# Synthesis and Microcontact Printing of Dual End-Functionalized Mucin-Like Glycopolymers for Microarray Applications

*Kamil Godula, David Rabuka, Ki Tae Nam, and Carolyn R. Bertozzi**

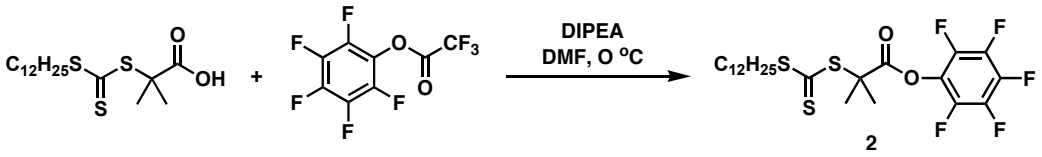
## Supporting Information

**Materials and General Procedures:**

All chemicals, unless stated otherwise, were purchased from Aldrich Chemicals. Texas Red (TR) maleimide conjugate was purchased from Invitrogen. The TR-conjugated lectin, *Helix Pomatia* agglutinin (TR-HPA), and the Fluorescein-conjugated lectin, *Bauhinia Purpurea* agglutinin (FITC-BPA), were purchased from EY Laboratories (San Mateo, CA). Solvents were purified on a Glass Contour solvent purification system. Column chromatography was performed on Biotage SP1 flash chromatography system. Distillations were performed on Kugelrohr short-path distillation apparatus (AC input 115 V) purchased from Aldrich. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Biospin Advance II 500 MHz High Performance NMR spectrometer with multinuclear CP-MAS probe. Spectra were recorded in $CDCl_3$ or $D_2O$ solutions at 293K and referenced to residual solvent peaks. Infra Red spectra were collected on Varian 3100 FT-IR spectrometer. ATR analyses were performed using Pike Technologies MIRacle$^{TM}$ single refraction ATR accessory (ZnSe crystal). UV-VIS spectra were collected on a Perkin Elmer Lambda 35 UV/VIS spectrometer. Size exclusion

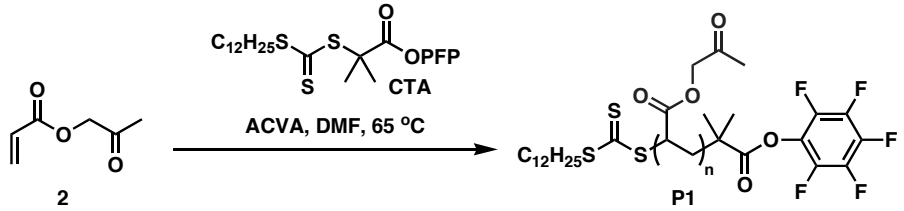
chromatography (SEC) was performed on Viscotek TDA 302 SEC system with triple detector array. For measurements in THF or DMF (0.2% LiBr), the instrument was equipped with 2 in-series Mixed Bed GMHHR-M columns, separation range 100-4M (30 cm x 7.8 mm i.d.) at 35 °C. SEC analyses of polymers **P3** and **P4** were performed on a Neutral PWXL GPC/SEC Column in a 0.1M PBS buffer (0.15M NaCl, pH= 7.2) mobile phase at 35 °C. 4" N/P <1-0-0> 10-20 OHM-CN, 525±25 μm PRIME 5000Å ± 5% thermal oxide silicon wafers were purchased from Silicon Quest International, Inc. Surface analyses were performed on PHI 5400 X-ray Photoelectron Spectroscopy (XPS) system with conventional (non-monochromatic) Al/Mg Dual-Anode X-ray source and Theromo Scientific Nicolet 6700 FT-IR spectrometer. Contact angle measurements were obtained on Krüss GmbH FM41 Easy Drop system at room temperature and ambient relative humidity using 18MΩ water according to the sessile drop method. Prof. Pil J. Yoo of Sungkyunkwan University, Korea, graciously provided PDMS stamps for micro-contact printing. Fluorescent images of wafers patterned with fluorescent polymer **P3a** were acquired on an Olympus CKX41 fluorescence microscope with QICAM Fast 1394 mono 12-bit camera. Fluorescence images of lectin stained micropatterns were acquired on a Zeiss Axiovert 200M inverted microscope (Carl Zeiss MicoImaging Inc., Thornwood NY) equipped with a 63 x 1.4 NA PlanApochromat oil immersion lens. Images were acquired using a CoolSNAP HQ CCD camera (Photometrics, Tucson, AZ). TEM analysis was performed on Jeol 2100F Transmission Electron Microscope operated at 200 kV.

## Part I: Glycopolymer synthesis.




***S*-Dodecyl-*S'*-(α,α-dimethylpentafluorophenyl acetate)trithiocarbonate (2).** A flame-dried Schlenk flask (25 mL) equipped with a magnetic stirring bar was charged with *S*-dodecyl-*S'*-(α,α-dimethylacetic acid)trithiocarbonate[1] (1.00 g, 2.74 mmol) under a stream of nitrogen. DMF (15 mL) was added. The flask was equipped with a rubber septum and the yellow solution was cooled to 0 °C. Diisopropylethylamine (955 μL, 5.48 mmol, 2.0 equiv.) was added followed by pentafluorophenyl trifluoroacetate (567 μL, 3.29 mmol, 1.2 equiv.). The reaction was stirred at 0 °C. After 1 hr, the acid ($R_f$ = 0.16) was consumed, as evidenced by TLC (silica, 20% ethyl acetate/hexanes). The reaction proceeded cleanly to give only one product ($R_f$ = 0.75). The reaction mixture was partitioned between ether and aqueous HCl (1N). The organic layer was washed (2x water, 1x brine), dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography (ethyl acetate/hexane, 5% → 40%) to give **2** as a yellow oil (1.39 g, 95%). *Note:* The chromatography purification must be performed swiftly, since the product is prone to hydrolysis on silica. $^1$H NMR ($CDCl_3$, 500 MHz) δ (ppm): 3.31 (t, *J* = 7.4 Hz, 2H), 1.86 (s, 6H), 1.68 (quin., *J* = 7.5 Hz, 2H), 1.39 (m, 2H), 1.33-1.20 (m, 16H), 0.88 (t, *J* = 7.0 Hz, 3H).$^{13}$C NMR ($CDCl_3$, 125 MHz) δ (ppm): 219.9, 169.6, 142.2,

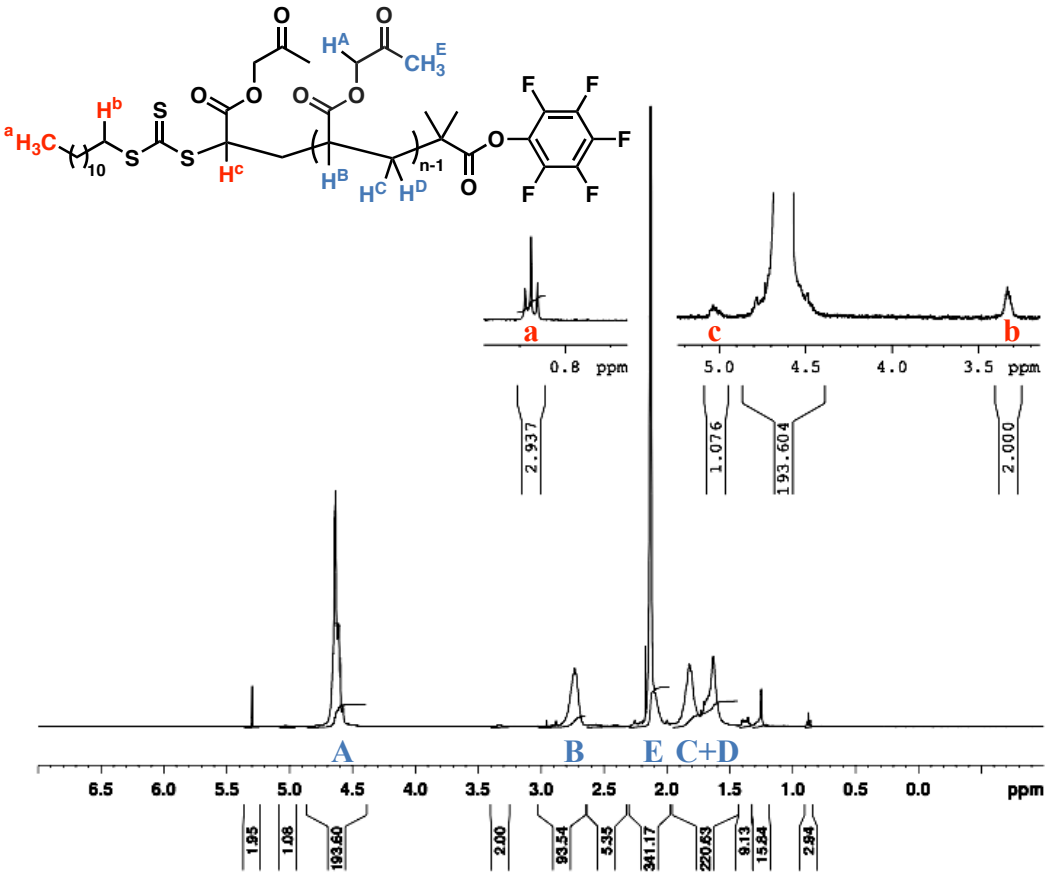
140.6, 140.2, 138.9, 138.5, 136.9, 125.2, 55.4, 37.2, 31.8, 29.6, 29.5, 29.4, 29.3, 29.1, 28.9, 27.8, 25.4, 22.7, 14.1. $^{19}$F NMR ($CDCl_3$, 470 MHz) δ (ppm): -151.5 (m, 2F), -157.7 (m, 1F), -162.3 (m, 2F). FT-IR (NaCl, $CH_2Cl_2$) ν ($cm^{-1}$): 2928, 1780, 1521, 1086, 995, 819. UV-VIS ($CHCl_3$) $\lambda_{max}$ (nm): 306. LRMS ($FAB^+$) *m/z*: calcd: 347 $(M\text{-}OC_6F_5)^+$, found: 347.



