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Glycocalyx scaffolding to control cell surface glycan displays

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Abstract

This unit describes a protocol for remodeling cells with synthetic glycoprotein and glycolipid mimetics that are functionalized with lipid anchors, allowing for cell surface display of specific glycan structures in predefined nanoscale arrangements. CHO-Lec2 cells are prepared as adherent monolayers and, after reaching confluence, are incubated with the glycomaterials. Synthetic glycopolymers bearing α 2,3-sialyllactose glycans are used to decorate cellular surfaces in the form of 3D multivalent ligands projecting away from the cell surface, while α 2,6-sialyllactose glycolipid conjugates are used to anchor glycans in dynamic 2D arrays proximal to the cell membrane. Following washing, mimetic incorporation and glycan display can be analyzed using lectins with specificity for α 2,3- or α 2,6-linked sialic acids. Flow cytometry data reveals that cell surface remodeling with either glycoconjugate mimetic occurs efficiently in a dose-dependent manner. Combinations of glycoconjugates can also be employed simultaneously to generate a mixed glycocalyx with tunable composition and organization.

Keywords

Glycocalyx; glycans; glycopolymers; cell surface; lectins

Glycocalyx scaffolding to modulate cell surface glycan displays

This unit describes a protocol for the remodeling of cell surfaces in a hierarchical manner with nanoscale synthetic glycopolymers or proximal glycolipid conjugates bearing specific glycan structures (Figure 1). Mammalian cells are prepared as adherent monolayers and incubated with the materials. After washing, cells can be analyzed for surface display of glycans. As a model system, this protocol employs CHO-Lec2 (Chinese Hamster Ovary) cells that are deficient in sialyllactose glycans (Stanley 1983), allowing convenient analysis of remodeled cell surface glycan levels.

This unit describes protocols for both the chemical synthesis of synthetic glycomimetics and cell surface remodeling to generate well-defined glycocalyx presentations (Figure 1). It begins with a method for the synthesis of synthetic glycoconjugates (Basic Protocol 1), followed by a method for the preparation of cells as adherent monolayers and their

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Conflict of Interest

There are no conflicts of interest.

subsequent incubation with the glycoconjugates (Basic Protocol 2), and is completed with a method for glycocalyx characterization via fluorescence microscopy or flow cytometry (Basic Protocol 3).

NOTE: All chemical procedures should be performed under the supervision of senior personnel knowledgeable about chemical hazards and proper safety procedures.

BASIC PROTOCOL 1: SYNTHESIS OF α 2,3-SIALYLLACTOSE GLYCOPOLYMER (5) AND α 2,6-SIALYLLACTOSE GLYCOLIPID (4)

This protocol describes the synthesis of a glycolipid conjugate bearing α 2,6-sialyllactose (4) and a glycopolymer bearing α 2,3-sialyllactose glycans (5). Both materials are first made as azido conjugates (1 and 2) (Figure 2) and subsequently conjugated with a dibenzocyclooctyne phospholipid *via* strain-promoted azide-alkyne click (SPAAC) chemistry (Figure 3). The synthetic glycopolymer is assembled using controlled polymerization of an acrylamide monomer to form a multivalent polymer backbone via the Radical Addition Fragment Transfer (RAFT) technique (Moad et al. 2009), and subsequent ligation with free reducing end sugars (Huang et al. 2015; Huang et al. 2016).

Materials

ADMP (2-azido-1,3-dimethylimidazolinium hexafluorophosphate)

AIBN (α,α,α' -azoisobutyronitrile)

AlexaFluor488-maleimide (Thermo Fisher Scientific # A10254)

Azido chain transfer agent (ACTA) synthesized as previously described (Huang et al. 2014, 2b)

Chlorotrimethylsilane

DSPE-PEG2000-DBCO (Avanti Lipids # 880229) re-suspended as a 10 mM DMSO solution.

