Tunable, biodegradable grafting-from glycopolypeptide bottlebrush polymers

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The cellular glycocalyx and extracellular matrix are rich in glycoproteins and proteoglycans that play essential physical and biochemical roles in all life. Synthetic mimics of these natural bottlebrush polymers have wide applications in biomedicine, yet preparation has been challenged by their high grafting and glycosylation densities. Using one-pot dual-catalysis polymerization of glycan-bearing $\alpha$-amino acid $N$-carboxyanhydrides, we report grafting-from glycopolypeptide brushes. The materials are chemically and conformationally tunable where backbone and sidechain lengths were precisely altered, grafting density modulated up to 100%, and glycan density and identity tuned by monomer feed ratios. The glycobrushes are composed entirely of sugars and amino acids, are non-toxic to cells, and are degradable by natural proteases. Inspired by native lipid-anchored proteoglycans, cholesterol-modified glycobrushes were displayed on the surface of live human cells. Our materials overcome long-standing challenges in glycobrush polymer synthesis and offer new opportunities to examine glycan presentation and multivalency from chemically defined scaffolds.
The surface of all cells are densely populated with a diverse array of glycolipids and glycoproteins that collectively form the glyocalyx. The extracellular matrix (ECM) is another region particularly rich in a glycoprotein subclass termed proteoglycans. Both glycoproteins and proteoglycans are composed of a polypeptide backbone with enzymatically grafted saccharide chains originating predominantly at serine (Ser), threonine, and asparagine residues. The resulting structures are glycosylated molecular bottlebrushes, or glycobrushes, where saccharides are the brush bristles. Glycoproteins contain one, few, or many saccharides of diverse identity, whereas proteoglycans contain amino sugars, have regularly repeating structures, and can grow to hundreds of glycan units. Overall, these structures encompass a complex variety of molecular shapes and sizes, with variation in length and composition of the polypeptide backbone, length and graft density of the saccharide bristles, saccharide identity, and charge. These factors play essential roles in hydration, lubrication, resistance to mechanical force, and regulating diffusion of small molecules and pathogens to the cell surface. For example, glycopolymer brushes have been used in arrays to form amido-amidate nickelacycles and yields a heterogeneous and complex mixture of structures. A general challenge facing all syntheses of bottlebrush polymers act as macromonomers to yield the backbone, whereas the saccharides bind copious water molecules and ions, as well as provide specific bioactivity.

Synthetic mimics of natural glycobrushes have extensive applications in biomedicine as components of lubricants, hydrogels, and ECM mimics, and have attracted attention as biolubricants to probe diverse cell surface events. Biosynthesis of proteoglycans and glycoproteins is controlled by 1000 enzymes and yields a heterogeneous and complex mixture of structures. Therefore, manipulation of individual molecular properties and study of their downstream biological effects have been hampered. Synthetic mimics offer opportunities to probe glyobiology in new ways. Glycan presentation, spacing, multivalency, and the brush architecture have all been shown to affect biological function. For example, glycopolymer brushes have been used in arrays to detect the specificity of lectins, have been employed in the study of viral adhesion, and investigated as antimicrobial, antiviral, or antifouling agents. Further, polymeric bottlebrushes are fascinating materials in their own right with properties that cannot be achieved by linear polymers. Due their large molecular size, anisotropic conformation, and reduced chain entanglement, such materials have been investigated for application in photonic materials, films for lithographic patterning, drug delivery, tissue engineering, and tumor detection and imaging. Glycans confer advantageous properties in such applications due to their non-ionic hydrophilicity and their biochemically active properties.

Here we describe a one-pot grafting-from approach to synthesize glycobrushes using α-amino acid N-carboxyanhydride (NCA) polymerization and dual transition metal catalysis. The method is precisely tunable within the same parameters as natural glycobrushes including polypeptide backbone composition and chain length, glyco-sidechain length and graft density, glycan identity, and chain conformation. To our knowledge, our glycobrushes are the first example of controlled polymerization of glycosylated monomers in a grafting-from approach based entirely on amino acids and saccharides.