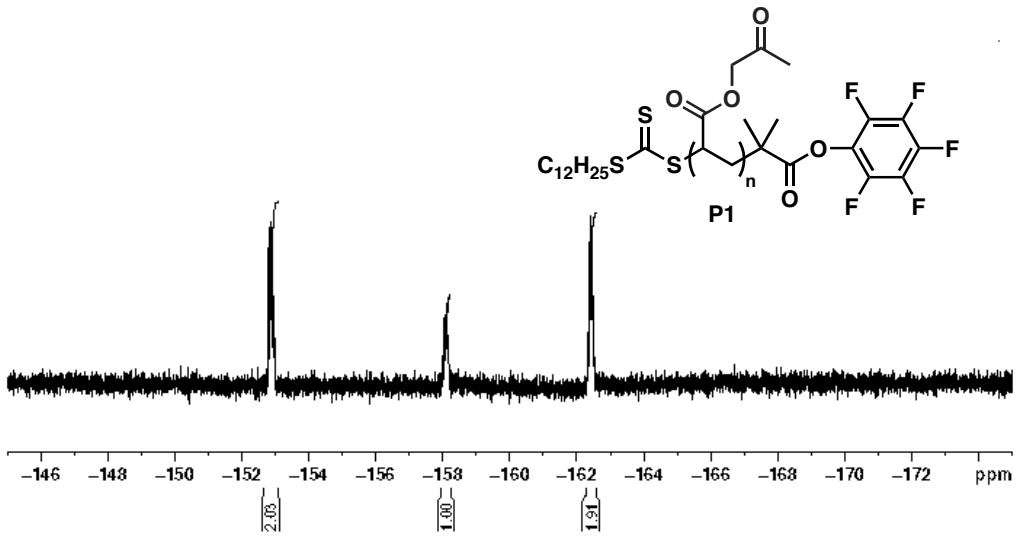

**A typical procedure for RAFT polymerization of (2-oxopropyl)acrylate[2] monomer (1) in the presence of chain transfer agent (2).** A flame-dried Schlenk flask (10 mL) equipped with a magnetic stirring bar was charged with **2** (27.5 mg, 0.05 mmol, 0.82 mol%), ACVA (4,4'-azobis(4-cyanovaleric acid), 5.8 mg, 0.02 mmol, 0.33 mol%), monomer **1** (800.7 mg, 6.25 mmol, filtered through basic alumina) and anhydrous DMF (800 mg). The flask was equipped with a rubber septum and attached to a Schlenk line. The yellow solution was thoroughly degassed by three freeze-pump-thaw cycles. After the final cycle, the flask was allowed to warm to room temperature and then immersed into an oil bath preheated to 65 °C. After 2 hrs, the mixture became viscose. A small sample was analyzed by $^1$H NMR spectroscopy. The monomer conversion was estimated at 90%. The reaction mixture was diluted with $CH_2Cl_2$ and precipitated into hexanes. The residue was re-dissolved in a minimal quantity of $CH_2Cl_2$ and precipitated again into hexanes with vigorous stirring. This was repeated twice more. The yellow polymer was

concentrated from $CH_2Cl_2$ three times to remove residual hexanes and dried under high vacuum overnight to give polymer **P1** as a pale yellow solid (680 mg, 82%). SEC (THF): $M_w$ = 12.21 kDa, $M_n$ = 11.41 kDa, DP = 91, PDI = 1.07. Estimated from $^1$H NMR: $M_w$ = 12.96 kDa, DP = 97. $^1$H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.80-4.60 (bm, 1H), 4.80-4.40 (bm, 194H), 3.37-3.28 (bm, 2H), 2.90-2.50 (bm, 97H), 2.32-1.95 (bm, 297H), 1.90-1.57 (bm, 99), 1.57-1.45 (bm, 97), 1.45-1.18 (bm, 18H), 0.88 (t, $J$ = 6.8 Hz, 3H). $^{19}$F NMR ($CDCl_3$, 470 MHz) δ (ppm): -152.9 (bm, 2F), -158.1 (bm, 1F), -162.4 (bm, 2F). FT-IR (NaCl, $CH_2Cl_2$) ν ($cm^{-1}$): 2939, 1730, 1420, 1371, 1273, 1157, 757. UV-VIS ($CHCl_3$) $\lambda_{max}$ (nm): 306.

