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Versatile Methodology for Glycosurfaces: Direct Ligation of Nonderivatized Reducing Saccharides to Poly(pentafluorophenyl acrylate) Grafted Surfaces via Hydrazide Conjugation

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Abstract

In this work, we report a convenient and versatile strategy for surface-grafted glycopolymer constructs with the goal of surface modification that controls the chemical presentation and grafting density of carbohydrate side chains. This approach employs a difunctional hydrazine linker, chemically modified to an active ester containing poly(pentafluorophenyl acrylate) grafted scaffold, to conjugate a variety of saccharides through the reducing end. The successive conjugation steps are carried out under mild conditions and yield high surface densities of sugars, as high as 4.8 mmol·cm⁻², capable of multivalency, with an intact structure and retained bioactivity. We also demonstrate that this glycosylated surface can bind specific lectins according to the structure of its pendant carbohydrate. To demonstrate bioactivity, this surface platform is used to study the binding events of a human respiratory tract pathogen, *Mycoplasma pneumoniae*, on surfaces conjugated with sialylated sugars.

Graphical Abstract

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Notes

The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b00779. Characteristics of surface grafted poly(PFPA); AFM images; 1-pyrenemethylamine and *p*-nitrobenzaldehyde hydrazone calibration curves; UV vis spectrum (PDF)

INTRODUCTION

In nature, a complex array of interactions between glycans and proteins are used to mediate and initiate physiological, cellular, and chemical events, such as those found in joint lubrication,¹ cell signaling,² pathogen recognition, and development of genetic disorders.³ In attempts to understand these individual interactions, many strategies have been developed for the attachment and modification of biomolecules to various substrates. Naturally occurring glycans are structurally complex making them difficult to purify or synthesize. Also, modification of these glycans with reactive functional moieties for site-specific conjugation is further challenging, as modifying these molecules may either require multiple orthogonal protecting groups, or use conditions that lead to significant structural changes which may alter biological function. Specific examples of covalent ligation chemistries for sugars include modification of the carbohydrate structure with amines,⁴ that then can be used to conjugate through amidation with activated ester substrates,⁵ oxidation to create aldehyde sites for linkage to amines via reductive amination,^{6,7} and reducing terminal aldehyde conjugation to amine, followed by reductive amination.^{8,9} Each of these techniques, however, involves several steps which can lead to difficult purification or changes in the shape and structure of the sugar, possibly affecting the biological activity due to alterations of recognition and binding affinities.^{10,11}

It has been argued that the 3D presentation, concentration, and multivalency of natural ligands are of great importance for binding events,^{12–14} and that polymer supports can effectively mimic glycans found on a cell surface or protein. Precise spatial control of these synthetic platforms allows the attachment of glycans with specific chemical presentation and functional densities for improved biological recognition and activity.^{2,15,16} This class of polymers, called glycopolymers, is of interest in development of biocompatible materials and can be generated through many different synthetic approaches. Some of these techniques include polymerization of glycomonomers,^{2,14,17} such as that of Kizhakkedathu et al., who polymerized monosaccharide based monomers using surface-initiated atom transfer radical polymerization. Another approach uses post-polymerization modification,

^{18,19} as the example from Haddleton et al., who used copper click to conjugate azide-modified sugars to an alkyne containing acrylate polymer.^{14,20}

Poly(acryloyl hydrazide) (PAH) has been demonstrated to be of great utility in material science due to its ability to undergo rapid and pH reversible reactions with a variety of functional groups. PAH has been utilized to create stimuli-responsive hydrogels,²¹ drug delivery systems,^{22,23} ion exchange resins,²¹ and glycopolymers.²⁴ The hydrazide moiety located along the backbone of the polymer is an effective conjugation site for many types of aldehyde-containing molecules, including sugars with a reducing end. Hydrazone formation is reversible, stable, and an efficient reaction used to form imines.²⁵ Because of these excellent properties, hydrazine is commonly used as a bifunctional linker for reversible cross-linking and conjugation. The synthetic routes to synthesizing PAH are often multistep and performed using solution polymerization of protected monomers. Kumar et al. synthesized PAH through modification of poly(methyl acrylate) with hydrazine, which required stirring for 12 h at 60 °C.²¹ Bertozzi et al. polymerized acetoxime acrylate through reversible addition–fragmentation chain transfer using a biotinylated chain transfer agent and then reacted the polymer with hydrazine to obtain PAH.²⁴