Glycans: 2,3-sialyllactose sodium salt and 2,6-sialyllactose sodium salt (Carbosynth # OS04397 and OS04398)

GPC (Gel permeation chromatography system, e.g. Hitachi Chromaster system equipped with an RI detector and a 5 μ m, mixed bed, 7.8 mm I.D. $\times$ 30 cm TSKgel column from Tosoh Bioscience)

Nitrogen line and high vacuum pump

N-Boc, *N*-methylaminoxypropylacrylamide monomer (2a)

n-butylamine in THF (20 mM)

PD-10 columns (GE Healthcare Life Sciences # 17085101)

Phenol

Quickspin columns (Roche Diagnostics # 11273913001)

Reaction buffer (1 M urea, 1 M NaOAc in water, pH 4.5)

Schlenk flask (10 mL capacity)

Semi-preparative RP-HPLC system equipped with a C12 column. For example, a Jupiter Proteo C12, 10×250 mm column. Eluent: 0.1% TFA/5% CH₃CN/H₂O.

Solvents: chloroform, diethyl ether, dichloromethane (DCM), n-hexanes, 1,4-dioxane

Trifluoroacetic acid

Synthesize the α 2-3 sialyllactose phospholipid glycopolymer (5; Figure 2)

- 1 Prepare a clean, flame-dried Schlenk flask that is equipped with a magnetic stirrer, an inlet tube with positive nitrogen (N₂) flow and another inlet tube with connection to a high vacuum pump.
- 2 Add the azido chain transfer agent (38.58 mg, 80.9 μ mol) and AIBN (11.25 mg, 68.5 μ mol) to the flask. Then, deliver the *N*-Boc, *N*-methylaminooxypropylacrylamide monomer (**2a**, 296 mg, 1.15 mmol) as a pre-prepared solution in anhydrous dioxane (420 μ L). Use extra anhydrous dioxane (167 μ L) to wash down reagents that are stuck on the sides into the bottom of the flask.
- 3 Degas the flask with multiple freeze-pump-thaw cycles. Repeat until the frozen solution no longer bubbles under negative pressure. Let the solution warm to room temperature.
- 4 Seal the inlets and let the mixture react in a pre-heated 65°C oil bath, ensuring the flask is submerged equally on all sides. Let the reaction proceed for seven hours, and quench the reaction by submerging the flask in a dry ice-acetone bath.
- 5 Dilute the reaction mixture in ether, and precipitate the solution three times in excess hexanes by vigorous stirring and centrifugation (1000 xg, 3-4 min). Concentrate the resulting residue in CHCl₃, and place it under high vacuum to dry.

Using this procedure, a white residue is isolated as the azido polymer (184 mg, 62% isolated yield) product. GPC analysis (isocratic 0.7 mL/min method in DMF with 0.2% LiBr at 70°C) indicates this polymer has a degree of polymerization (*n*) of 22, and a dispersity index (*M_w*/*M_n*) of 1.136, *M_w* = 6824, *M_n* = 6010. ¹H NMR (CDCl₃, 300 MHz): δ (ppm): 3.90-3.65 (bs, 2H), 3.35-2.80 (bm, 5H), 1.80-1.05 (bm, 16H).

- 6 Conjugate a fluorophore to the polymer backbone by first releasing the trithiocarbonate end group (**2c**) that is incorporated from the chain transfer agent, then letting the exposed free thiol (**2d**) react with the AlexaFluor488-maleimide fluorophore.
- 6a Charge a Schlenk flask with a stir bar and the azide polymer isolated from the previous step. Add a 100 mg/mL equivalent of a degassed solution of n-

butylamine in THF (20 mM). Let the solution react for 2 hrs on an ice bath. Dilute the reaction in ether and precipitate into excess hexanes. Repeat this step three times, isolating the residue at each step. Concentrate the end-deprotected polymer to remove residual hexanes.

