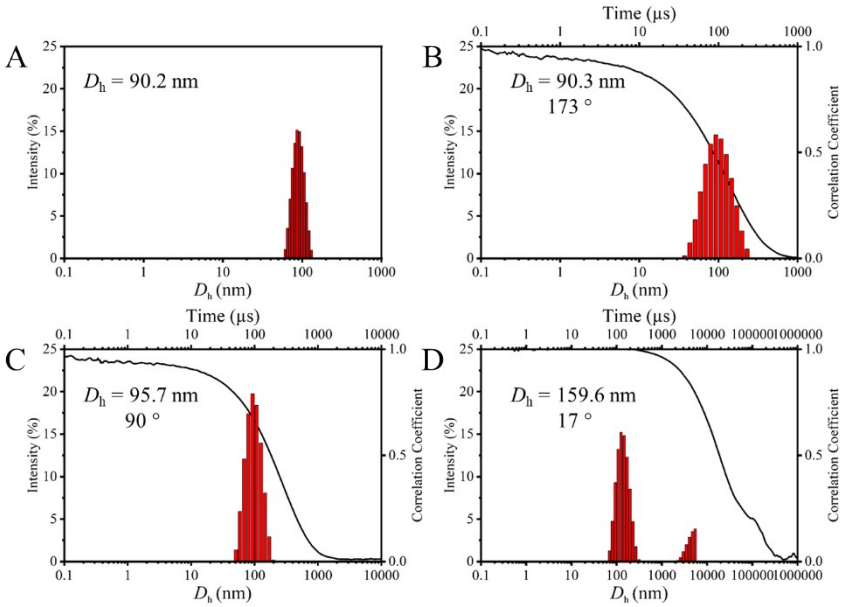


Figure S7-4. (A) Integrated DLS intensity plot (bars) of GPM_4. Individual DLS intensity plot (bars) correlation coefficient (lines) from three scattering angles (B) back angle (173°), (C) side angle (90°) and (D) front angle (17°).

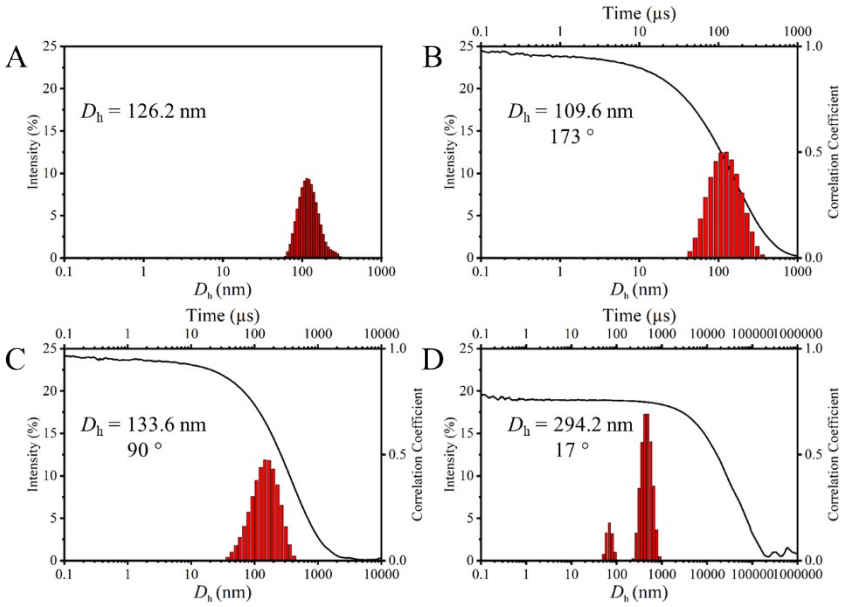


Figure S7-5. (A) Integrated DLS intensity plot (bars) of GPM_5. Individual DLS intensity plot (bars) correlation coefficient (lines) from three scattering angles (B) back angle (173°), (C) side angle (90°) and (D) front angle (17°).