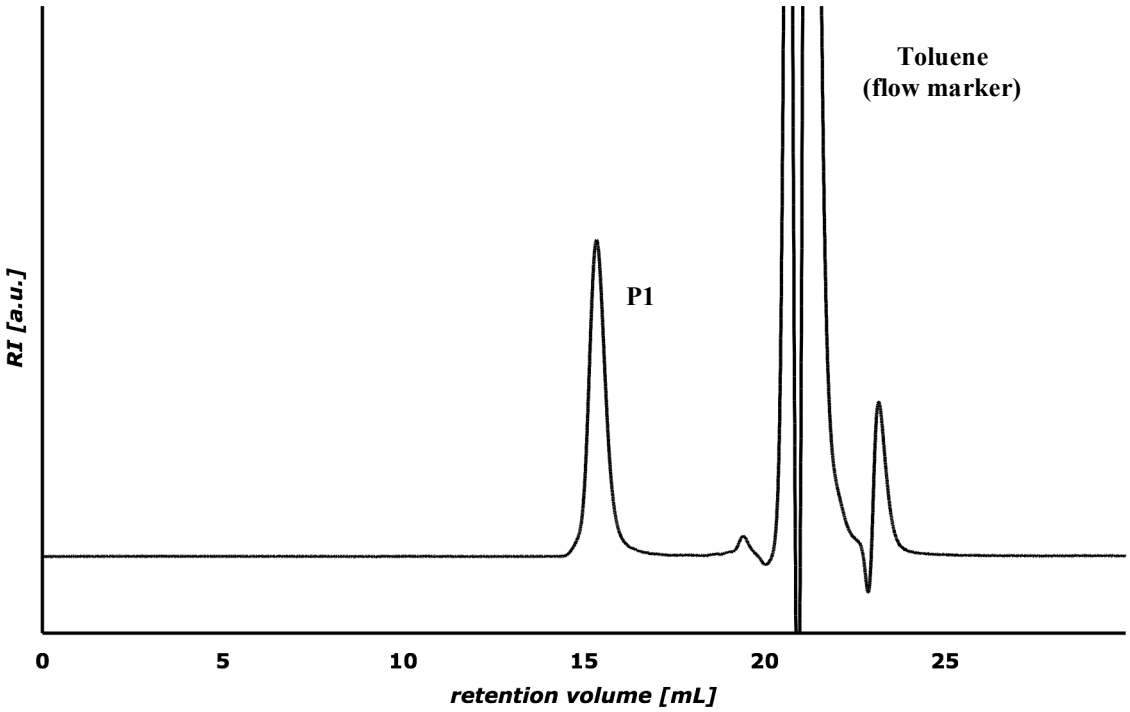
**$^1$H NMR ($CDCl_3$, 500 MHz):**



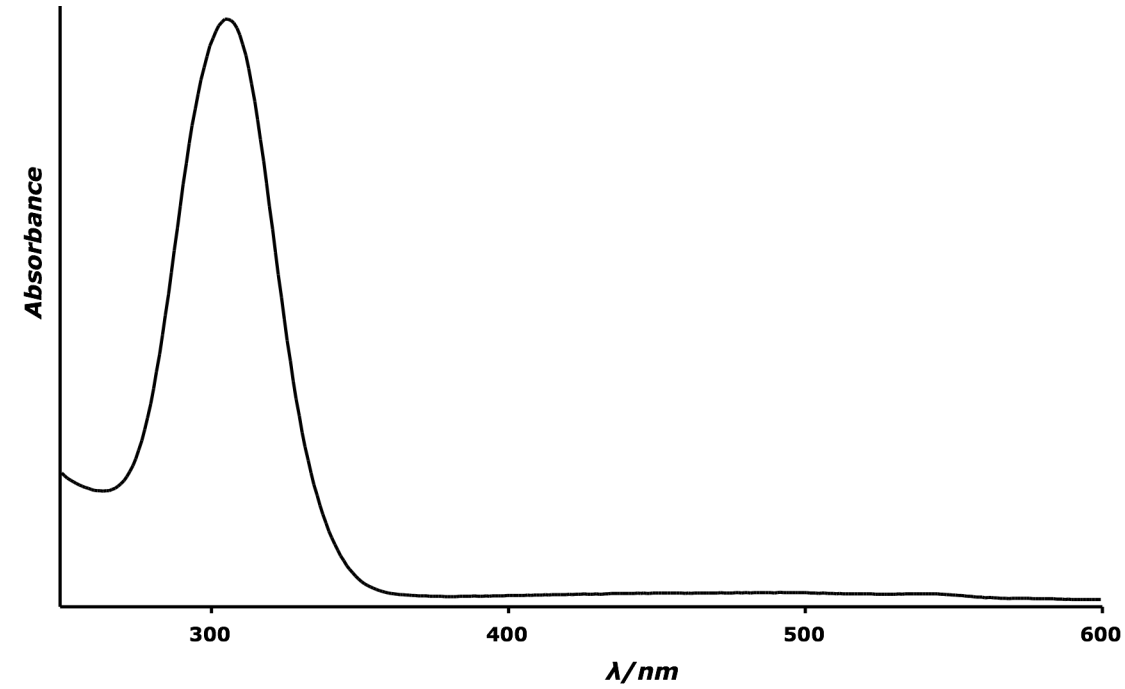
**$^{19}$F NMR ($CDCl_3$, 470 MHz):**

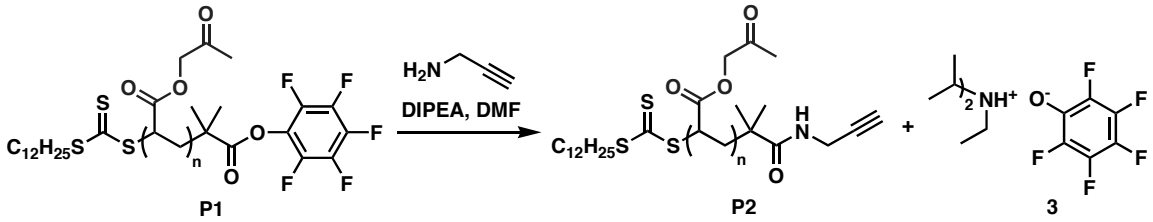



**GPC (THF):**



**UV-VIS :**

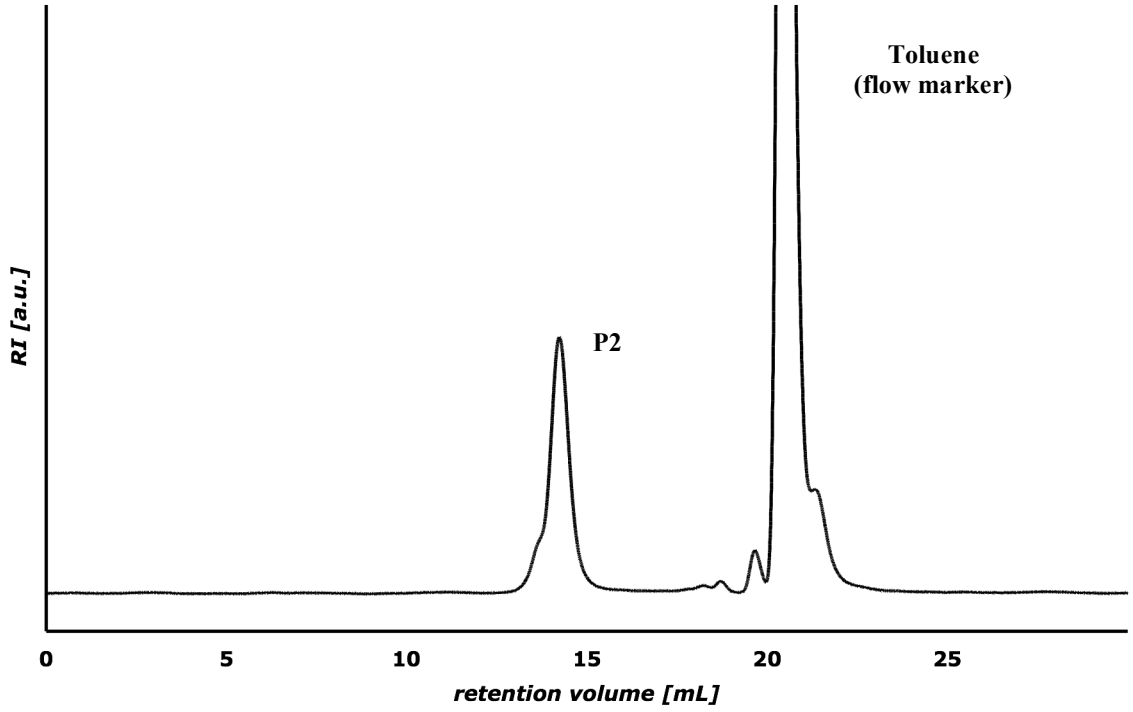


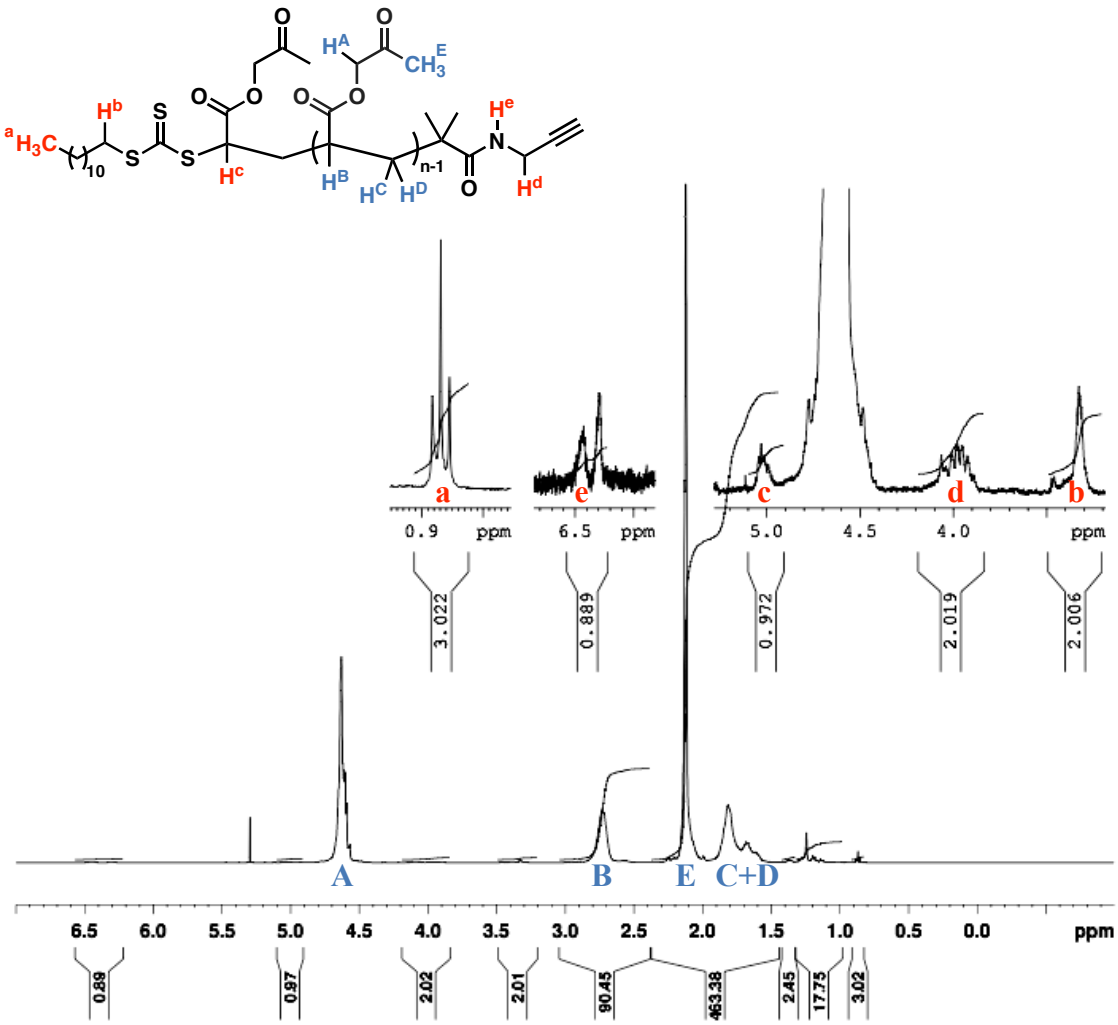

**Synthesis of alkyne terminated polymer P2.** A vial equipped with a magnetic stirring bar was charged with polymer **P1** (20.0 mg, DP = 97, $M_w$ = 12.96 kDa, PDI = 1.07, ~1.5 μmol of pentafluorophenyl ester end-groups) and a stock solution (154 μL) of propargyl amine (50 mM) and diisopropylethylamine (100 mM) in DMF was added giving a polymer solution with end-group concentration of 10 mM. The vial was filled with nitrogen and capped. The solution was stirred at room temperature for 4 hrs. After this time, the solvent was removed under reduced pressure and the residue was analyzed by $^{1}H$ and $^{19}F$ NMR. The crude product was dissolved in $CH_2Cl_2$ and precipitated into

methanol under vigorous stirring. The precipitate was collected, re-dissolved in $CH_2Cl_2$ and precipitated into hexanes under vigorous stirring. This was repeated twice. The final precipitate was concentrated from $CH_2Cl_2$ three times to remove residual hexanes and dried under vacuum to give yellow polymer **P2** (20.0 mg, quantitative). **Crude reaction mixture:** $^{19}F$ NMR ($CDCl_3$, 470 MHz) δ (ppm): -163.0 (m, 2F), -165.2 (m, 2F), -171.4 (bm, 1F). **Isolated product:** SEC (DMF): $M_w$ = 12.42 kDa, $M_n$ = 11.09 kDa, DP = 97, PDI = 1.12. $^1H$ NMR ($CDCl_3$, 500 MHz) δ (ppm): 6.50-6.28 (bm, 1H, NH), 5.60-4.80 (bm, 1H), 4.90-4.40 (bm, 194H), 4.15-3.85 (bm, 2H), 3.50-3.25 (bm, 2H), 3.00-2.40 (bm, 98H), 2.30-1.90 (bm, 291H), 1.90-1.50 (bm, 202H), 1.45-1.10 (bm, 18H), 0.87 (bt, *J* = 6.9 Hz, 3H). FT-IR (NaCl, $CH_2Cl_2$) ν ($cm^{-1}$): 2936, 1730, 1421, 1372, 1274, 1157, 758. UV-VIS ($CHCl_3$) $\lambda_{max}$ (nm): 306.