Several techniques have been used to covalently attach carbohydrates to surfaces. Commonly, surfaces are functionalized with a reactive small molecule that can react with the complementary functional group on the carbohydrate.^{24,26,27} The most common example is the use of N-hydroxysuccinimide (NHS) modified surfaces that can react with amine containing small molecules.⁵ Pentafluorophenyl acrylate (PFPA) is an activated ester that has been demonstrated to have greater hydrolytic stability and faster reaction kinetics toward primary amines than NHS. PFPA is easily synthesized through a one-step procedure and can be polymerized to form poly(PFPA) through radical polymerization. High-density polymer brushes (as high as 2.6 chains-nm⁻²) of poly(PFPA) have been reported using surface initiated polymerization,²⁸ and lower density brushes (ranging from 0.005 to 0.05 chains-nm⁻²) can be formed using a combination of spin-coating and annealing.^{29,30} Poly(PFPA) can be easily grafted to many types of surfaces as well, allowing for use in a wide variety of applications.³¹ Once immobilized to a solid support, a one-step post-polymerization modification of poly(PFPA) with hydrazine allows the fabrication of PAH. This then allows for a simple platform to conjugate aldehydes to polymers, nano-particles, surfaces, and scaffolds for use in patterning, microarrays, and forming gradients of desired functionalities.

Herein, we present the fabrication of stable, grafted-to polymeric surfaces that can be modified in two steps for the immobilization of carbohydrates with a high degree of control over functional density and carbohydrate ratios. This direct conjugation does not require any synthetic preparation or purification of the carbohydrate, and only simple modification including substrate rinsing. With the expense of purified carbohydrates, this method employs small volumes and low concentrations, and offers a more cost-effective approach to carbohydrate conjugation. *Mycoplasma pneumoniae* is a human respiratory pathogen and a major cause of community acquired respiratory disease.³² *M. pneumoniae* recognizes sialylated glycoproteins as receptors for attachment to host cells.^{33,34} The proposed carbohydrate conjugation strategy is employed to decorate surfaces with specific sialylated

sugars in order to study the interaction of *M. pneumoniae* as well as illustrate possible applications for this proposed methodology.

EXPERIMENTAL SECTION

Materials

Wheat Germ Agglutinin Alexa Fluor 555 conjugate was purchased from Life Technologies. Silicon wafers (orientation (101), native oxide) were purchased from University Wafer. Quartz microscope slides were purchased from AdValue Technology. 3'-Sialyllactose sodium salt was purchased from Carbosynth and used as received. Lactose was purchased from Aqua Solutions. All other chemicals were purchased from TCI, Alfa Aesar, Oakwood Chemical, or Aldrich and used without further purification.

Amine Monolayer Formation

All substrates (glass, silicon, and quartz) were solvent cleaned using hexane, isopropyl alcohol, acetone, and water by sonication for 5 min in each. After drying, the substrates were plasma cleaned for 5 min (Harrick Plasma PDC-32G at 0.80 mbar). Monolayers were formed by placing substrates into a solution of 3 mM 3-aminopropyltrimethoxysilane in absolute ethanol overnight, followed by 10 min of sonication in fresh ethanol.

PFPA Free Radical Polymerization

Pentafluorophenyl acrylate monomer, synthesized using previously reported methods,²⁹ was dissolved in anhydrous dioxane (3 M) and then subjected to three cycles of freeze, pump, thaw to degas the solution. Azodiisobutyronitrile (AIBN) (0.1 mol %) was added under positive nitrogen pressure, and the flask was placed in a preheated oil bath (70 °C) overnight. The flask was then removed from heat, and the residual dioxane was removed by rotary evaporation. The remaining glassy polymer was dissolved in minimal tetrahydrofuran, then precipitated in cold methanol and filtered. The precipitation was then repeated to further purify polymer. Analysis by gel permeation chromatography showed a M_n of 97 600 g·mol⁻¹ with a PDI of 2.33.

Surface Fabrication

A solution of 20 mg·mL⁻¹ poly(PFPA) in dry chloroform was prepared and filtered to remove any large particulates. Substrates with freshly prepared amine monolayers were spin coated with PFPA at 2000 rpm for 15 s. The films were then annealed at 150 °C in a glovebox for 1 h. The resulting films were rinsed and sonicated in tetrahydrofuran for 15 min to remove any physisorbed polymer, dried under a stream of nitrogen, and cut to the desired size.

Hydrazine Post-Polymerization Modification

Substrates containing grafted-to poly(PFPA) brushes were placed into a flask and purged with nitrogen 3 times. Hydrazine monohydrate (0.3 mmol) was dissolved in 3 mL dry dimethylformamide (DMF) along with triethylamine (2 equiv, 0.6 mmol). The solution was

injected into the flask containing the substrates and allowed to stir for 1 h. The substrates were rinsed with DMF, water, and DMF again and then dried under a stream of nitrogen.

p-Nitrobenzaldehyde Conjugation

p-Nitrobenzaldehyde (0.03 mmol) was dissolved in 3 mL DMF/water (1:1) and then aniline (0.30 mmol) and drops of 2 M HCl were subsequently added to obtain a solution with a pH 4.5. Hydrazine modified substrates were placed in the solution and allowed to react for 2 h with stirring. After 2 h, the substrates were removed and then rinsed with DMF, water, DMF again, and then dried under a stream of nitrogen.