S8: Transmission electron microscopy images of the glycosylated polyplex micelles

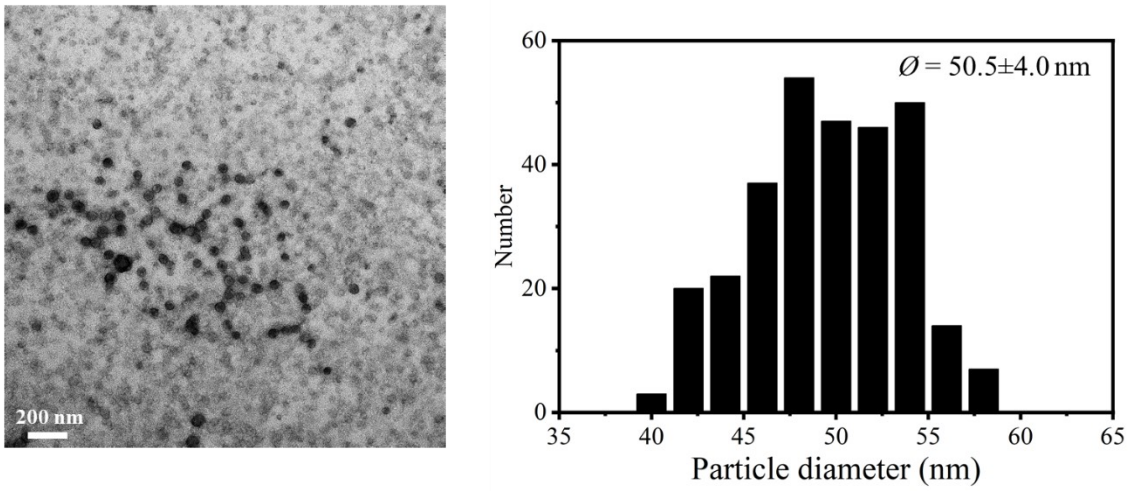


Figure S8-1. TEM images of uranyl acetate-stained glycosylated nanoparticles ($PMETAI_{41}/PGlcMA_{72}$-*b*-$PMAA_{85}$, GPM_2) in a 10 mM KNO_3 solution (left) and statistical analysis of glycosylated nanoparticles (data extracted from 300 particles in several images) (right).

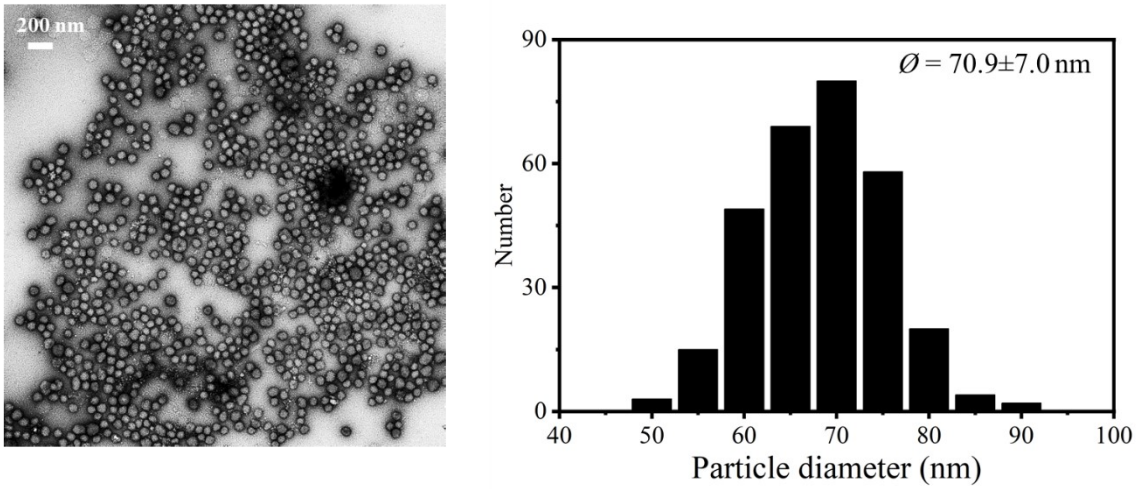


Figure S8-2. TEM images of uranyl acetate-stained glycosylated nanoparticles ($PMAA_{74}/PGlcMA_{72}$-*b*-$PMETAI_{71}$, GPM_3) in a 10 mM KNO_3 solution (left) and statistical analysis of glycosylated nanoparticles (data extracted from 300 particles in several images) (right).