**GPC (DMF, 0.2% LiBr):**

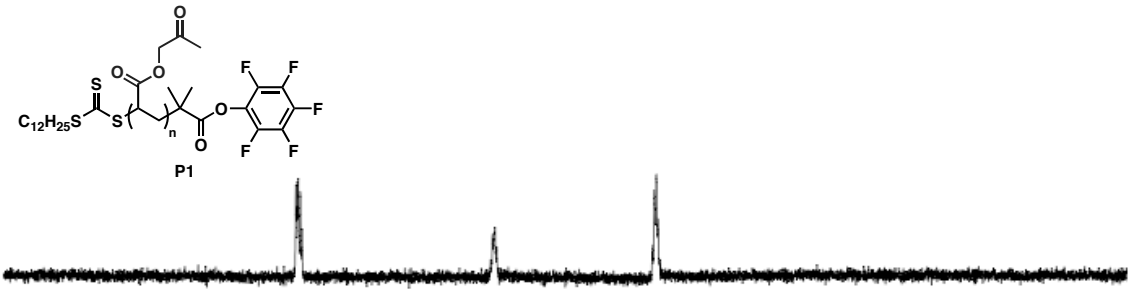


**$^1$H NMR ($CDCl_3$, 500 MHz):**

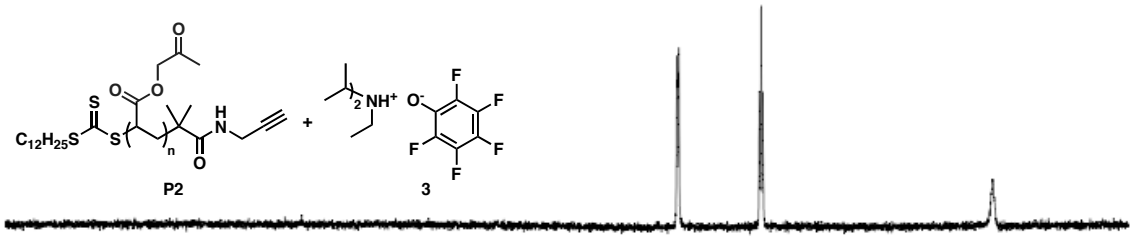


**$^{19}$F NMR ($CDCl_3$, 470 MHz):**

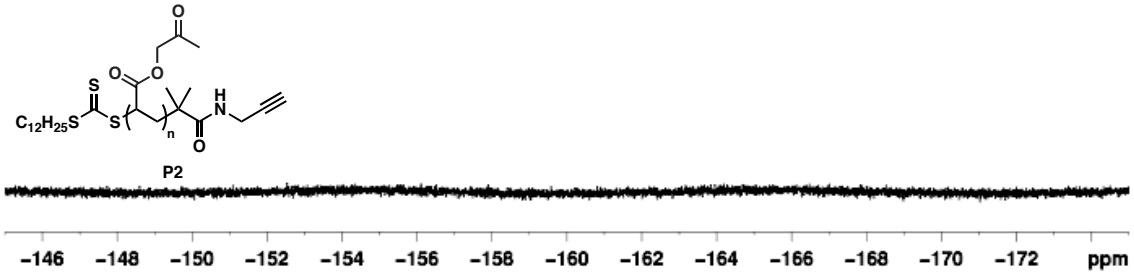
**A) T = 0 hrs**



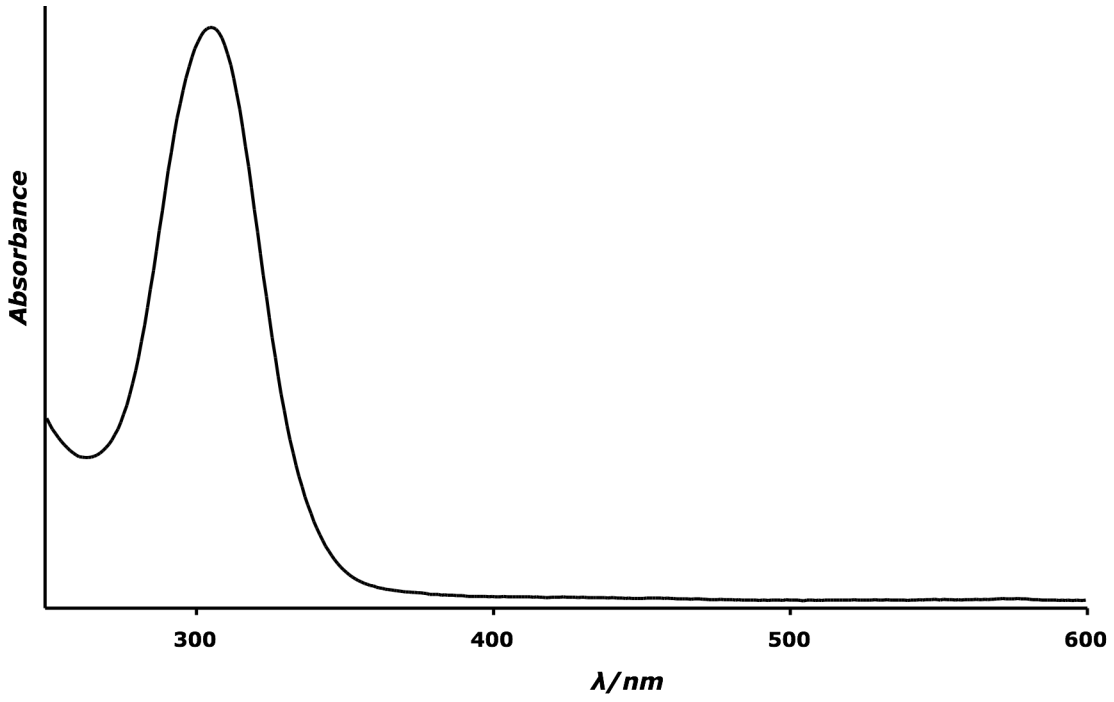

**B) T = 4 hrs**

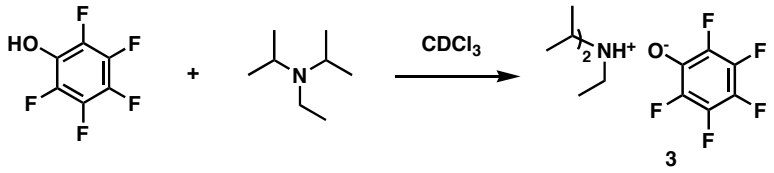



**C) Isolated polymer**



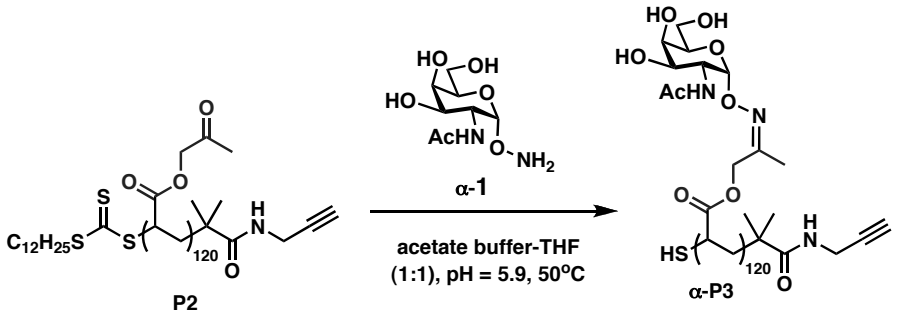
**UV-VIS:**



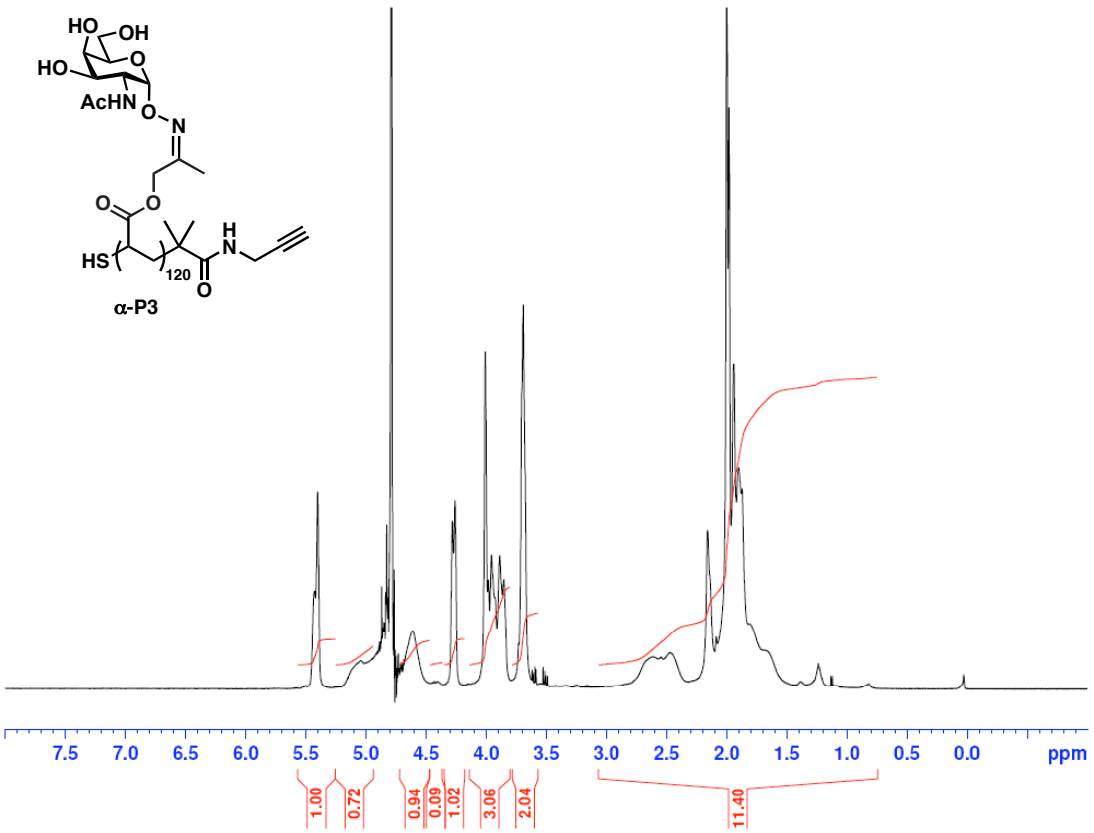

**Preparation of a sample of diisopropylethylammonium pentafluorophenolate (3).** To a solution of pentafluorophenol (39 mg, 0.21 mmol) in $CDCl_3$ (0.75 mL) in an NMR tube was added diisopropylethylamine (37 μL, 0.21 mmol, 1 equiv.). Diisopropylethylammonium pentafluorophenolate formed instantaneously and the salt was characterized by NMR spectroscopy. $^{1}H$ NMR ($CDCl_3$, 500 MHz) δ (ppm): 3.43 (sept. $J$ = 6.7 Hz, 2H), 2.88 (q, $J$ = 7.4 Hz, 2H), 1.33-1.26 (m, 15 H). $^{13}C$ NMR ($CDCl_3$, 125 MHz) δ (ppm): 141.8, 141.4, 140.0, 139.4, 137.4, 52.1, 40.8, 18.2, 12.4. $^{19}F$ NMR

($CDCl_3$, 470 MHz) δ (ppm) [3]: -167.2 (m, 2F), -169.4 (m, 2F), -183.3 (bm, 1F). FT-IR (NaCl, $CH_2Cl_2$) ν ($cm^{-1}$): 2997, 2695 (broad), 1534, 1501, 1463, 1443, 1399, 1356, 1276, 1135, 991, 617.