- 6b** Dissolve the residue in AlexaFluor488-maleimide (1.1 eq., 2 mM in DMF). Degas the solution with three freeze-pump-thaw cycles, and let the reaction proceed overnight in the dark. Dilute the reaction in ether and precipitate in excess hexanes as before. The dried polymer (**2e**) can be stored in the freezer until ready for next use.

For small quantities, centrifugation at 1000 xg, 3-4 minutes is a more efficient way to isolate the polymer compared to the vortexing method.

- 7** To remove the Boc protecting groups from the polymer side chains, add a freshly prepared solution (0.5 mL) of TMS-Cl (1M) and phenol (3M) in anhydrous DCM. Stir for 2 hours, RT, in the dark. Precipitate the polymer by adding ether (20 mL) and centrifugation (1000 xg, 3-4 min), and decantation. Repeat three times, then briefly dry the residue under a stream of nitrogen.
- 8** Dissolve the residue in water and purify using a PD-10 column. Collect the first-eluting colored fraction (the later eluting colored fraction is the unreacted fluorophore), freeze, and lyophilize to dry.

Isolate a small quantity of this material (~ 1 mg) and dissolve in a known amount (e.g. 0.3 mL) of water. Collect UV-Vis absorbance readings at 495 nm and use the Beer-Lambert law ($A = \epsilon bc$, where A is the absorbance at 495 nm, b is the pathlength, and ϵ is the AlexaFluor 488 extinction coefficient $71000 \text{ M}^{-1} \text{ cm}^{-1}$) to calculate the concentration of the AlexaFluor488 fluorophore in this solution. Determine fluorophore labeling efficiencies by calculating the ratio of concentration of fluorophore and concentration of polymer (by weight)³

- 9** Re-dissolve the polymer (**2f**, 2.69 mg) in reaction buffer such that the side chains are in 200 mM final concentration. Vortex and rock until the sticky polymer is completely dissolved. Add the desired amount of glycan (e.g. α 2,3-sialyllactose), such that there is 1.1 molar equivalents of the glycan relative to each side chain. Vortex and spin to dissolve. Let the solution react for 72 hr at 50°C. Isolate the glycopolymer (**2**) using the Quickspin DNA columns. Dilute with deuterated phosphate buffer for ^1H NMR and UV-Vis analysis.

To calculate polymer/side chain concentrations, use the isolated weight of polymer **2f** and the molecular weight of the polymer (determined as the product of n , the number of repeating units, and the molecular weight of the repeating unit, 158.2). For example, 2.69 mg of **2f** ($n = 22$) with a molecular weight of 3480.4 ($158.2 * 22$) yields 7.7×10^{-4} mmol of polymer or 1.7×10^{-2} mmol of side chains. To generate a 200 mM by side chain solution, this amount of polymer was dissolved in 85 μL of reaction buffer. 9.12 mg of α 2,3-sialyllactose (FW 655.53, 0.014 mmol) requires 63

μL of the polymer in reaction buffer to achieve a 1.1 molar equivalent of glycan per reactive side chain. Ligation efficiencies of 50-70% are routinely obtained using this procedure for both glycans. Determine polymer concentrations using absorbance values at 495 nm and the pre-calculated fluorophore labeling efficiencies (from step 8).

- 10 In a small Eppendorf tube, react the azido glycopolymer (2, e.g. 300 μL of a 900 μM solution) with DBCO-PEG(2000)-DSPE (1.1 eq., 29.2 μL of 10 mM DMSO solution) overnight at RT. The crude reaction mixture can be used for cellular remodeling.

Synthesize the α 2-6 sialyllactose glycolipid (4)

- 11 Generate a fresh batch of the ADMP (2-azido-1,3-dimethylimidazolinium hexafluorophosphate) reagent according to previously published procedures. (Kitamura et al. 2015)

This reference is an excellent resource for the preparation of ADMP.

It lists commercial sources for starting materials and includes pictures for reaction set-up and crystallization procedures.