Carbohydrate Conjugation

Carbohydrate conjugations were performed using 10 mM carbohydrate and 100 mM aniline in 100 mM acetate buffer (pH 4.5). Solution was placed onto hydrazine modified substrates and allowed to react in a moisture chamber for 24 h. The substrates were then thoroughly rinsed with water then dried under a stream of nitrogen.

Microcapillary Printing

PDMS stamps with channels of 250 μm made using conventional lithographic methods were used to pattern substrates. The stamps were first plasma cleaned for 1 min to allow wicking of aqueous solution. The stamps were then placed on substrates and 3 μL of 3'-sialyllactose solution wicked into channels through capillary action. The setup was placed into a moisture chamber and allowed to react for 24 h before removing the stamp and rinsing with water. 100 μL of lactose solution was then placed on the substrate previously patterned with 3'-sialyllactose. The setup was also placed into a moisture chamber and allowed to react for 24 h before rinsing with water.

Wheat Germ Agglutinin Staining

Sialic acid residues were stained using wheat germ agglutinin Alexa Fluor 555 conjugate (WGA). The lectin was dissolved in phosphate-buffered saline (PBS, pH 7.4) at a concentration of 1 $\text{mg}\cdot\text{mL}^{-1}$. Small aliquots were placed onto substrate surfaces to cover patterned regions and allowed to incubate in the dark at room temperature for 15 min. The substrates were then rinsed with PBS and dried.

***Mycoplasma pneumoniae* Culture Preparation**

Wild-type *M pneumoniae* (strain M129) was cultured in 30 mL of SP4 growth medium³⁵ for 72 h at 37 °C in tissue culture flasks. Growth medium was decanted, flasks washed with 30 mL PBS, and cells scrapped into PBS. Cells were collected by centrifugation (20 000 $\times g$ for 25 min at 4 °C), suspended in PBS, collected by centrifugation (20 000 $\times g$ for 20 min at 4 °C), and suspended in modified SP4 growth medium³⁵ (without fetal bovine serum or phenol red and supplemented with 1% ovalbumin and 3% gelatin). Cells were dispersed by passage through a 22.5 gauge needle ten times and filtered twice (0.44 μm) to remove clumps of cells.

Live *M. pneumoniae* Imaging and DAPI Staining

Cell suspensions were incubated on each substrate using a PDMS mold for 1 h at 37 °C. Unbound cells were then removed, surfaces were washed three times with modified SP4 medium, and fresh modified SP4 medium was added to the substrates. Substrates were imaged with live *M. pneumoniae* using a Leica DM IL inverted microscope (Leica Microsystems, Buffalo Grove, IL) with a digital charge-coupled-device (CCD) camera (Hamamatsu Photonics K.K., Hamamatsu City, Japan) and analyzed using Openlab version 5.5.0 (PerkinElmer, MA). After imaging live *M. pneumoniae*, cells were fixed with 2% formaldehyde, 0.5% glutaraldehyde in modified SP4 growth medium for 15 min at room temperature, permeabilized with Triton-X100, labeled with the fluorescent stain DAPI for 15 min at room temperature in the dark, washed with water, and dried. Cells were then examined at 100× to visualize DAPI labeling.

Surface Characterization

The thickness of silicon substrate grafted functionalities were measured after each step using spectroscopic ellipsometry (J.A. Woollam, M-2000 V) then the data were fit using a Cauchy model within the CompleteEASE software. Static water contact angle measurements were taken using drop shape analysis (DSA100, Krüss USA, Matthews, NC, DSA3 software) by placing drops of water onto three different spots of each substrate and taking the average angle. Reaction completion was confirmed by utilizing grazing-angle attenuated total reflectance Fourier transform infrared spectroscopy (GATR-FTIR) (Thermo Scientific, Nicolet 6700/Harrick VariGATR). Each of the quartz substrates was analyzed by UV–visible spectrophotometry (Varian, Cary 50Bio UV–visible spectrophotometer). Surface morphologies were examined by atomic force microscopy via the Scanasyt program on Bruker Multimode AFM (Scanasyt-AIR, $k = 0.4 \text{ N}\cdot\text{m}^{-1}$, resonant frequency (f_0) = 50–90 kHz).

RESULTS AND DISCUSSION

Surface Functionalization

The strategy used for carbohydrate conjugation is shown in Scheme 1. Clean substrates were first functionalized with (3-aminopropyl)-trimethoxysilane (APTMS), and then poly(PFPA) was spin-cast onto substrates, where some of the ester residues on the backbone react with the amine-bearing monolayer, forming a stable amide bond that prevents delamination. The grafting density of poly(PFPA) was estimated at $0.1 \text{ chains}\cdot\text{nm}^{-2}$ (Table S1 in SI). Hydrazine was then reacted with the remaining activated esters on the surface. Hydrazine was chosen as the bifunctional linker because primary amines have a fast reaction rate with poly(PFPA)²⁹, and the formed PAH can conjugate a variety of reducing glycans ranging from simple monosaccharides and disaccharides to relatively complex oligosaccharides.