### Results and discussion

#### Design, synthesis, and analysis of tunable glycobrushes

There are three synthetic routes to bottlebrush polymers: grafting-to, where pre-existing backbone and sidechain polymers are ligated; grafting-from, where sidechain polymerization is initiated from backbone polymers; and grafting-through, where sidechain polymers act as macromonomers to yield the backbone chain. A general challenge facing all syntheses of bottlebrush polymers is steric hindrance at the backbone due to dense grafting, resulting in low molecular weight (MW) chains and low grafting efficiencies. Compared to grafting-through and grafting-to strategies, the grafting-from method offers improved alleviation of steric hindrance due to the gradual growth of the sidechains. Synthesis of glycobrushes has focused mainly on the grafting-to approach, which is convenient, as both backbone and sidechains can be individually synthesized and characterized. However, this method typically suffers from low graft density and incomplete functionalization. Although much beautiful work has been done to optimize grafting-from systems utilizing controlled radical, ring-opening metathesis, and NCA polymerizations, there are few reports on the use of glyco-monomers and prior work has focused exclusively on reversible deactivation radical polymerization. This method yields hydrocarbon backbone polymers incapable of the hydrogen bonds crucial to protein conformation and function, requires non-native glycan structures, and, to date, initiation inefficiency has limited the grafting density, as only low MW sidechains were produced. In addition, such materials would not be substrates for natural proteases and their degradation products would yield biological foreign materials.

Inspired by the work of Rhodes and Deming who synthesized bottlebrushes based entirely on polypeptides, we sought to develop tunable glycobrushes based entirely on amino acid and saccharide building blocks, which we expect to be biodegradable. Toward this end, we employed two-step NCA polymerization in a one-pot dual-catalysis system to form glycobrushes with graft density up to 100% if desired, high MW chains, and tunable sidechain morphology and composition. For the backbone polypeptide, an allyloxyacarbonyl (Alloc) functionalized lysine (Lys) NCA was utilized, as the Alloc group is inert to established Co(0) polymerization initiators but reacts with electron-rich NPs species to form amido-amidate nickelacycles. These nickelacycle species are known initiators of NCA polymerization and served as sites for growth of glycopolypeptide branches. Isoleucine (Ile) or methionine (Met) were used as linkers between the Alloc and Lys, to generate two activatable monomers Alloc-Met-Lys (AMK) NCA and Alloc-Ile-Lys (AIL) NCA. We chose Ile, as it confers good organic solubility, and Met, as peptide bonds can be selectively cleaved at Met sites using cyanogen bromide (CNBr). This feature liberates the sidechains from the backbone, enabling separate analyses of their properties. Therefore, most initial polymerization studies were conducted with AMK NCA. In addition, Met residues could later serve as substrates for selective bioconjugation of desired functional molecules or as a site of oxidation to manipulate backbone hydration.

Graft density was readily modulated by stoichiometric copolymerization of activatable AMK or AIL NCAs and non-activatable NCAs (Table 1 and Fig. 1b). For non-activatable NCAs, we chose N-ε-carbobenzoxy-o-L-lysine (ε-BnE), γ-benzyl-γ-glutamate (BnE), γ-tart-butyl-γ-glutamate (tBuE), and diethylene glycol functionalized Lys (EGK) NCAs. We chose these, as ε-BnE, BnE, and tBuE are well-established monomers for NCA polymerization and as non-ionic EGK requires no deprotection chemistry and offers both aqueous and organic solubility. In addition, polymers of these structures are all known to form α-helices, resulting in ordered display of the Alloc groups. NCAs were polymerized in various relative ratios from 25% to 100% AMK, and at varied monomer to initiator (M : I) ratios with (PMe3)4Co initiator at room temperature in tetrahydrofuran (THF). As expected, polymer MWs were higher than predicted from monomer to initiator stoichiometry, which is due to the known incomplete efficiency of (PMe3)4Co initiation in THF. High MW homo- or co-polypeptide AMK or AIL backbones up to degrees of polymerization (DPs) ca. 300, could typically be formed within 1–2 h.
Fig. 1 Cartoon representations of native proteoglycans vs. our synthetic glycobrushes, which are prepared via two-step, one-pot NCA polymerization.