- 12 Add α 2,6-sialyllactose (28.4 mg, 0.0433 mmol) and triethylamine (30 μL, 0.212 mmol) and 1 mL of a 4:1 D₂O:CH₃CN solvent mixture into a round-bottom flask equipped with a magnetic stirring bar. Stir in an ice bath (0°C for 5 minutes).
- 13 Add freshly re-crystallized ADMP (37 mg, 0.130 mmol) to the stirring solution and let the mixture react for 3 hours.
- 14 Dilute the reaction mixture with 0.1% TFA/water (4 mL) and purify the product via semi-preparative RP-HPLC. Isolate the relevant fractions, freeze, and lyophilize to yield colorless crystals.

A 1.8 mL aliquot used for RP-HPLC preparation yields 5.54 mg of the purified ADMP reagent. ¹H NMR analysis (500 MHz, D₂O); δ (ppm) 5.34 (s, 1H) 4.67 (d, J = 7.88, 1H), 4.40 (d, J = 7.88 Hz, 1H), 4.04 (d, J = 7.88 Hz, 1H), 3.92 - 3.44 (m, 16H), 3.27 (t, J = 8.95 Hz, 1H), 3.13 (q, J = 7.30 Hz, 6H), 2.68 - 2.61 (m, 1H), 1.96 (s, 3H), 1.77 - 1.63 (m, 1H), 1.21 (t, J = 7.40 Hz, 9H). Mass Spectrometry (ESI-MS) calculated for C₂₉H₅₂N₅O₁₈ [M-Et₃N]¹⁻, m/z 657.21, found [M-Et₃N]¹⁻, m/z 658.31; [M+H-Et₃N-N₃]¹⁻, m/z 615.36

- 15 React the azido conjugate (1.4 mg, 2.6 mg/mL solution in water) with DBCO-PEG(2000)-DSPE (1.1 eq. in 10 mM DMSO) overnight at RT. The crude reaction mixture can be used for cellular remodeling.

BASIC PROTOCOL 2: PREPARATION OF CELLS AS ADHERENT MONOLAYERS AND INCUBATION OF CELLS WITH GLYCOPOLYMERS

This section describes the culture and maintenance of CHO-Lec2 cells, and their subsequent treatment with the phospholipid glycopolymers synthesized from Basic Protocol 1. The phospholipid groups on the glycopolymers allow their passive incorporation into the cellular membranes (Rabuka et al. 2008).

Materials

CHO-Lec 2 cells (ATCC # CRL-1736)

Complete growth medium: 10% fetal bovine serum (FBS) in MEM α

Dulbecco's phosphate-buffered saline (DPBS)

MEM α , no nucleosides (Life Technologies # 12561056)

Tissue-culture treated plastic tubes and dishes

0.05% Trypsin-EDTA solution (Life Technologies # 25300120)

4% (w/v) para-formaldehyde/PBS

1% (w/v) para-formaldehyde/PBS

NOTE: All solutions or equipment that come in contact with live cells must be kept sterile. Proper aseptic techniques should be observed when handling live cells.

NOTE: The subsequent procedures should be performed in a biological hazard laminar-flow hood. Obtain proper safety approvals prior to commencing work.

Culture CHO-Lec2 Cells and Remodel the Surface with Synthetic Glycoconjugates

1. Transport the frozen cryovial of CHO-Lec2 cells in dry ice into the tissue culture facility. While frozen, wipe the outside of the cryovial with a paper towel covered with 70% ethanol to sterilize. Slowly open the cryovial cap inside the biosafety cabinet to release built-up pressure. Re-cap the cryovial and thaw it in a warm sterile water bath. Submerge the vial only until the water bath meets the bottom of the opening for about 1-2 mins.
2. While there is still a small amount of ice left, transfer the cell suspension into a conical tube with 4 mL of pre-warmed complete media. Centrifuge the conical tube at 300 xg for 3-4 mins. Remove the supernatant and pipet 1 mL complete media to relieve the cell pellet. Transfer the cell suspension into one well of a six-well dish containing 2 mL complete media.
3. Incubate the plate at 37°C, 5% CO₂. Monitor cell health and growth the next day. If a lot of floating cell debris is present the next day, wash the cells once with DPBS and add fresh complete media.
4. When the cells are ~70% confluent and ready to be split, wash the cells once with DPBS, and incubate with pre-warmed 0.5 – 1.0 mL of trypsin-EDTA for