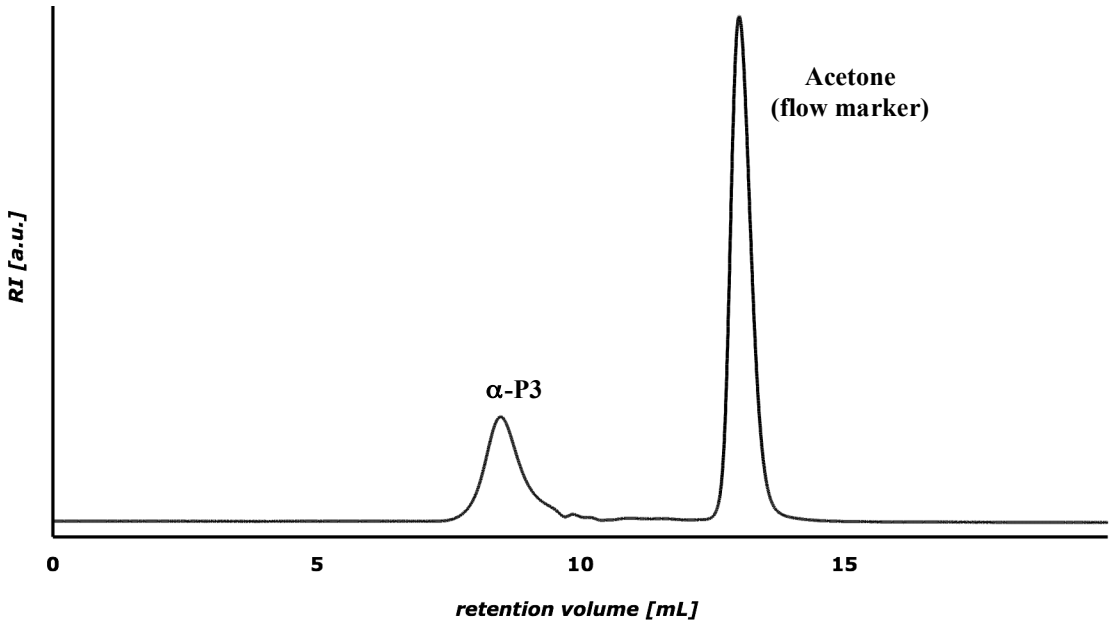


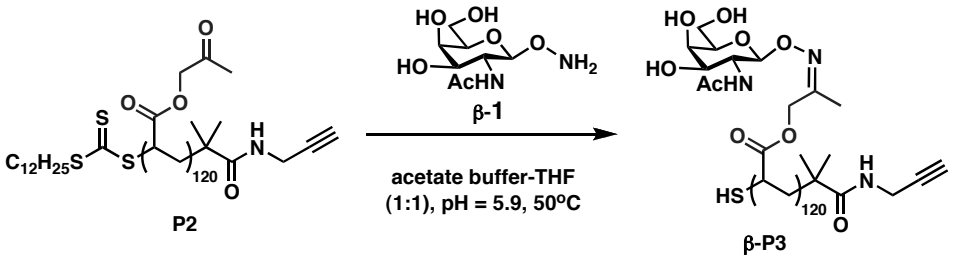

**Synthesis of a non-fluorescent glycopolymer α-P3.** A vial (4 mL) equipped with a magnetic stirring bar charged with alkyne-terminated polymer **P2** (5.0 mg, DP = 120, $M_w$ = 15.76 kDa, PDI = 1.18, 38.1 μmol of keto-groups) and α-aminooxy-GalNAc (**α-1**, 9.9 mg, 41.9 μmol, 1.1 equiv.) was added THF (140 μL). The polymer was allowed to dissolve, and then 50 mM acetate buffer (140 μL, pH = 5.5) was added. The final pH of the reaction mixture was 5.9. The headspace was filled with nitrogen and the vial was capped. The reaction mixture was heated at 50 °C for 18 hrs. After this time, the solvents were removed and the crude product was dialyzed against distilled water for 24 hours with the water being changed periodically. The product was obtained after lyophilization as a fluffy white solid (13.3 mg, 94%). Estimated from $^1$H NMR: 76% GalNAc incorporation, $M_w$ = 35.46 kDa, DP = 120. SEC (0.10 M PBS buffer, 0.15 M NaCl ): PDI = 1.21. $^1$H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.50-5.35 (bm, 1H), 5.15-4.50 (bm, 2H), 4.30-4.20 (bm, 1H), 4.05-3.77 (bm, 3H), 3.64-3.78 (bm, 2H), 2.80-0.80 (bm, 11.40H). ATR (ZnSe) ν ($cm^{-1}$): 3340, 2938, 1731, 1647, 1551, 1435, 1374, 1158, 1115, 1051, 930, 808.

**$^1$H NMR ($CDCl_3$, 500 MHz):**



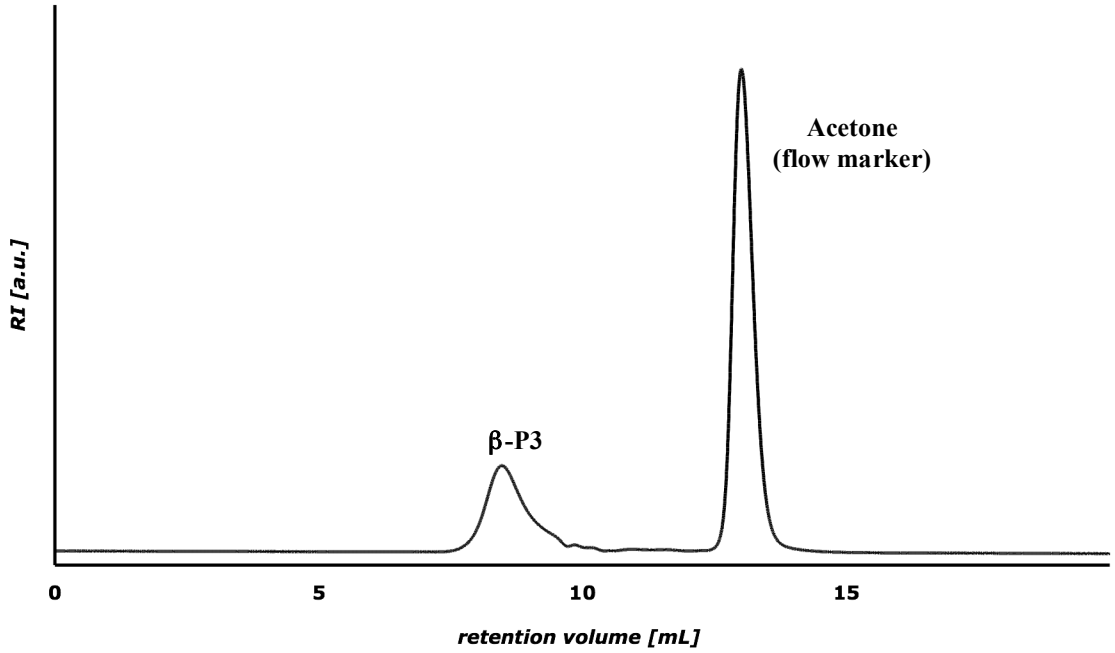

**GPC (0.10 M PBS buffer, 0.15M NaCl, pH=7.2):**



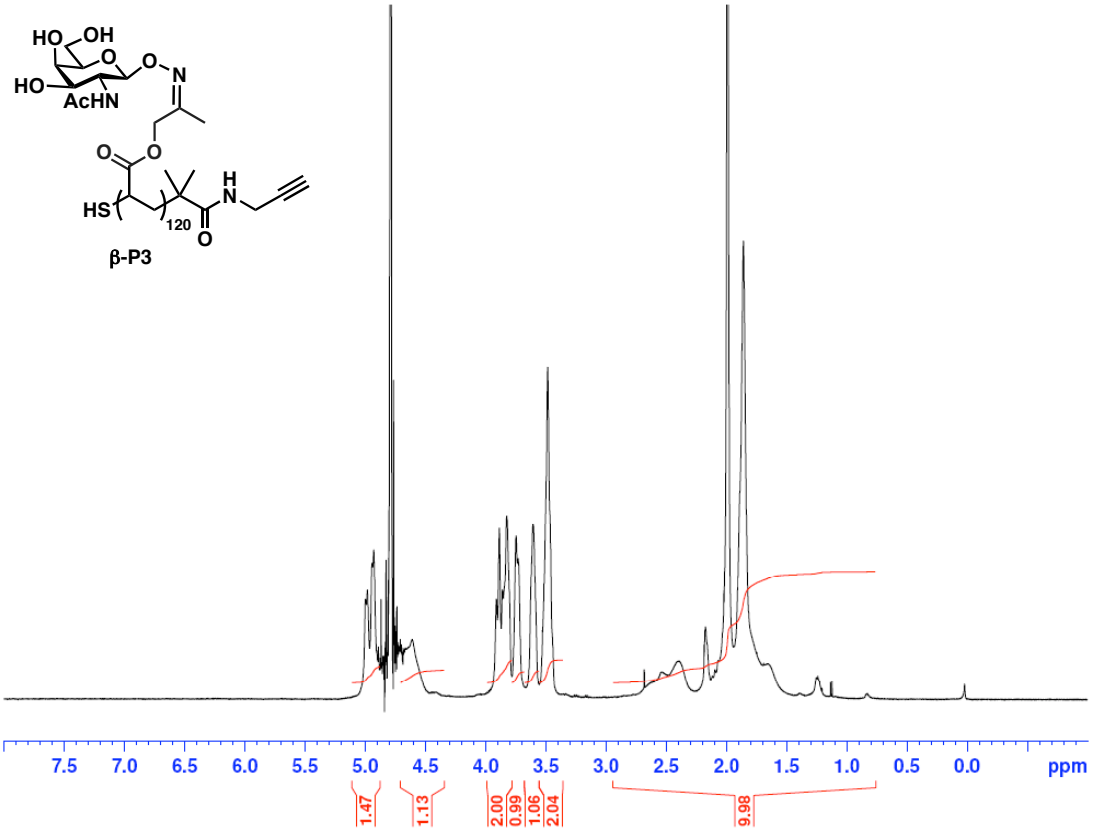


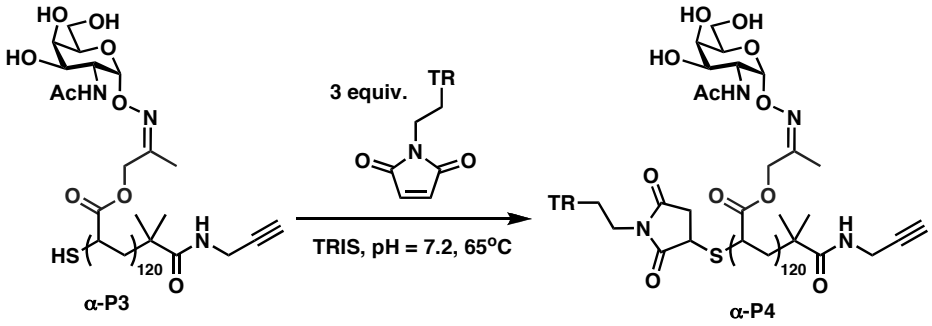

**Synthesis of a non-fluorescent glycopolymer β-P3.** This polymer was prepared in a manner identical to that for the preparation of **α-P3**, except that β-aminooxy-GalNAc (**β-1**, 9.9 mg, 41.9 μmol, 1.1 equiv.) was used, to yield a fluffy white solid (12.6 mg, 100%). Estimated from $^1$H NMR: 86% GalNAc incorporation, $M_w$ = 38.08 kDa, , DP = 120. SEC (0.10 M PBS buffer, 0.15 M NaCl): PDI = 1.18. $^1$H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.05-4.50 (m, 3H), 4.06-3.90 (m, 2H), 3.82-3.68 (m, 4H), 2.80-0.80 (m, 9.98). ATR (ZnSe) ν ($cm^{-1}$): 3297, 2935, 1736, 1646, 1554, 1375, 1156, 1063, 922, 788.