a Comparison of a representative native proteoglycan bottlebrush (i.e., aggrecan with polypeptide backbone and chondroitin sulfate, and keratin sulfate polysaccharide chains) with our synthetic glycobrushes. b Synthetic route to chemically tunable glycobrush-based dual-catalysis, one-pot NCA polymerization. Chain length, graft density, glycosylation density, and pattern are tuned via NCA monomer feed ratios and equivalents of transition metal catalysts. R' = CH2CH2SCH3 for Met-linked AMK or CH(CH3)CH2CH3 for Ile-linked AIK. c Functional Ni0 catalysts used in this study to install chemical groups of interest at the chain initiation site.
The Alloc-containing polypeptides were then directly treated with 1,2-bis(dimethylphosphino)ethane nickel cyclooctadiene (dmpeNi(COD)) at 80 °C in dimethylformamide (DMF), to generate amido-amidate nickelacycle-initiating groups along the backbones. These macroinitiators were combined with various glycan-bearing NCAs to form tunable glycobrushes. We chose Ser (S) NCAs bearing α-N-acetylgalactosamine (GalNAcS) or disaccharide β-lactose (LacS), as these native structures play important roles in cell surface biology. Polymers of these two structures differ in that poly(GalNAcS) forms highly rigid rods, whereas poly(LacS) forms disordered structures. To expand the biological target space, we included Thr (T) NCAs bearing α-glucose-modified Lys (GlcK) NCAs, which are known to form α-helical structures when polymerized. GlcK is also attractive, as it features an anomerically C-linkage that has been shown to bind biological targets with affinities equal to O-linkages, but that is resistant to acidic hydrolysis and enzymatic deglycosylation. Similar to modulation of backbone composition, sidechain glycosylation density was readily modulated by stoichiometric incorporation of BnE NCAs (Table 1, entries 7–9).

Polymerization reactions were monitored by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) via observation of NCA absorbances at ca. 1850 and 1790 cm⁻¹ vs. polypeptide absorbances at ca. 1650 and 1540 cm⁻¹ (Fig. 2a). After complete conversion of NCA to glyobrush, polymers were analyzed by a combination of ¹H nuclear magnetic resonance (NMR) and gel permeation chromatography coupled with tandem RI analysis of backbone and backbone polymers. We were delighted to observe quantitative conversion of monomer to polymer with linear and predictable glycopolypeptide sidechain growth even at 100% graft density (Table 1, Entries 1–9). Remarkably, sidechains of 100% glycosylation density could be grown up to ca. 117 residues. Clear shifts in MW were observed by GPC/MALS/RI analysis for glycobrushes prepared with varied M : I ratios and at varied graft densities (Fig. 2b and SI Fig. 10). For some high MW samples with 100% branch density, we observed slightly viscous DMF solutions. GPC/MALS/RI analysis revealed a MALS shoulder on the elution peak, indicating the presence of some aggregates in organic solvent. As even very low-concentration aggregates can strongly scatter light, we examined the RI trace of these samples. RI analysis indicates very little mass in the region we attribute to aggregation, which rationalizes the low calculated dispersities (see SI for representative RI trace). We did not observe such aggregation for lower graft density glycobrushes, nor the backbones themselves (Fig. 2f and SI Fig. 10). Further, no aggregation was observed for 50% grafting density brushes after deprotection and dissolution in aqueous solution as evidenced by dynamic light scattering (DLS) (Fig. 3c).