3-5 minutes, or until the cells detach. Neutralize the trypsin with an equal volume of complete media. Split the cells at defined ratios (e.g., 1:5, 1:10, 1:20) into new wells. Observe the cells every day to determine the number of days and splitting ratios that cause cell confluence.

Typically, CHO-Lec2 cells become confluent in two days at a 1:10 splitting ratio, similar to wild-type CHO cells.

5. Split the cells once or twice from being thawed to ensure optimal cell health and recovery.
6. To prepare for the remodeling experiment, seed the CHO-Lec2 cells to 70% confluency 18-24 hours in tissue-culture treated 24-well plates in complete growth medium.
7. Next day, check that the cells are healthy, adherent, and confluent. Wash the cells once with pre-warmed DPBS, and add pre-prepared solutions of the α 2,6-sialyllactose lipid (4) and α 2,3-sialyllactose lipid polymer (5) in MEM α . Incubate for desired periods of time (e.g. 1 hr) at 37°C.

Dilutions (0-1000 mM by glycan) of both lipid conjugates in MEM α were successfully incorporated into CHO-Lec2 cells. Cells may be incubated with the individual materials separately or in defined mixtures of both materials. At minimum, duplicate technical replicates are required. Biological replicates are highly recommended. See Figures 3-6 for expected results.

8. Wash the cells twice with pre-warmed DPBS. For microscopy analysis, fix the cells by incubating with 4% PFA/PBS for 10 min at RT, and washing twice with DPBS. For flow cytometry analysis, detach the cells from the plate by incubating with 0.05% trypsin-EDTA for 3-5 mins. Neutralize the suspension by adding an equal volume of complete media. Collect the cells into an Eppendorf tube, centrifuge at 300 xg, 3-4 min. Wash twice with DPBS, and incubate with 1% PFA/PBS for 30 mins on ice. Wash twice with DPBS and re-suspend in 1% BSA/DPBS. Both the fixed plate and cell suspensions can be stored at 4°C.

Fixation of cellular samples is not an absolute necessity for the analysis of polymer incorporation, if analysis can be performed shortly (within 1 hr) of incorporation. We have observed a natural turn-over of the polymers from the cell surface (half-life ~ 8 hours, Huang et al. 2014) that should be noted. Should live cell analysis be desired, storage of cellular samples under ice is recommended.

BASIC PROTOCOL 3: ANALYSIS OF REMODELED CELLS BY FLOW CYTOMETRY OR MICROSCOPY

In this method, the remodeled CHO-Lec2 cells are analyzed for the amount of glycopolymer incorporated into their cellular membranes, as well as for the glycans on their cell surfaces. The latter is performed using lectins, glycan-binding proteins that show preference for various carbohydrate structures.

Materials

Benchtop microcentrifuge (e.g., Beckman Coulter Microfuge 20)

Biotinylated *Maackia amurensis* lectin (bMALII) (Vector Labs # B-1265, 1 mg/mL)

Cy5-streptavidin (Molecular Probes # SA1011)

Dylight 649 conjugated *Sambucus nigra* lectin (Dy649-SNA, EY Labs # Dy649-6802-1)

Fluorescence microscope (e.g. Zeiss Axiovert A.1 equipped with GFP/AlexaFluor488 and Cy5/Dylight649 filters)

Flow cytometer (e.g., BD Accuri C6 equipped with GFP/AlexaFluor488 and Cy5/Dylight649 filters)

1 % BSA/DPBS (+Ca, +Mg)

Lectin staining

- 1 The preserved cell suspensions can be stained for α 2,3-sialyllactose by incubation with bMAL II (1:100, 10 μ g/mL) in 1% BSA/DPBS overnight at 4°C with gentle agitation (i.e. end-over-end mixing or rocking).