**GPC (0.10 M PBS buffer, 0.15 M NaCl, pH=7.2):**



**$^1$H NMR ($CDCl_3$, 500 MHz):**

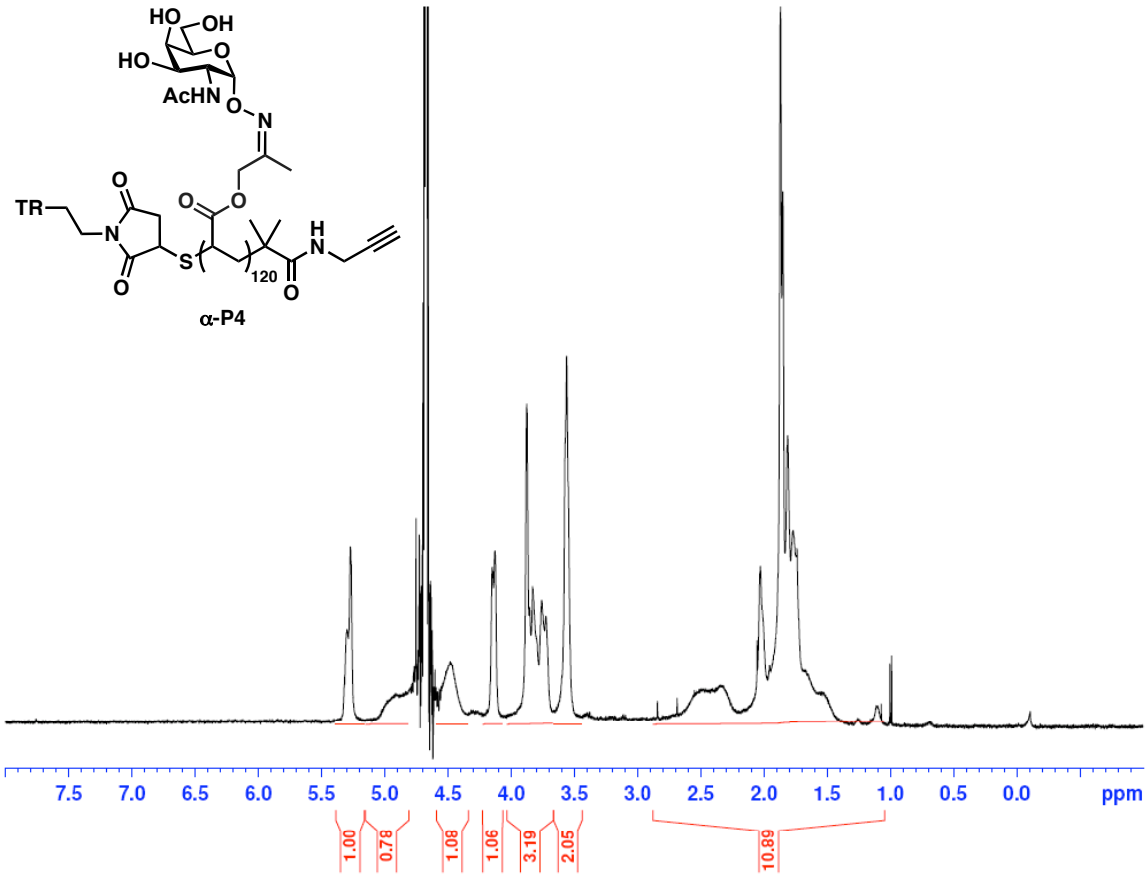


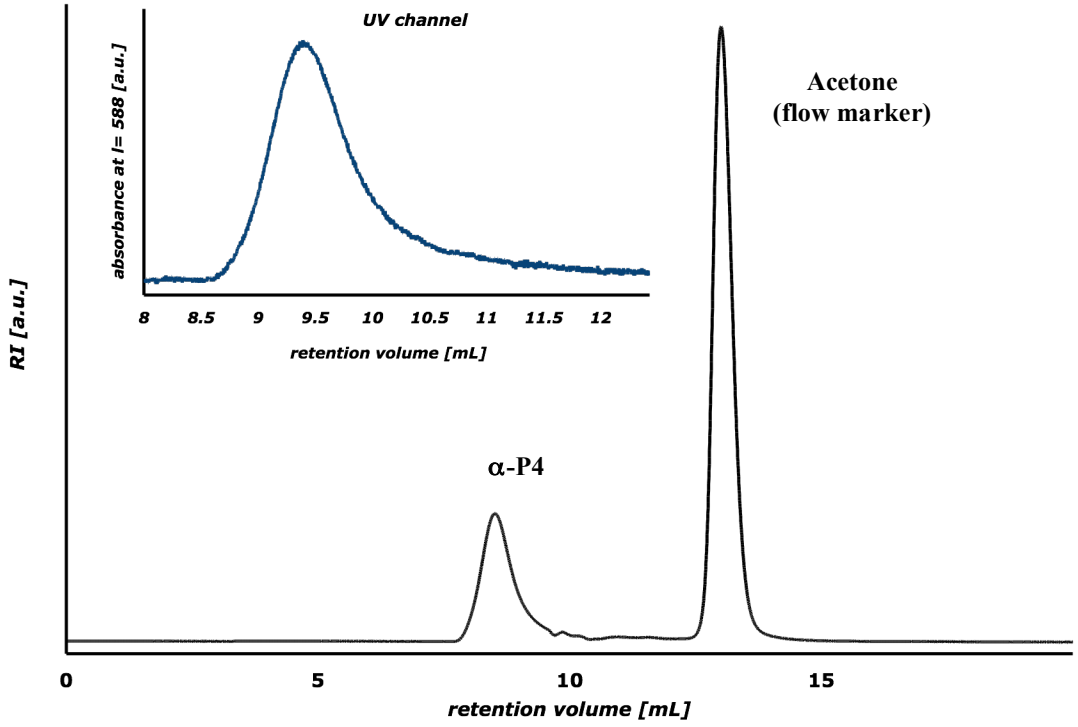

**Synthesis of TR-labeled glycopolymer α-P4.** In a vial (4 mL) equipped with a magnetic stirring bar, polymer **α-P3** (2.02 mg, DP = 120, $M_w$ = 35.46 kDa, PDI = 1.21, 5.7 x $10^{-5}$ mol of thiol groups) was dissolved in 25 mM TRIS buffer, 150 mM NaCl (210 μL). A DMSO solution of Texas Red-maleimide (17.1 μL, c = 10 mM, 3.0 equiv.) was added.

The headspace was filled with nitrogen, the vial was capped and the content was heated at 65 °C for 15 hrs. After this time, the crude reaction mixture was transferred onto a Sephadex G25 column and the purple TR-labeled polymer was eluted with DI water. The pure polymer was obtained after lyophilization as a fluffy purple solid (1.4 mg, 70%). $M_w$ = 35.80 kDa, DP = 120. SEC (0.10 M PBS buffer, 0.15 M NaCl): PDI = 1.22. $^1$H NMR ($CDCl_3$, 500 MHz) δ (ppm): 5.50-5.35 (bm, 1H), 5.15-4.50 (bm, 2H), 4.30-4.20 (bm, 1H), 4.05-3.77 (bm, 3H), 3.64-3.78 (bm, 2H), 2.80-0.80 (bm, 10.89H). ATR (ZnSe) ν ($cm^{-1}$): 3336, 2938, 1727, 1650, 1548, 1429, 1374, 1157, 1114, 1050, 928, 806. UV-VIS ($CHCl_3$) $\lambda_{max}$ (nm): 600, 556.