A reducing sugar, such as lactose, has a dynamic equilibrium (Scheme 2) between an open-chain form bearing an aldehyde group, which typically exists less than 0.1% in the equilibrium, and a cyclic pyranose (and furanose) form with a free hemiacetal group.³⁶ Using the open-chain form of the reducing sugar, the carbohydrate can be conjugated to the hydrazine moiety through the formation of hydrazone and hydrazide, with high

chemoselectivity and high yields. Even though such conjugated compounds are thermally and hydrolytically stable under biological conditions, the formation of these conjugates usually has slow kinetics.³⁷ One methodology to realize a higher reaction rate is to employ an organocatalyst such as aniline, which can increase the overall reaction rate up to 20-fold.³⁸ The aniline attacks the carbonyl group of the reducing sugar to form an imine intermediate (Scheme 2) which is thought to be more rapidly protonated than the carbonyl species, facilitating the subsequent attack by hydrazine, along with regeneration of the aniline catalyst.

Surface Characterization

In order to monitor and confirm substrate functionalization, spectroscopic ellipsometry and drop shape analysis were used to characterize thickness and contact angle changes. These results are shown in Table 1. On average, the amine monolayer formed with APTMS was 1.2 nm in thickness with a contact angle of 43°. After spin-coating poly(PFPA), the thickness and contact angle increased to 11 nm and 100°, respectively. Upon reacting the substrate with hydrazine, the thickness of the film decreased to 6.6 nm, and the hydrophilicity of the surface changed drastically from hydrophobic (100°) to nearly wetting (14°). The decrease in film thickness correlates well to the decrease in molecular weight of the polymer side chain, as we have observed in previous studies.^{29,39} After hydrazide formation, we examined carbohydrate conjugation using a variety of substrates, ranging from simple monosaccharides to more complex polysaccharides. Table 1 demonstrates that upon carbohydrate conjugation, the thickness of the films increased by ~3–4 nm and the surface remained hydrophilic with low contact angles, all below 20°. The morphologies of each surface after each transformation were also measured by AFM (Figure S1 in SI), all of which show a smooth, nearly featureless topology with an RMS roughness less than 1.5 nm.

We also monitored the surface reactions using GATR-FTIR for each reaction step (Figure 1). The disappearance of the ester carbonyl stretch at 1785 cm⁻¹ and the fluorinated aromatic ring C=C, stretch at 1525 cm⁻¹ upon post-polymerization modification with hydrazine indicates the formation of a hydrazide linkage with clear amide peaks observed at 1658 and 1540 cm⁻¹. After conjugation with the carbohydrates listed in Table 1, however, there were no obvious changes in the FTIR spectrum because of overlapping peaks. In order to further confirm carbohydrate conjugation, we used wheat germ agglutinin staining and *M. pneumoniae* binding to test the biological activity of conjugated carbohydrates as discussed below.

Surface Conjugated Carbohydrates Quantification

The aminolysis of grafted poly(PFPA) with 1-pyrenemethylamine (AMP), a UV-active dye, was used to determine the total surface functional group density of the poly(PFPA) brushes. The functional group density was calculated using the absorbance value of conjugated AMP at λ_{max} . The extinction coefficient for AMP was $35133 \pm 252 \text{ M}^{-1}\cdot\text{cm}^{-1}$ at 345 nm, measured by a calibration curve in acetonitrile (Figure S2). Using eq 1, where d is the functional density in mol·cm⁻², A is absorbance, and ϵ is extinction coefficient of the chromophore at λ_{max} , the functional group density of the brushes can be calculated. This

calculation method is used under the assumption that the difference in extinction coefficient between AMP in solution and AMP conjugated to the polymer film.

$$d = \frac{A}{\varepsilon \cdot 1000} \quad (1)$$

Figure 2a shows FTIR spectrum differences on the grafted surface before and after AMP modification, where amide bond formation is evident with the appearance of the amide I stretch at 1660 cm^{-1} and amide II stretch at 1520 cm^{-1} . The UV-vis absorbance of conjugated AMP shown in Figure 2b was 0.186 ± 0.0012 at 350 nm, which was used to calculate a total functional group density of $5.29 \pm 0.034 \text{ nmol cm}^{-2}$.