Make sure to prepare control samples, including 1) cells that are not treated with polymer, 2) cells that are stained only with Cy5-streptavidin, and 3) cells that are neither treated with the polymer nor any of the staining reagents (“cells only”). At minimum, technical duplicate samples are recommended and the experiment should be conducted with at least two biological replicates.
- 2 Wash the cells twice by centrifugation at $300 \times g$, 3–4 minutes and resuspension in 1% BSA/DPBS.
- 3 Next day, incubate the cells with Cy5-Streptavidin (1:500) in 1% BSA/DPBS for 1 hr at 4°C. Wash cells twice as before and re-suspend in 1% BSA/DBPS.
- 4 Cells can also be stained for cell surface α 2,6-sialyllactose glycans using Dy649-SNA (1:25, 40 mg/mL) in 1% BSA/DPBS overnight at 4°C. with gentle agitation. After washes and re-suspension in 1% BSA/DPBS, cells can be imaged directly by fluorescence microscopy or analyzed by flow cytometry.

Analyze by flow cytometry

- 5 Prepare the flow cytometer for analysis by running 0.22- μ m purified water (e.g. Milli-Q water) prior to the samples.
- 6 Analyze the “cells only” control to generate the appropriate forward and size-scattering gates to exclude debris.
- 7 Analyze the AlexaFluor488 and the Dylight 649 or Cy5 fluorescence signal of subsequent samples based on the appropriate scattering gates determined above, including live cells and excluding debris. Collect at least 5-10,000 events within

this gate. The former signal (AlexaFluor488) results from polymer incorporation and the latter (Dylight649 or Cy5) from staining with the lectins.

- 8 Using the AlexaFluor488 or the Dylight649 fluorescence geometric mean of each sample, determine the average and standard deviation values of technical replicates. Plot the data as desired.

REAGENTS AND SOLUTIONS

1. The DBCO-PEG(2000)-DSPE reagent can be prepared as a 10 mM solution in DMSO (or d₆-DMSO for convenient NMR analysis) and stored at -20°C indefinitely. A glass vial container is recommended for storage.
2. The lectins may be purchased as lyophilized powders or solutions. In the former case, follow manufacturer's instructions for reconstitution. The lectin solutions are stable in short term at 4°C or at -20°C for prolonged storage.
3. Complete media for the culture of CHO-Lec2 cells: MEMα + 10% (v/v) FBS. Solutions can be filtered through a 0.22-μm membrane for additional sterilization. Complete media can be stored at 4°C for short-term storage.

COMMENTARY

Background Information

This procedure is performed to display sialyllactose glycans on cellular surfaces in a nanoscale, hierarchical manner, similar to the canopy of carbohydrates formed by native glycoproteins and glycolipids. In this protocol, this display is achieved with a multivalent α 2,3-sialyllactose glycopolymer (**5**) that can extend several tens of nanometers away from the cellular membrane and with α 2,6-sialyllactose glycolipids (**4**) that are anchored proximally to the cell surface. Both materials are equipped with a phospholipid tail that allows for passive incorporation into cell membranes (Rabuka 2008). The central advantage of the glycopolymer technique is its ability to display multiple copies of the glycan in a multivalent manner.

CHO-Lec2 cells are mutants of the well-known CHO cell line (Stanley 1983). CHO-Lec2 cells exhibit defects in the ability to transport the activated nucleotide sugar precursor, CMP-sialic acid. Thus, they have low levels of sialic acid glycans, common terminal sugars found on glycoproteins and glycolipids.

Critical Parameters and Troubleshooting

1. RAFT polymerization is sensitive to air and the presence of peroxides. Should polymerization be difficult to achieve, check that glassware and tubing produce good seals, and that vacuum pumps produce high vacuum to degas the reaction mixture.
2. RAFT polymerization can be improved with the use of fresh dioxane without stabilizers and filtering over basic alumina to remove peroxides.