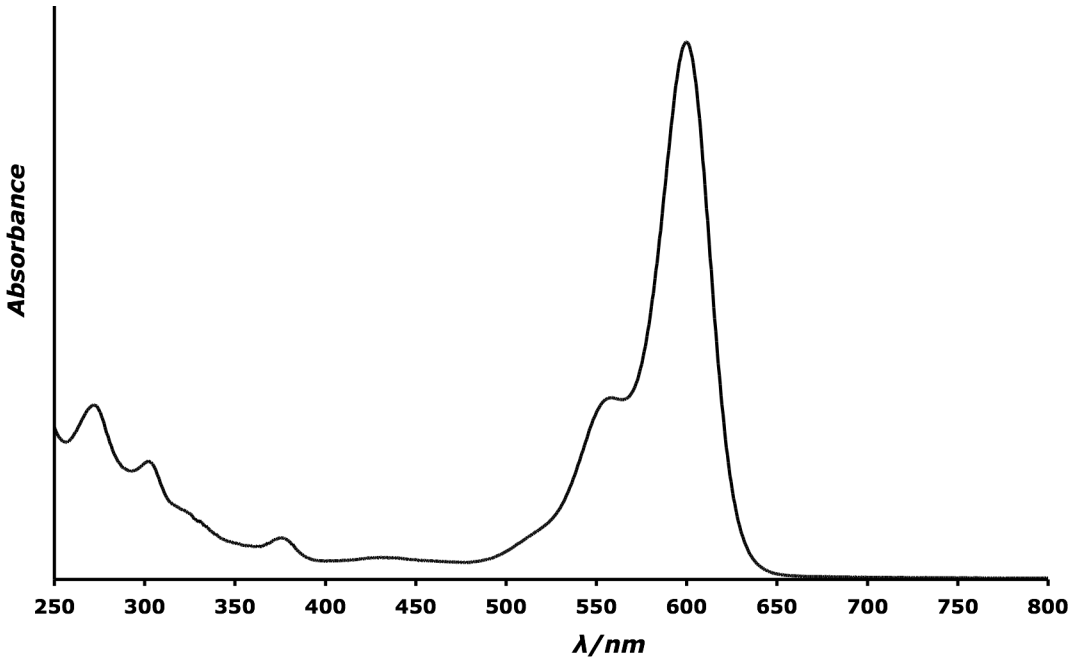
**$^1$H NMR ($CDCl_3$, 500 MHz):**



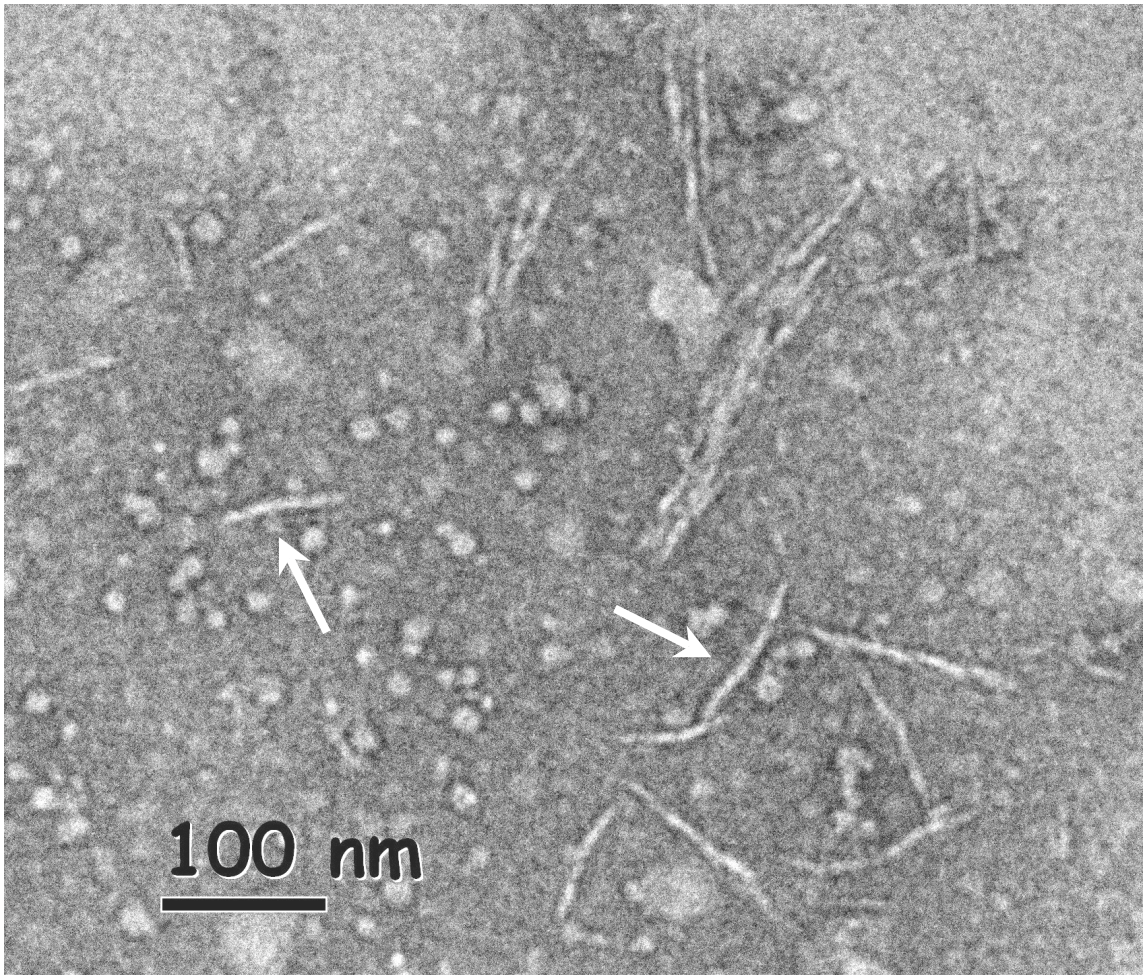
**GPC (0.10 M PBS buffer, 0.15 M NaCl, pH=7.2):**



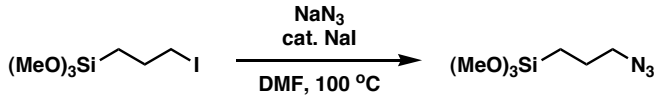

**UV-VIS:**



**TEM:** Polymer was deposited on a carbon grid from a solution (0.1 mg/mL) in 0.1 M PBS buffer (0.15 M NaCl, pH = 7.2) over a period of 30 min. The grid was stained with 1% solution of phosphotungstic acid (PTA) for 20 min, washed, dried and imaged (*note*: prolonged staining with PTA was necessary to obtain reasonable TEM contrast. We believe the high background is a result of that).



## Part II: Wafer modification and glycopolymer printing.

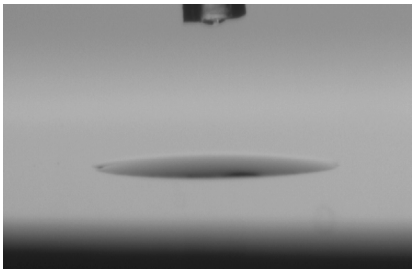



**Synthesis of (3-azidopropyl)trimethoxy silane (4).** A flame-dried round-bottom flask was charged with (3-chloropropyl)trimethoxy silane (12 mL, 65.3 mmol), sodium azide (6.38 g, 98.1 mmol, 1.5 equiv.), and sodium iodide (100 mg). Dry DMF was added. The flask was furnished with a rubber septum and immersed in a 100 ºC warm oil bath. The reaction was stirred at this temperature for 16 hrs. After this time, the reaction mixture was partitioned between ether and water. The organic layer was washed (three times with water and once with brine), dried ($MgSO_4$), concentrated, and purified by Kügelrohr distillation. The product was collected at 100 ºC (1.5 mm Hg) as a clear oil (9.32 g, 70%). $^1$H NMR ($CDCl_3$, 500 MHz) δ (ppm): 3.57 (s, 9H), 3.26 (t, $J$ = 7.0 Hz, 2H), 1.71 (m, 2H), 0.70 (m, 2H). $^{13}$C NMR ($CDCl_3$, 125 MHz) δ (ppm): 53.7, 50.6, 22.4, 6.3. FT-IR (NaCl, $CH_2Cl_2$) ν ($cm^{-1}$): 2946, 2100, 1195, 1088, 818. These spectral data matched those reported previously.[4]

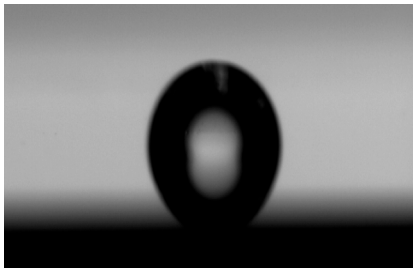
**Functionalization of silicon oxide wafers.** Silicon wafers with a 5000Å layer of thermal oxide were treated with piranha solution (conc. $H_2SO_4$/ 30% $H_2O_2$ 3:1) for 3 hours. After this time, the wafers were thoroughly washed with DI water. The clean wafers were placed in a flask containing a toluene solution of (3-azidopropyl)trimethoxy silane (10 mM). After assuring that all wafers were completely immersed, the flask was heated at

110 ºC for 18 hrs. After this time, the wafers were removed and washed thoroughly with acetone, ethanol and water. The wafers were dried in a stream of nitrogen. Contact angles of a water droplet on the plasma-treated and the modified wafers were determined to be $\alpha_{oh}$ =5º and $\alpha_{N3}$ = 85º, respectively.