However, we still wondered whether minor aggregation or other factors could result in inconsistent growth of sidechains for 100% graft density brushes. Therefore, we desired analysis of the sidechains separated from the backbone. To achieve this, AMK-based glycobrushes were treated with CNBr to cleave the branches from the backbone at the Met sites (Fig. 2e) and the cleavage reaction was directly analyzed by GPC/MALS/RI. Figure 2f is a representative GPC/MALS/RI trace of the backbone, a glycobrush, and cleaved branches. We again observed very low levels of aggregates, which had negligible mass by RI (see SI for representative RI traces). This we attribute to backbone structures, as we directly analyzed aliquots of the branch cleavage reaction. Figure 2g indicates linear sidechain growth for 50% glycosylated sidechains and Fig. 2h indicates linear sidechain growth of 100% glycosylated sidechains, and remarkably low dispersities ranging from 1.03 to 1.28. Both samples are 100% grafting density where every backbone monomer unit has a sidechain. Table 1, Entries 1–9, contains the polymer data for these samples (see SI for additional GPC data, SI Fig. S10). Previously reported atom transfer radical polymerization grafting from glycobrushes suffered from inefficient initiation ranging from 23% to 38%, necessitated monomer conversions of <11%, and used non-native glycans and polymer backbones. Our system overcomes these challenges offering quantitative initiation and conversion, up to 100% graft density, complete tunability in
chain length, glycan identity and density, native components, and MWs on par with native proteoglycan and glycoprotein structures.

**Kinetics of glycobrush growth.** During the course of this work, we observed a striking rate acceleration for growth of GlcK brushes as compared to GalNAcS and LacS brushes. Conversion from monomers to glycobrushes were monitored by ATR-FTIR as previously described and data were normalized to the absorbance intensities of acetate functional groups that remain constant throughout the course of the reactions (see SI Figs. S1–9). Remarkable 20- and 59-fold rate enhancements were observed for
polymerization of GlcK brushes as compared to GalNAcS and LacS, respectively (Table 2). We believe this phenomenon is due to the resulting secondary structure of the branches where polyGlcK forms α-helices, whereas polyGalNAcS is extended and rod-like and polyLacS forms disordered structures. Prior work from Cheng and colleagues30 indicated rate acceleration for growth of neighboring α-helical polymers due to cooperative interactions of macrodipoles (vide infra). To confirm these secondary structures, we performed conformational analyses of glycobrushes and linear glycopolymerides by circular dichroism (CD) spectroscopy. As expected from previously reported data on GlcK homopolymers42, strong negative absorbances at 208 and 222 nm were observed for both linear chains and GlcK brushes, indicating classical α-helices (Fig. 3a, b). GalNAcS linear polymers and brushes display a positive maxima at 218 nm, negative minima at 202 nm, and positive maxima at 194 nm, which indicate a uniquely rigid, extended structure similar to a polyproline helix where the glycosyl amide hydrogen bonds to the peptide backbone40. For the LacS linear polymers, we observed a fairly weak absorbance and minima at 198. This CD pattern likely indicates a disordered structure. The CD of LacS glycobrushes is dominated by the strong absorbance of the helical polypeptide backbone.

In the work by Cheng and colleagues30, polynorbornene macroinitiators bearing varied densities of trimethylsilylamine groups were used to initiate polymerization of BnE NCA into helical polyBnE bottlebrushes. They reported a dramatic rate enhancement for growth of the neighboring α-helical polymers. Linear polymer chains will likely grow as individual units in solution, but by comparison, brush sidechains will grow in a sterically crowded environment where they can interact. Cheng and colleagues30 rationalized the growth rate acceleration of helix-forming brush sidechains as compared to disordered chains, due to the cooperative interactions of helix macrodipoles. Although the polymerization chemistry and backbone/branch polymer structures are very different than those we describe here, we observed the same phenomena. Steric effects are also a probable factor in the kinetics we observed, as reducing graft density to 25% increased the speed of polyGlcK brush growth 2.3-fold. However, considering that GalNAcS and GlcK are both monosaccharide-bearing structures, the 59-fold rate enhancement for 100% density GlcK vs. GalNAcS brushes is most likely a conformational effect rather than a steric effect. Polymerization kinetics of GalNAcS and several other glycoNCAs were reported previously, and was determined to be essentially the same as a modified Lys NCA39,41, so kinetics of individual NCAs are not a likely source of such dramatic rate enhancements. We believe we have now independently confirmed the effect of cooperative interactions of α-helical macrodipoles in neighboring polymers in our transition metal-catalyzed system with glycobrushes.