After hydrazine conjugation with PFPA, *p*-nitrobenzaldehyde (NBA) was used to quantify the total number of hydrazide groups available for reaction with the carbohydrates used in this study. The extinction coefficient ε used in the calculation was estimated using the experimentally verified extinction coefficient of *p*-nitrobenzaldehyde hydrazone (NBAH), $23283 \pm 59 \text{ M}^{-1} \cdot \text{cm}^{-1}$ at 330 nm in DMF (Figure S3). Through imine formation, UV-vis spectroscopy can then be used to estimate NBA functional density using the same equation as above. The PAH surface was first immersed into a solution of NBA for 15 min. The observed UV-vis spectrum revealed the functional group density of hydrazide, which is the total number of reactive sites available for carbohydrate conjugation. The absorbance value of conjugated NBA shown in Figure 3b was 0.125 ± 0.0135 at 330 nm, indicating the total hydrazide functional group density of $5.37 \pm 0.579 \text{ nmol cm}^{-2}$, which was close to the total PFPA functional group density after aminolysis, and implies little to no cross-linking between adjacent hydrazide and pentafluorophenol ester moieties. The FTIR spectra in Figure 3a shows the disappearance of two amide peaks for PAH as well as the appearance of nitro group stretches at 1518 and 1345 cm^{-1} further confirming the presence of the nitro containing chromophore on the surface. Next, this NBA conjugation was repeated on substrates that had previously reacted with carbohydrates to reveal the density of hydrazide groups remaining after carbohydrate conjugation, which is the yield of the carbohydrate surface reaction. Different carbohydrates substrates were tested and compared to fully functionalized PAH in order to obtain carbohydrate coverages. By comparing the absorbance of NBA between PAH and carbohydrate substrates shown in Figure 3b,c and Figure S4, the surface coverage can be estimated. For example, the absorbance of conjugated NBA on substrate functionalized with 3'-sialyllactose (Figure 3c) was 0.0165 ± 0.0012 measured by three trials, indicating that 13% of the hydrazide groups remain after carbohydrate conjugation. Therefore, the 3'-sialyllactose surface coverage is estimated at 87%, which gives a carbohydrate surface density of $4.67 \pm 0.048 \text{ nmol cm}^{-2}$. Table 2 shows the thickness, contact angle, and carbohydrate coverage of different carbohydrate substrates, indicating nearly 80% surface coverage for all of the sugars studied. Using this method, a carbohydrate coverage of 4.0 nmol cm^{-2} was achieved in a $\sim 10 \text{ nm}$ film, which was enough to facilitate multivalent effects observed in nature.

Wheat Germ Agglutinin Staining

As mentioned above, FTIR, DSA, and SE did not yield direct evidence of the successful conjugation of carbohydrates onto the PAH platform, even though the thicknesses increased and contact angle decreased. Glycopolymers can strongly interact with saccharide recognition proteins (lectins) by multivalent effects.⁴⁰ Sialyllactose contains a 3-acetylneuraminic acid moiety which firmly binds to the lectin, wheat germ agglutinin (WGA). Here, WGA Alexa Fluor 555 was utilized as a fluorescent probe to visualize the presence of sialyllactose conjugated to PAH films. We used reactive microcapillary printing to create 250- μm -wide channels of 3'-sialyllactose on a grafted PAH substrate, which was subsequently reacted with lactose after the stamp was removed. After subsequent staining with WGA, Figure 4 shows the 3-sialyllactose patterns that mimicked the channel dimensions, where distinct contrast between the patterned lines of lactose and 3'-sialyllactose are easily observed. The 3'-sialyllactose conjugated channel fluoresced bright green, while the lactose channel was dark, which demonstrates WGA binding selectively to the 3'-sialyllactose-functionalized regions. This staining technique provides indirect evidence for conjugation and confirms the carbohydrate functionalization.

Live *M. pneumoniae* Imaging and DAPI Staining

The retention of biological activity is of great importance for glycoconjugated substrates. To test the preservation of bioactivities of conjugated carbohydrates using this method, glass coverslips were used to fabricate sialyllactose surfaces, where the interaction of incubated *M. pneumoniae* with the surface was observed by phase contrast microscopy. Glycans containing terminal 2,3-sialyl residues have been previously shown to be receptors for *M. pneumoniae* binding to host cell surfaces.³³ Figure 5a shows many mycoplasma cells with a characteristic, extended cell morphology bound to the sialylated surface. DAPI staining confirms these as attached cells on the functionalized surface. As a control for specificity we also examined mycoplasma binding to PAH substrates. Figure 5b shows much less mycoplasma binding to PAH than that observed with the sialylated surface, confirming that binding specificity was retained with this surface modification technique.