3. For proper comparison of dose-dependent polymer incorporation by fluorescence microscopy, imaging settings, including exposure times and brightness/contrast settings, must be kept constant across different samples.

Anticipated Results

1. Both the α 2,3-sialyllactose phospholipid glycopolymer (**5**) and the α 2,6-sialyllactose glycolipid (**4**) were effective in remodeling the surface of CHO-Lec2 cells (Figure 4).
2. CHO-Lec2 cells remodeled with the α 2,3-sialyllactose phospholipid glycopolymer (**5**) display significant dose-dependent increases in AlexaFluor488 fluorescence when compared to its azide precursor (Figure 5). Some non-specific background binding of the azido glycopolymer is observed, but phospholipid incorporation into the cellular membranes displays much higher fluorescence.
3. Both the α 2,3-sialyllactose phospholipid glycopolymer (**5**) and α 2,6-sialyllactose glycolipid (**4**) exhibit dose-dependent incorporation into CHO-Lec2 membranes (Figure 6A). Note that in the former case, the number of glycans introduced to the cell surface (detected by lectin staining) reached saturation.
4. To generate a “mixed glycocalyx,” defined ratios of the glycopolymer and the glycolipid can be pre-made and introduced to cells. CHO-Lec2 cells treated in this manner display dose-dependent increases in lectin staining of the appropriate glycan (Figure 6B).

Time Considerations

The entire procedure, including chemical synthesis and incubation with cells, can be completed within one week's time, with access to the monomer (Huang et al. 2014). Synthesis and purification of the α 2,6-sialyllactose azido conjugate can be accomplished in three days, and synthesis of the α 2,3-sialyllactose azide glycopolymer can be performed simultaneously. The subsequent conjugation to the cyclooctyne-phospholipid is then performed overnight, and the crude products can be directly introduced into cells without purification. Incubation with cells and preparation of the fixed cell samples can be performed in one day. Because the plate containing the remodeled cells (for microscopy analysis) or the cell suspensions (for flow cytometry analysis) can be preserved well at 4°C, this stage is a convenient stopping point. Lectin staining and analysis by flow cytometry and fluorescence microscopy can then be performed at a later time. Steps that can be lengthened include the incubation with lectins, as long as the samples are left at 4°C.

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Significance statement

The cellular surface exhibits a complex system of glycoconjugates termed the glycocalyx. Therein, glycans presented in nanoscale arrangements in distinct regions of the glycocalyx harbor binding sites for glycan receptors involved in processes ranging from cell adhesion and migration to signaling and differentiation. For instance, glycoconjugates such as glycolipids are found proximal to the cell membrane, whereas glycoproteins can project up to hundreds of nanometers from the surface. This complexity within the natural glycocalyx obscures systematic analysis of how the presentation of glycans influences their biological functions. In the absence of molecular biology tools for spatiotemporal control of glycosylation, chemical methods for glycan engineering, such as the one described here, provide powerful alternatives that may enable such investigations.

Figure 1.
The chemical synthesis of, and cell surface remodeling with, membrane-targeting synthetic glycoconjugates.

Figure 3.
Installation of azide precursors with DBCO-PEG(2000)-DSPE to generate phospholipid conjugates for anchoring to cellular membranes.

Figure 4. Synthetic glycolipids (left panel) or glycopolymers (right panel) are synthesized and introduced into the surfaces of mammalian cells. Subsequent analysis can be performed to determine the extent of incorporation.

Figure 5. Flow cytometry comparison of cellular fluorescence after treatment with the α 2,3-sialyllactose azido or the phospholipid glycopolymer.

Figure 6.

A) Dose dependent membrane incorporation of **4** and **5**. B) Dose dependent membrane incorporation of **4** and **5** to form a mixed glycocalyx.