**Aqueous morphology.** Our glycobrushes were readily decayinglized by treatment with K2CO3 in methanol/water to yield watersoluble glycobrushes with native glycans. To characterize the conformation and morphology of our glycobrushes, we analyzed 50% graft density GlcK glycobrushes with varied sidechains and backbone DPs by DLS and atomic force microscopy (AFM). DLS of (PEG2K0.5-PAMK0.5)300-PGlcK45 and (PEG2K0.5-PAMK0.5)300-PGlcK45 revealed uniform hydrated particle sizes of 125 ± 8 nm and 161 ± 16 nm, respectively (Fig. 3c). AFM imaging of the glycobrushes was performed on freshly cleaved mica in tapping mode. Similar to native proteoglycans, our structures were prone to intermolecular association, so low-concentration 5 nM solutions were utilized to enhance observation of single molecules. Representative images of (PEG2K0.5-PAMK 0.5)150-PGlcK45 and (PEG2K0.5-PAMK0.5)300-PGlcK45 are shown in Fig. 3e, f. As expected from the DLS data, we observed spherical and ellipsoid particles. Based on peptide bond lengths, we had estimated the ideal dimensions of (PEG2K0.5-PAMK 0.5)150-PGlcK45 as 23 × 15 nm and (PEG2K0.5-PAMK0.5)300-PGlcK45 as 46 × 15 nm. Resolution of less than ca. 15 nm is challenging due to the convolution of the AFM tip; however, we did observe a clear statistical difference in particle sizes from AFM images of 150mer vs. 300mer backbone glycobrushes with similar sidechain lengths as indicated in the violin plots shown in Fig. 3d. All particles deemed aggregates were excluded and only volumes of single particles were included in the analysis. Particle volumes expected by conversion of DLS-obtained diameters (V = 4πr3/3) are larger than those observed by AFM, but this is most likely due to the hydration of the glycobrushes in aqueous solution vs. dehydration and kinetic trapping on the mica surface.

**Biodegradation and toxicity assays.** To investigate the utility of our glycobrushes as surrogates for native proteoglycans, we examined their effect on cell viability, their biodegradability, and their ability to be displayed on the surface of live cells. A commercial Cell Counting Kit-8 (CCK-8) colorimetric assay for the determination of cell proliferation and cytotoxicity was conducted in human embryonic kidney cells (HEK293T) after 24 h treatment with glycobrushes bearing either GalNAcS or GlcK. The phosphate-buffered saline (PBS) control was normalized to 100% and other samples are reported relative to that value. Triton x100 was used as a positive control to kill cells and ensure functional assay conditions. A one-way analysis of variance test was conducted and compared to controls, revealing our glycobrushes had no statistically significant effect on viability (Fig. 4a).