CONCLUSIONS

Here, we have reported a strategy to conjugate carbohydrates to surfaces using poly(PFPA) brushes reacted with hydrazine. This simple and straightforward approach is both highly efficient and convenient, and allows conjugation of simple and complex carbohydrates. By backfilling with a NBA chromophore, we confirmed a ligation efficiency of about 80% for various carbohydrates, which is comparable to that observed in solution. Using a minimal amount of carbohydrate, we were able to effectively produce surface grafted glycopolymers where all the conjugation is through the reducing end, which mimics cell-surface glycans. This method also retains biological activity of the conjugated saccharides, using only mild processing conditions. The biorecognition and bioactivity as well as evidence for successful conjugation were confirmed through fluorescent tagged WGA binding and *M. pneumoniae* attachment to the sialyllactose conjugated surface. This methodology offers a simplified approach to fabricate carbohydrate micro-arrays using diverse sugar structures for high

throughput screening without laborious derivatization and purification steps of the carbohydrate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Lienkamp K, Noe L, Breniaux MH, Lieberwirth I, Groehn F, Wegner G. Synthesis and Characterization of End-Functionalized Cylindrical Polyelectrolyte Brushes from Poly(styrene sulfonate). *Macromolecules*. 2007; 40:2486–2502.
2. Brown JM, Xia J, Zhuang BQ, Cho KS, Rogers CJ, Gama CI, Rawat M, Tully SE, Uetani N, Mason DE, Tremblay ML, Peters EC, Habuchi O, Chen DF, Hsieh-Wilson LC. A Sulfated Carbohydrate Epitope Inhibits Axon Regeneration after Injury. *Proc Natl Acad Sci U S A*. 2012; 109:4768–4773. [PubMed: 22411830]
3. Freeze HH. Genetic Defects in the Human Glycome. *Nat Rev Genet*. 2006; 7:537–551. [PubMed: 16755287]
4. Bohorov O, Andersson-Sand H, Hoffmann J, Blixt O. Arraying Glycomics: A Novel Bi-functional Spacer for One-step Microscale Derivatization of Free Reducing Glycans. *Glycobiology*. 2006; 16:21C–27C.
5. Gerland B, Goudot A, Ligeour C, Pourceau G, Meyer A, Vidal S, Gehin T, Vidal O, Souteyrand E, Vasseur JJ, Chevotot Y, Morvan F. Structure Binding Relationship of Galactosylated Glycodusters toward *Pseudomonas aeruginosa* Lectin LecA Using a DNA-Based Carbohydrate Microarray. *Bioconjugate Chem*. 2014; 25:379–392.
6. Ruhela D, Riviere K, Szoka FC Jr. Efficient Synthesis of an Aldehyde Functionalized Hyaluronic Acid and Its Application in the Preparation of Hyaluronan-Lipid Conjugates. *Bioconjugate Chem*. 2006; 17:1360–1363.
7. Rinaudo M. Periodate oxidation of methylcellulose: Characterization and Properties of Oxidized Derivatives. *Polymers*. 2010; 2:505–521.
8. Bigge JC, Patel TP, Bruce JA, Goulding PN, Charles SM, Parekh RB. Nonselective and Efficient Fluorescent Labeling of Glycans using 2-aminobenzamide and Anthranilic Acid. *Anal Biochem*. 1995; 230:229–238. [PubMed: 7503412]
9. Moroney SE, D'Alarcao LJ, Goldmacher VS, Lambert JM, Blatder W. A Modification of the Binding Site(s) of Lectins by an Affinity Column Carrying an Activated Galactose-terminated Ligand. *Biochemistry*. 1987; 26:8390–8398. [PubMed: 3442663]
10. Flinn NS, Quibell M, Monk TP, Ramjee MK, Urch CJ. A Single-Step Method for the Production of Sugar Hydrazides: Intermediates for the Chemoselective Preparation of Glycoconjugates. *Bioconjugate Chem*. 2005; 16:722–728.
11. Wang S, Oommen OP, Yan H, Varghese OP. Mild and Efficient Strategy for Site-Selective Aldehyde Modification of Glycosaminoglycans: Tailoring Hydrogels with Tunable Release of Growth Factor. *Biomacromolecules*. 2013; 14:2427–2432. [PubMed: 23721079]
12. Wang Z, Chinoy ZS, Ambre SG, Peng W, McBride R, de Vries RP, Glushka J, Paulson JC, Boons GJ. A General Strategy for the Chemoenzymatic Synthesis of Asymmetrically Branched N-Glycans. *Science*. 2013; 341:379–383. [PubMed: 23888036]
13. Kilcoyne M, Gerlach JQ, Kane M, Joshi L. Surface Chemistry and Linker Effects on Lectin-carbohydrate Recognition for Glycan Microarrays. *Anal Methods*. 2012; 4:2721–2728.