**CONTACT ANGLE:**

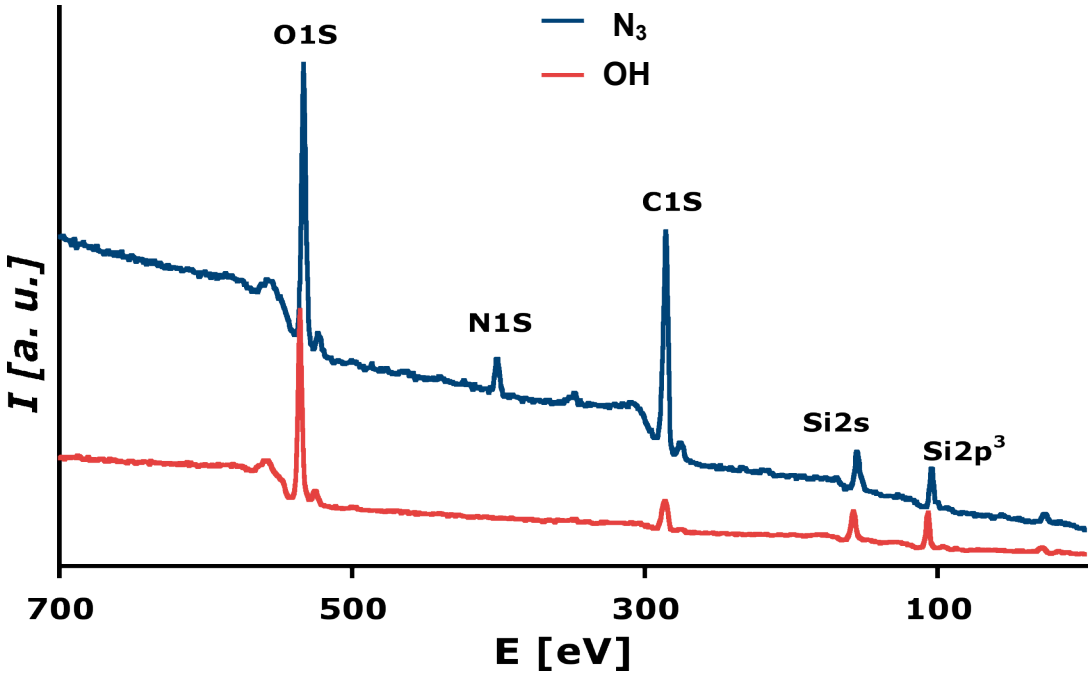


**plasma treated wafer**
**$\alpha_{OH}$ = 5 º**

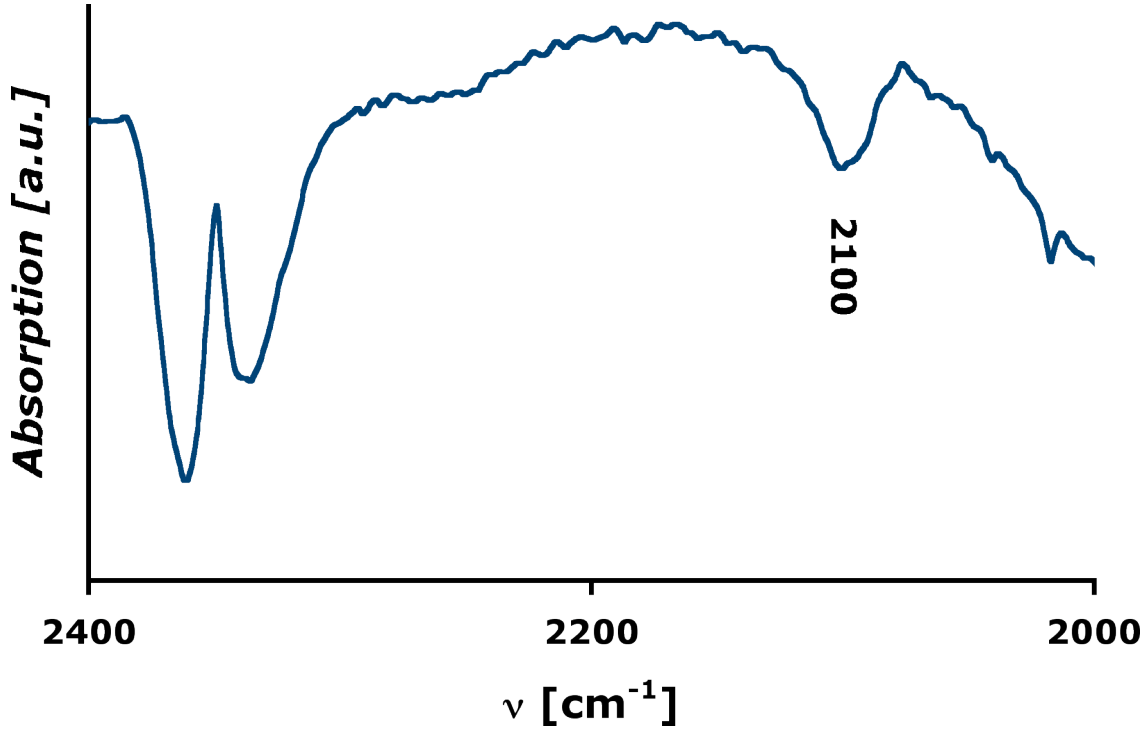


**wafer treated with 4**
**$\alpha_{N3}$ = 85 º**

**XPS:**

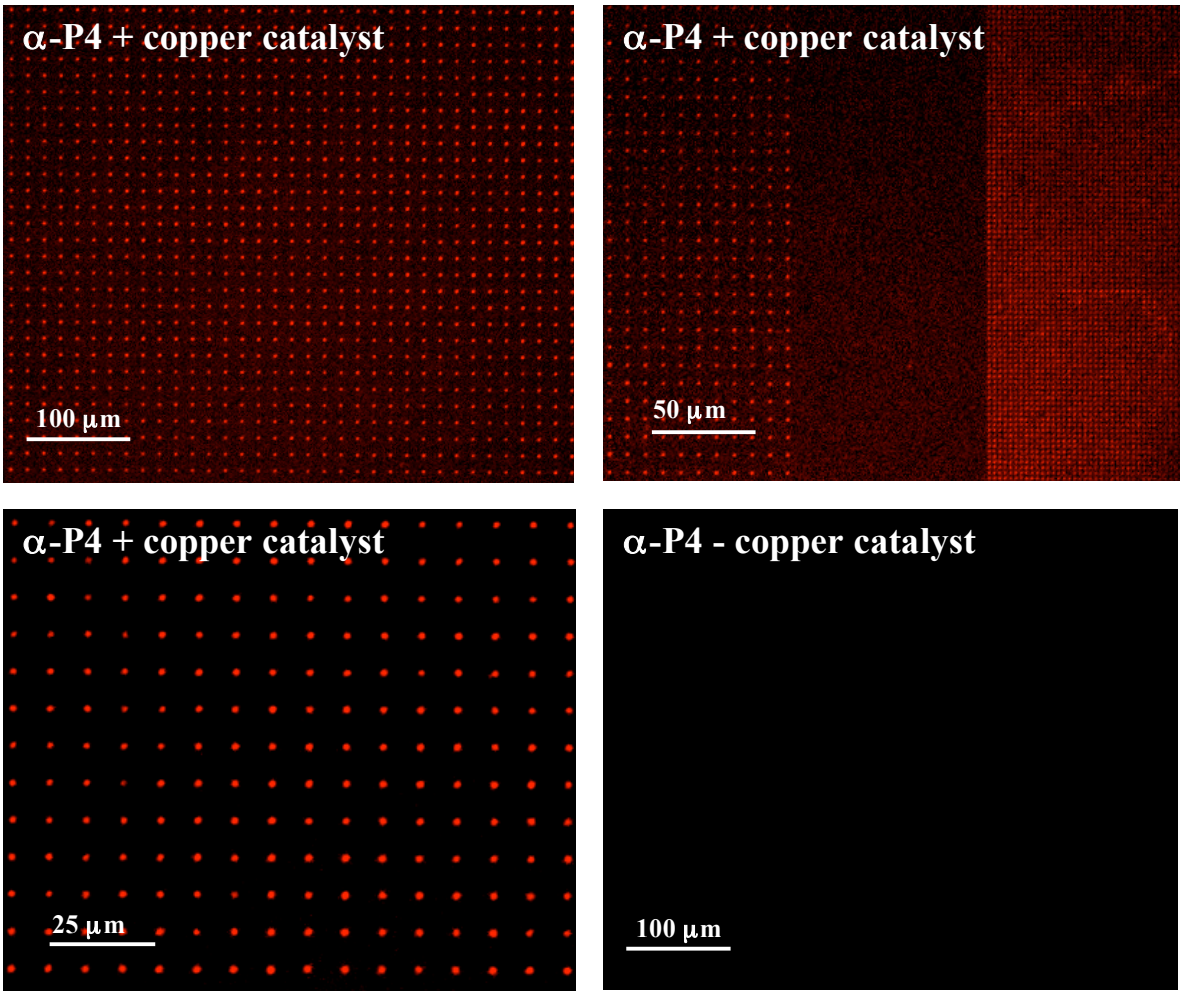


**FTIR:**



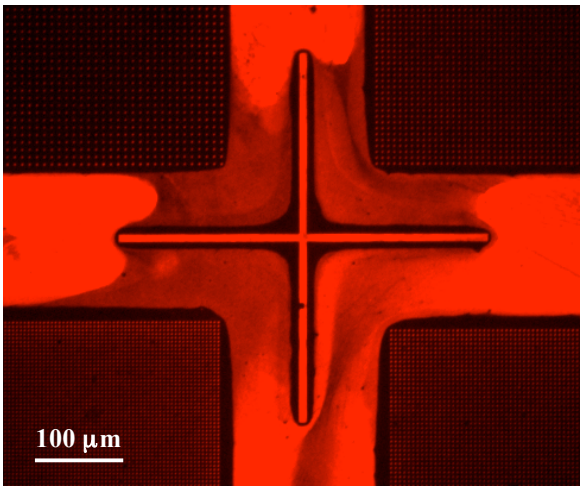

**General procedure for micro-contact printing of polymers P3 on azide-modified silicon oxide wafers.** In a small Eppendorf tube, polymer **P3** (0.2 mg) was dissolved in aqueous sodium ascorbate solution (200 μL, c = 625 μM), DMSO (200 μL), solution of tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]]amine (TBTA) in DMSO (50 μL, c = 500 μM), and aqueous $CuSO_4 \cdot 5H_2O$ (50 μL, c = 500 μM) were added. The resulting mixture was vortexed and a portion of the solution (5 μL) was transferred onto a PDMS stamp (treated with air plasma for 3 min). A cover slip was placed over the ink and wiggled gently to remove all bubbles. The stamp was allowed to incubate for 30 min. Then, the cover slip was removed and the excess of ink was gently washed off with DI water. The stamp was dried in a stream of nitrogen and carefully placed onto an azide-modified wafer. A 35 g weight was applied for 30 min. *Note*: In order to obtain high quality

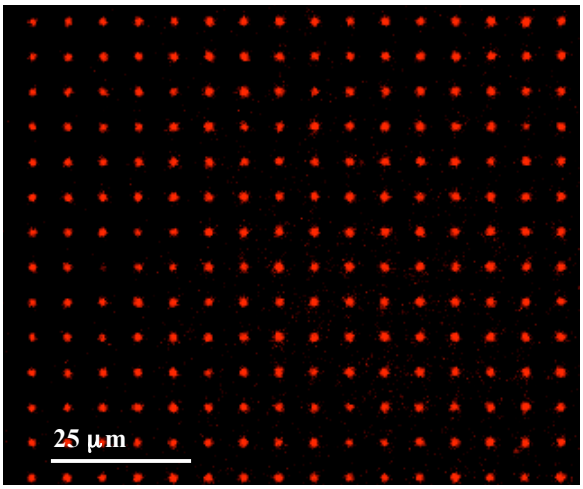
patterns, it is important to assure that during this process the PDMS stamp does not slide over the wafer surface. After this time, the stamp was removed and the surface was washed thoroughly with distilled water and placed into a solution of Bovine Serum Albumin (BSA, c = 50 μg/mL) in PBS buffer (pH = 7.2) for 1 hr. The resulting wafers were washed with DI water and dried in a stream of nitrogen.



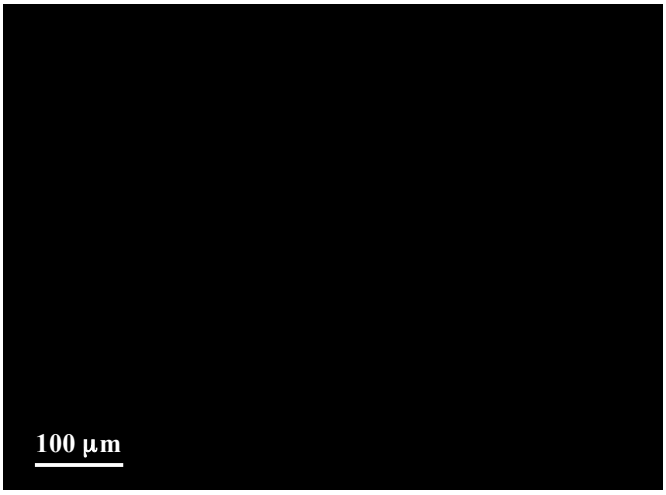
## Part III: Staining of Printed Mucin Mimics with TR-HPA Conjugate.

**General protocol for lectin binding experiments:** Wafers containing covalently attached micro-patterns of non-fluorescent glycopolymers **P3** and passivated with BSA were immersed in a plastic microscope well containing TRIS buffer (pH = 7.2, 4 mL). Texas Red-labeled HPA lectin was added (final concentration = 0.25 μg/mL) and the wafers were incubated in dark at room temperature for 30 min. After this time, the wafers were washed thoroughly with DI water to remove any unbound or non-specifically bound lectins, dried in a stream of nitrogen and imaged using a fluorescence microscope.



**Competitive binding experiement:** To a plastic microscope well containing 200 mM solution of *N*-acetylgalactosamine in TRIS buffer solution (pH = 7.2, 4 mL) was added Texas Red-labeled HPA lectin (final concentration = 0.25 μg/mL). The lectin was allowed to incubate in dark at ambient temperature for 30 min. After this time, a wafer containing patterns of **α-P3** and treated with BSA as described above was submerged in the lectin/GalNAc solution and incubated in dark for additional 30 min. After this time, the wafer was washed with DI water, dried, and images were captured on a fluorescence microscope.




## References.

[1] Lai, J. T.; Filla, D.; Shea, R. *Macromolecules* **2002**, *35*, 6754-6756.
[2] Lee, J.-Y.; Padias, A. B.; Hall, H. K. *Macromolecules* **1991**, *24*, 17-19.
[3] $^{19}$F NMR chemical shifts are strongly dependent on the concentration of **3**.
[4] Verkade, J. G.; Shang-Yi, V.; Sarkar, A. U.S. Pat. Appl. Publ. 2005176978, **2005**