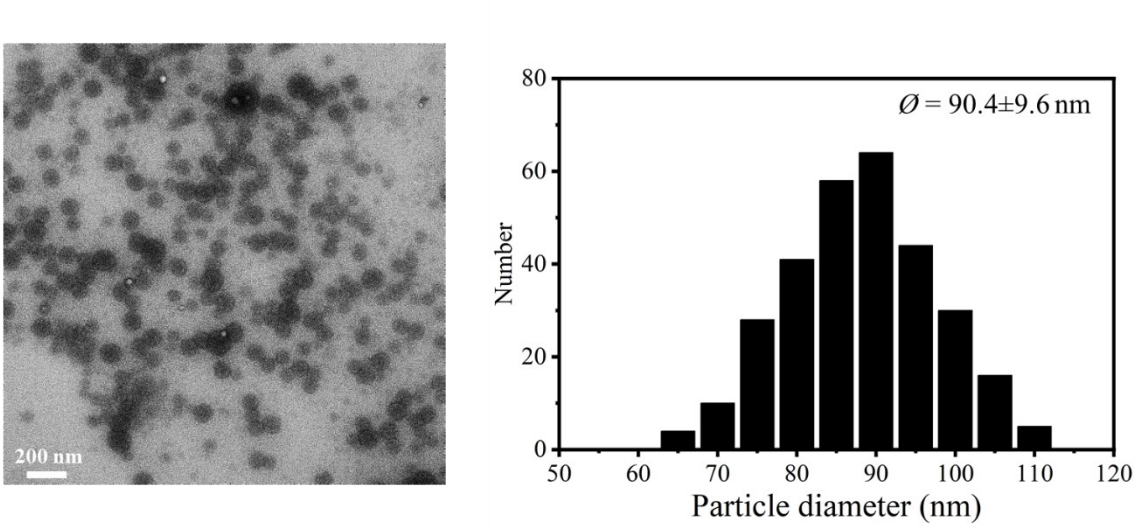


Figure S8-3. TEM images of uranyl acetate-stained glycosylated nanoparticles (PSPMA-Na_{114}/$PGlcMA_{72}$-*b*-$PMETAI_{71}$, GPM_4) in a 10 mM KNO_3 solution (left) and statistical analysis of glycosylated nanoparticles (data extracted from 300 particles in several images) (right).

S9: Charge balance of GPM in PGlcMA$_{72}$-b-PMETAI$_{71}$ /PSPMA-Na$_{114}$ system

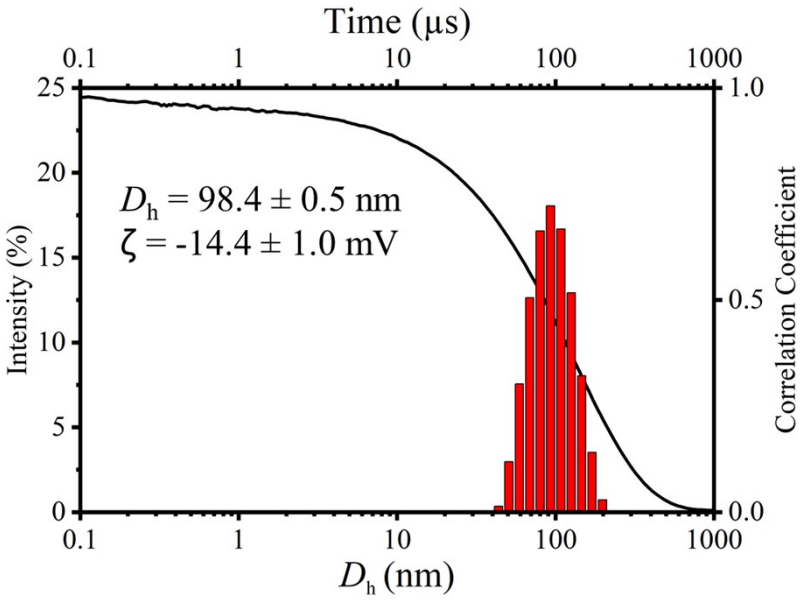


Figure S9-1. DLS intensity plots (bars), correlation coefficient (lines) and zeta potential values of PSPMA-Na$_{114}$/PGlcMA$_{72}$-*b*-PMETAI$_{71}$ (0.75/1).