14. Yu K, Lai BFL, Foley JH, Krisinger MJ, Conway EM, Kizhakkedathu JN. Modulation of Complement Activation and Amplification on Nanoparticle Surfaces by Glycopolymers Conformation and Chemistry. *ACS Nano*. 2014; 8:7687–7703. [PubMed: 25106451]
15. Godula K, Bertozzi CR. Density Variant Glycan Microarray for Evaluating Cross-Linking of Mucin-like Glycoconjugates by Lectins. *J Am Chem Soc*. 2012; 134:15732–15742. [PubMed: 22967056]
16. Appelhans D, Klajnert-Maculewicz B, Janaszewska A, Lazniewska J, Voit B. Dendritic Glycopolymers Based on Dendritic Polyamine Scaffolds: View on their Synthetic Approaches, Characteristics and Potential for Biomedical Applications. *Chem Soc Rev*. 2015; 44:3968–3996. [PubMed: 25519948]
17. Chen Q, Han BH. Prepolymerization and Postpolymerization Functionalization Approaches to Fluorescent Conjugated Carbazole-based Glycopolymers via “Click Chemistry”. *J Polym Sci, Part A: Polym Chem*. 2009; 47:2948–2957.
18. Wang X, Wang L, Yang S, Zhang MM, Xiong Q, Zhao H, Liu L. Construction of Multifunctionalizable, Core-Cross-Linked Polymeric Nanoparticles via Dynamic Covalent Bond. *Macromolecules*. 2014; 47:1999–2009.
19. Martinez G, Fernandez-Garcia M, Sanchez-Chaves M. Synthesis and Characterization of N-vinylpyrrolidone-tert-butyl Methacrylate-methacrylic Acid Terpolymers having Amino Sugar or Bioactive Amino Side Compounds. *J Polym Sci, Part A: Polym Chem*. 2005; 43:18–27.
20. Ladmiral V, Mantovani G, Clarkson GJ, Cauet S, Irwin JL, Haddleton DM. Synthesis of Neoglycopolymers by a Combination of “Click Chemistry” and Living Radical Polymerization. *J Am Chem Soc*. 2006; 128:4823–4830. [PubMed: 16594719]
21. Kumar A, Ujjwal RR, Mittal A, Bansal A, Ojha U. Polyacryloyl Hydrazide: An Efficient, Simple, and Cost Effective Precursor to a Range of Functional Materials through Hydrazide Based Click Reactions. *ACS Appl Mater Interfaces*. 2014; 6:1855–1865. [PubMed: 24397622]
22. Bae Y, Fukushima S, Harada A, Kataoka K. Design of Environment-sensitive Supramolecular Assemblies for Intracellular Drug Delivery: Polymeric Micelles that are Responsive to Intracellular pH Change. *Angew Chem, Int Ed*. 2003; 42:4640–4643.
23. Ganguly T, Kasten BB, Bucar DK, MacGillivray LR, Berkman CE, Benny PD. The Hydrazide/hydrazone Click Reaction as a Biomolecule Labeling Strategy for M(CO)₃ (M = Re, ^{99m}Tc) Radiopharmaceuticals. *Chem Commun*. 2011; 47:12846–12848.
24. Godula K, Bertozzi CR. Synthesis of Glycopolymers for Microarray Applications via Ligation of Reducing Sugars to a Poly(acryloyl hydrazide) Scaffold. *J Am Chem Soc*. 2010; 132:9963–9965. [PubMed: 20608651]
25. O’Shannessy DJ, Wilchek M. Immobilization of Glyco-conjugates by their Oligosaccharides: Use of Hydrazido-derivatized Matrixes. *Anal Biochem*. 1990; 191:1–8. [PubMed: 2127657]
26. Zhi ZL, Powell AK, Turnbull JE. Fabrication of Carbohydrate Microarrays on Gold Surfaces: Direct Attachment of Nonderivatized Oligosaccharides to Hydrazide-Modified Self-Assembled Monolayers. *Anal Chem*. 2006; 78:4786–4793. [PubMed: 16841896]
27. Hoypierres J, Dulong V, Rihouey C, Alexandre S, Picton L, Thebault P. Two Methods for One-Point Anchoring of a Linear Polysaccharide on a Gold Surface. *Langmuir*. 2015; 31:254–261. [PubMed: 25496460]
28. Brooks K, Razavi MJ, Wang X, Locklin J. Nanoscale Surface Creasing Induced by Post-polymerization Modification. *ACS Nano*. 2015; 9:10961–10969. [PubMed: 26493442]
29. Arnold RM, Sheppard GR, Locklin J. Comparative Aminolysis Kinetics of Different Active Ester Polymer Brush Platforms in Postpolymerization Modification with Primary and Aromatic Amines. *Macromolecules*. 2012; 45:5444–5450.
30. Bielecki RM, Doll P, Spencer ND. Ultrathin, Oil-Compatible, Lubricious Polymer Coatings: A Comparison of Grafting-To and Grafting-From Strategies. *Tribol Lett*. 2013; 49:273–280.
31. Arnold RM, McNitt CD, Popik VV, Locklin J. Direct Grafting of Poly(pentafluorophenyl acrylate) onto Oxides: Versatile Substrates for Reactive Microcapillary Printing and Self-sorting Modification. *Chem Commun*. 2014; 50:5307–5309.
32. Waites K, Atkinson TP. The Role of Mycoplasma in Upper Respiratory Infections. *Curr Infect Dis Rep*. 2009; 11:198–206. [PubMed: 19366562]