As our materials are composed entirely of sugars and amino acids, we explored their susceptibility to natural proteases. We selected a sample with 100% graft density and 100% glycosylation, PAMK0.5-g-PGalNAcS30, as this structure is quite sterically hindered. If this sample can be digested by proteases, then we can safely extrapolate that our lower graft and glycosylation density samples will as well. Previous research on related linear GalNAcS glycopolymerides indicated that this material is a substrate for both a general protease (trypsin) and a glycoprotein-specific protease (secreted protease of C1 esterase inhibitor)30, so we included these alongside general protease Proteinase K and Met-specific protease Met aminopeptidase 2 (MetAP2). Over a 48 h time period, we observed minor degradation by trypsin, MetAP2, and StcE. Proteinase K treatment, however, resulted in nearly
complete degradation of the glycobrush (Fig. 4b). Although a full study of the degradation properties of various brush compositions is outside the scope of this work, we have demonstrated that these materials are protease resistant but are substrates for natural proteases and will eventually degrade.

Glycocalyx engineering. A subset of native proteoglycans are directly tethered to the cell surface through lipid anchors. In a biomimetic strategy, we utilized terminal cholesterol groups to tether our glycobrushes to the surface of live human epithelial cells (Fig. 5a). This group was installed on every chain end via our

Fig. 3 Analyses of glycobrush conformations and morphologies. a CD spectra of various glycobrushes. b CD spectra of linear glycopolypeptide branches. c Hydrodynamic size distribution determined via DLS. d Glycobrush particle volume analysis from AFM images. ****P-value < 0.0001 from a two-sided Mann-Whitney test. n = 30 for (PAMK0.5-s-PEG2K0.5)g-PGlcK45 and n = 44 for (PAMK0.5-s-PEG2K0.5)150-g-PGlcK45. e AFM image of (PAMK0.5-s-PEG2K0.5)150-g-PGlcK45. f AFM image of (PAMK0.5-s-PEG2K0.5)300-g-PGlcK45.
Table 2  Second-order kinetic parameters for NCA polymerization and growth of glycopolypeptide branches with various secondary structures and at varied graft densities.

| Polymer                               | $k_2$ (M⁻¹·h⁻¹) | Branch conformation |
|---------------------------------------|-----------------|---------------------|
| PAMK₆₃₋₉₋P GlcK₉,₇                  | 823             | 1120                |
| (PAMK₀.₂₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅-_content...
a need for well-defined synthetic materials that harness their properties.

Methods

Full experimental details, characterization of compounds, and additional data can be found in the SI.

Polypeptide backbone synthesis. In an N2 atmosphere glove box, 6.4 mg of AMK NCA (0.0165 mmol) was dissolved in anhydrous THF. Ten microliters of a 30 mg/mL solution (0.826 μmol) of (PMe3)4Co in anhydrous THF was added to the NCA solution corresponding to the intended backbone : initiator ratio ([M]:[I]). Polymerizations were monitored by ATR-FTIR.

Glycobrush synthesis. In an N2 atmosphere glove box, 2.16 mg of Ni(COD)2 (1 eq) was added to 51.4 μL of anhydrous THF. To the solution, 2.6 μL dmpe (2 eq.) was added and allowed to mix for 10 min. PAMK containing 7.85 μmol of activatable groups was dissolved in 131 μL of anhydrous DMF. The dmpeNi(COD) solution was added to the PAMK solution and the mixture was heated for 16 h at 80 °C. The activated backbone solution was cooled. NCAs used for the sidechain growth were dissolved at 50 mg/mL in anhydrous DMF. A volume of activated backbone solution was added to the NCA solution corresponding to the intended monomer : initiator ratio ([M]:[I]). Polymerizations were monitored by ATR-FTIR.

Glycobranch cleavage. From the crude glycobrush polymerization reaction, a volume containing 2 mg of glycobrush was removed. To this solution, 0.5 mL of 40 mg/mL CNBr in 5 : 4 : 1 acetonitrile : acetic acid : water was added. The reaction was sealed and allowed to stand overnight. The reaction was evaporated to dryness. The solids were redissolved in 0.1 M LiBr in DMF at 3 mg/mL polymer and analyzed by GPC/LS.