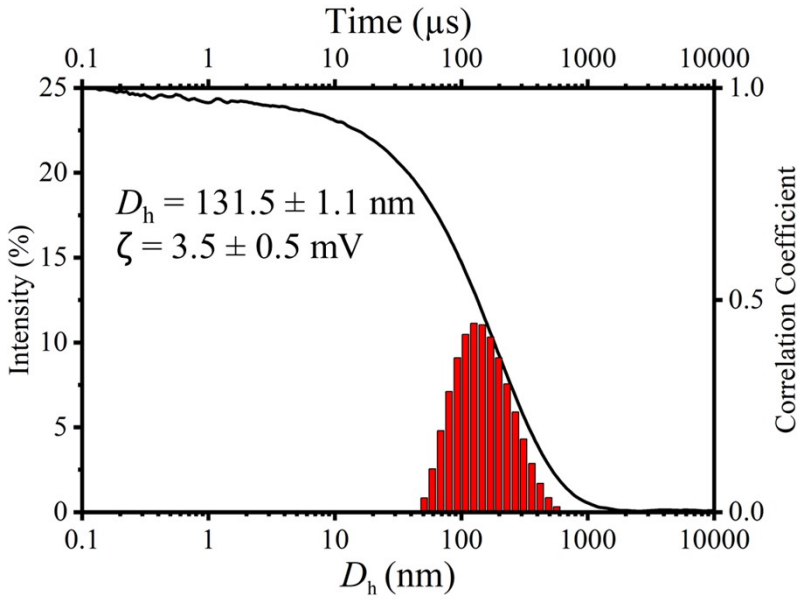


Figure S9-2. DLS intensity plots (bars), correlation coefficient (lines) and zeta potential values of PSPMA-Na$_{114}$/PGlcMA$_{72}$-*b*-PMETAI$_{71}$ (0.4/1).

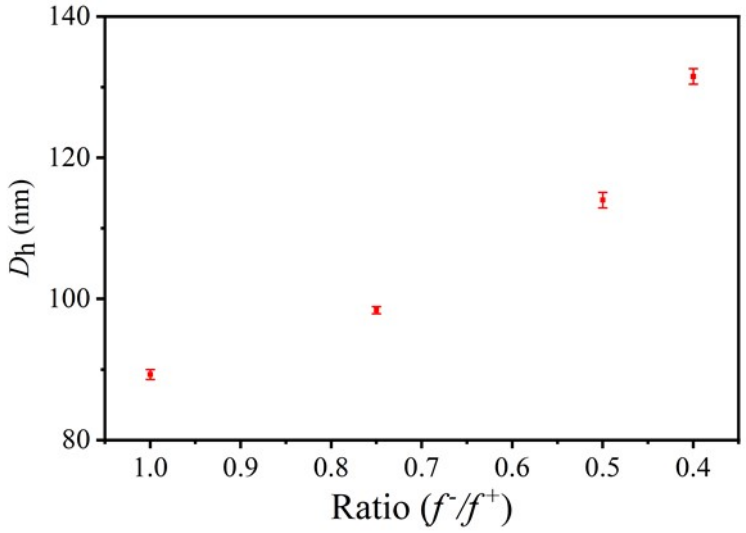


Figure S9-3. Hydrodynamic radii of GPMs in PSPMA-Na_{114}/$PGlcMA_{72}$-*b*-$PMETAI_{71}$ (f^-/f^+) system.

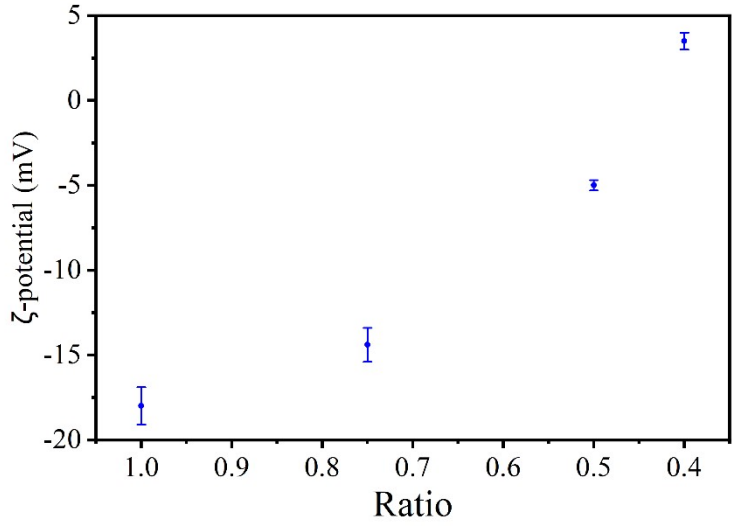


Figure S9-4. ζ-potential values of GPMs in PSPMA-Na_{114}/$PGlcMA_{72}$-*b*-$PMETAI_{71}$ (f^-/f^+) system.

Supporting references

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