33. Roberts DD, Olson LD, Barile MF, Ginsburg V, Krivan HC. Sialic Acid-Dependent Adhesion of *Mycoplasma Pneumoniae* to Purified Glycoproteins. *J Biol Chem.* 1989; 264:9289–9293. [PubMed: 2470754]
34. Kasai T, Nakane D, Ishida H, Ando H, Kiso M, Miyata M. Role of Binding in *Mycoplasma mobile* and *Mycoplasma pneumoniae* Gliding Analyzed through Inhibition by Synthesized Sialylated Compounds. *J Bacteriol.* 2013; 195:429–435.
35. Tully JG, Whitcomb RF, Clark HF, Williamson DL. Pathogenic Mycoplasmas: Cultivation and Vertebrate Pathogenicity of a New *Spiroplasma*. *Science.* 1977; 195:892–894. [PubMed: 841314]
36. Zhu Y, Zajicek J, Serianni AS. Acyclic Forms of [1–¹³C] Aldohexoses in Aqueous Solution: Quantitation by ¹³C NMR and Deuterium Isotope Effects on Tautomeric Equilibria. *J Org Chem.* 2001; 66:6244–6251. [PubMed: 11559169]
37. Dirksen A, Dirksen S, Hackeng TM, Dawson PE. Nucleophilic Catalysis of Hydrazone Formation and Transimination: Implications for Dynamic Covalent Chemistry. *J Am Chem Soc.* 2006; 128:15602–15603. [PubMed: 17147365]
38. Thygesen MB, Munch H, Sauer J, Clo E, Jørgensen MR, Hindsgaul O, Jensen KJ. Nucleophilic Catalysis of Carbohydrate Oxime Formation by Anilines. *J Org Chem.* 2010; 75:1752–1755. [PubMed: 20131837]
39. Orski SV, Fries KH, Sheppard GR, Locklin J. High Density Scaffolding of Functional Polymer Brushes: Surface Initiated Atom Transfer Radical Polymerization of Active Esters. *Langmuir.* 2010; 26:2136–2143. [PubMed: 20099926]
40. Miura Y. Synthesis and biological application of glycopolymers. *J Polym Sci, Part A: Polym Chem.* 2007; 45:5031–5036.

Figure 1.

FTIR spectra of the individual steps in the carbohydrate conjugation, including amine monolayer, poly(PFPA) brush, poly-(acryloyl hydrazide), and sialyllactose conjugated to the surface as a representative spectrum of a conjugated carbohydrate.

Figure 2. FTIR spectra (a) and UV vis spectrum (b) of conjugated 1-pyrenemethylamine on poly(PFPA) coated substrate.

Figure 3.

(a) FTIR spectra of conjugated NBA on a poly(acryloyl hydrazide) and 3'-sialyllactose surface; (b) UV-vis spectrum of the fully functionalized PAH substrate reacted with NBA (in triplicate); (c) UV-vis spectrum of NBA conjugated to 3'-sialyllactose substrate (in triplicate).

Figure 4.
3'-Sialyllactose and lactose patterned on a PAH surface stained with fluorescent WGA to indicate successful immobilization of carbohydrates.

Figure 5. Live *M. pneumoniae* cells adhered to (a) 3'-sialyllactase conjugated and (b) PAH grafted coverslips; and (c) DAPI staining on 3'-sialyllactose conjugated coverslips.

Scheme 1.

General Carbohydrate Functionalization Strategy Using Post-Polymerization Modification

Scheme 2.
Pathway for Carbohydrate Hydrozone/Hydrazide Formation

Table 1

Thickness and Contact Angle Data for Each Functionalization Step

	thickness (nm)	contact angel (deg)
APTMS	1.2 ± 0.1	43 ± 0.3
poly(PFPA)	11.3 ± 0.4	100 ± 0.6
PAH	6.6 ± 0.1	14 ± 0.7
Xylose	9.4 ± 0.2	15 ± 0.3
Glucose	9.1 ± 0.5	9 ± 0.6
galactose	9.4 ± 0.5	8 ± 0.9
Lactose	10.0 ± 0.3	12 ± 0.7
Maltose	9.1 ± 0.2	13 ± 0.3
3'-sialyllactose	9.2 ± 0.3	19 ± 0.6

Table 2

Surface Characterization after NBA Functionalization and Calculated Amount of Carbohydrate on the Surface

	carbohydrate coverage	# carbohydrate molecule (nmol·cm ⁻²)
Xylose	73 ± 3.2%	3.92 ± 0.172
Glucose	88 ± 0.3%	4.73 ± 0.016
galactose	86 ± 1.6%	4.62 ± 0.086
Lactose	90 ± 0.7%	4.83 ± 0.038
Maltose	91 ± 2.6%	4.89 ± 0.140
3'-sialyllactose	87 ± 0.9%	4.67 ± 0.048