Cellular cytotoxicity assays. HEK293T cells were cultured in Dulbecco’s modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS), 2 mM l-glutamine, and 100 U/mL penicillin. Upon reaching sufficient confluency, cells were trypsinized and suspended in medium. Cells were loaded 5 × 10^4 per well in a clear, flat-bottom 96-well plate coated with poly-L-Lys. Twenty-four hours after plating, cells were treated with 0.25 g/L polymer for 24 h, then analyzed using a CCK-8 assay from Dojingo Molecular Technologies, Inc. The CCK-8 reagent was allowed to incubate with cells for 3 h prior to absorbance reading.

Glycocalyx engineering. AF594-labeled glycopolyelectrolytes were dissolved at 15 μM in complete media (DMEM with 10% FBS, pen/strep, and l-glutamine) and sterile filtered through a 0.2 μm membrane. HEK293T cells were trypsinized and neutralized with complete media. The cells were pelleted by centrifugation at 100 × g for 5 min. Media was removed and the cells were resuspended in media containing polymer. Cells were incubated in the media + polymer for 2 h at room temperature. Incubation could be conducted in the centrifuge tube, but transfer to a culture dish was preferred for improved surface area. Cells did not adhere at room temperature. Post incubation, treated cells were resuspended and centrifuged, washed with PBS, resuspended in complete media (lacking polymer), and plated. Untreated control cells were plated on a separate 24-well plate. All cells were left to grow at 37 °C. Cells were Hoescht stained and imaged under a fluorescence microscope (Laxco LMI-6000) at timepoints from 24 to 96 h following polymer treatment. See SI for studies with transferrin.

Protease digestions. Next, 0.05% Gibco Trypsin was used at an E : S of 1 : 10 in a reaction buffer of 50 mM NH4HCO3, pH 8. METAP2 (from R&D Systems) was used at an E : S of 1 : 20 in a reaction buffer of 50 mM Heps, 100 mM NaCl, 0.1 mM CoCl2, at pH 7.4. StcE was a gift from the lab of Carolyn Bertozzi. Protease K was obtained from ThermoFisher (#AM2542). Digestions with StcE and Protease K were performed in 1× PBS pH 7.4 with E : S of 1 : 10. All digestions were performed at 37°C for 2 h. Media was removed, and the cells were pelleted by centrifugation at 300 × g for 5 min, washed with PBS, and resuspended in DMEM containing polymer for 2 h at room temperature. Incubation could be conducted in the centrifuge tube, but transfer to a culture dish was preferred for improved surface area. Cells did not adhere at room temperature. Post incubation, treated cells were resuspended and centrifuged, washed with PBS, resuspended in complete media (lacking polymer), and plated. Untreated control cells were plated on a separate 24-well plate. All cells were left to grow at 37 °C. Cells were Hoescht stained and imaged under a fluorescence microscope (Laxco LMI-6000) at timepoints from 24 to 96 h following polymer treatment. See SI for studies with transferrin.

Fig. 5 Robust and prolonged glycocalyx engineering using Chol-terminal GalNAcS- glycobrushes. a Reaction scheme for conjugation of AF594-NHS to glycobrush (PLG0.8-s-PAIK0.2)50-g-GalNAcS13-Chol amino termini, followed by engineering of the glycocalyx of live epithelial cells. Cell images in b, c are 24 h after incubation b with 15 μM AF594-glycobrush-N3 or c with 15 μM AF594-glycobrush-Chol. d Flow cytometry data of HEK293T cells untreated (blue) or treated with AF594-glycobush-N3 (magenta) or AF594-glycobrush-Chol (black). Cell images in e–g are paraformaldehyde-fixed HEK293T cells that were incubated with 15 μM AF594-glycobrush-Chol and 30 μg/mL CF488A-transferrin, where e is imaging for AF594-glycobrush-Chol, f is imaging for CF488A-transferrin, and g is the overlay of the images in e and f. Scale bars are 50 μm. Images in b, c, e–g are representative of four separate experiments.
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Competing interests

The authors declare no competing interests.

Additional information

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