Cell surface glycan engineering reveals that matriglycan alone can recapitulate dystroglycan binding and function

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α-Dystroglycan (α-DG) is uniquely modified on O-mannose sites by a repeating disaccharide (-Xylα1,3-GlcAβ1,3-)n termed matriglycan, which is a receptor for laminin-G domain-containing proteins and employed by old-world arenaviruses for infection. Using chemoenzymatically synthesized matriglycans printed as a microarray, we demonstrate length-dependent binding to Laminin, Lassa virus GP1, and the clinically-important antibody IIH6. Utilizing an enzymatic engineering approach, an N-linked glycoprotein was converted into a IIH6-positive Laminin-binding glycoprotein. Engineering of the surface of cells deficient for either α-DG or O-mannosylation with matriglycans of sufficient length recovers infection with a Lassa-pseudovirus. Finally, free matriglycan in a dose and length dependent manner inhibits viral infection of wildtype cells. These results indicate that matriglycan alone is necessary and sufficient for IIH6 staining, Laminin and LASV GP1 binding, and Lassa-pseudovirus infection and support a model in which it is a tunable receptor for which increasing chain length enhances ligand-binding capacity.
Dystroglycan (DG) is a highly glycosylated receptor involved in physiological processes such as maintenance of skeletal muscle-cell membrane integrity, signal transduction, brain development, and preservation of neuronal synapses. It is post-translationally cleaved into an extracellular α-subunit (α-DG) that is non-covalently linked to a transmembrane β-subunit (β-DG). The intracellular domain of β-DG interacts with several cytosolic proteins, most notably with the structural protein, dystrophin, which in turn binds the actin cytoskeleton. Through their participation in what is known as the dystrophin-glycoprotein complex (DGC), α-DG and β-DG provide a critical glycosylation-dependent link between the extracellular matrix (ECM) and the actin cytoskeleton, especially in muscle tissue.

Specific O-glycans on α-DG serve as receptors for laminin-G (LG) domain-containing (ECM) proteins such as laminin, agrin, perlecan and neurexin. Improper glycosylation of α-DG, due to mutations in genes encoding the involved glycosyltransferases or the enzymes associated with sugar-nucleotide donor biosynthesis, leads to multiple forms of congenital muscular dystrophies collectively referred to as secondary or tertiary dystroglycanopathies, respectively. Furthermore, certain arenaviruses, such as Lassa virus (LASV), have evolved cell surface glycoproteins with LG-domains that utilize the same O-glycan structures on α-DG as a receptor to gain entry into host cells. LASV causes severe hemorrhagic fever in humans with a mortality rate approaching 15 to 20% in hospitalized patients resulting in thousands of deaths each year in West Africa. The virus is carried by rodents of the Mastomys genus, and human infection occurs mainly via reservoir-to-human transmission. Due to the high fatality rate, lack of a vaccine, and limited therapeutic options, LASV is considered an important emerging pathogen. Under myogenesis, the molecular weight of dystroglycan (α-DG) contains a mucin-like domain rich in O-glycans on α-DG, due to the terminal ribitol-5-phosphate resulting in a GlcA-phospho-trisaccharide is further modified by the enzyme HNK-1ST, preventing further extension. The resulting phospho-trisaccharide is further modified by the Golgi-resident enzymes fukutin (FKTN) and fukutin-related protein (FKRP) to form the phospho-monosaccharide destined to be extended and contain laminin-binding sites arenaviruses such as lymphocytic choriomeningitis virus (LCMV) and Lassa virus (LASV). Importantly, cells defective in any of the post-ribitol glycosyltransferases have reduced molecular weight, no longer bind IIH6, and have a complete loss of laminin binding.

The length of matriglycan varies in a developmental and tissue-specific manner, as shown by marked differences in α-DG apparent molecular weight on reducing SDS-PAGE. During myogenesis, the molecular weight of α-DG and the expression levels of DAG1 and LARGE1 increase at the same time. In both cultured cells and mice, ectopic expression of LARGE1 leads to substantial increases in the level of glycosylation of α-DG, which in turn increases the potential to bind ligands of the ECM. Alternatively, the non-reducing end GlcA of matriglycan can be capped by sulfation by the enzyme HNK-1ST, preventing further extension. In brain, matriglycan has the smallest number of repeating units and the highest ratio of expression of HNK-1ST to LARGE1. Collectively, these findings support a model in which the expression of LARGE1 and HNK-1ST controls the length of matriglycan.

The functional glycosylation of α-Dystroglycan involves the three post-ribitol enzymes needed for priming and synthesis of matriglycan shown. Carbohydrate symbol representation is consistent with Symbol Nomenclature for Graphical Representations of Glycans. Endogenous α-DG was examined in human HAPII cells (HAPII-WT) as well as HAPII cells with genetic defects in the post-ribitol enzymes RXYLT1, B4GAT1, or LARGE1. The molecular weight of α-DG was greatly diminished in the cells lacking the post-ribitol glycosyltransferases and was no longer reactive with the IIH6 antibody. α-DG from the three cell lines lacking these enzymes also displayed a complete loss of binding to laminin in an overlay assay. These results highlight the importance of post-ribitol glycosyltransferases for functional glycosylation of α-DG. Three independent experiments were performed with similar results each time.

**Fig. 1 Functional Glycosylation of α-Dystroglycan.** 
**a.** Cartoon representation of the fully elaborated O-mannose M3 glycan that is present on 2 sites of α-dystroglycan with the three post-ribitol enzymes needed for priming and synthesis of matriglycan shown. Carbohydrate symbol representation is consistent with Symbol Nomenclature for Graphical Representations of Glycans. 
**b.** Endogenous α-DG was examined in human HAPII cells (HAPII-WT) as well as HAPII cells with genetic defects in the post-ribitol enzymes RXYLT1, B4GAT1, or LARGE1. The molecular weight of α-DG was greatly diminished in the cells lacking the post-ribitol glycosyltransferases and was no longer reactive with the IIH6 antibody. α-DG from the three cell lines lacking these enzymes also displayed a complete loss of binding to laminin in an overlay assay. These results highlight the importance of post-ribitol glycosyltransferases for functional glycosylation of α-DG. Three independent experiments were performed with similar results each time.
matriglycan, which in turn, regulates the binding of LG domain-containing proteins.

Despite these observations, it has not been established how many repeating units are needed to bind LG domain-containing proteins. It is also not known whether the protein component of α-DG or the underlying M3 glycan are required for all its functions. One study demonstrated, however, that high molecular weight synthesized LARGE-glycan chains, but not low, are capable of binding laminin-111 and the antibody IIH6, while another more recent study found that a pentamer based on the non-reducing end of matriglycan is capable of binding to laminin-a2 LG 4–5. Other studies have utilized overexpression of LARGE, the matriglycan polymerase, in various knock-out cell lines to demonstrate that other glycoproteins containing O-GalNAc and/or complex N-linked glycans can become IIH6 reactive. These observations indicate that the protein and underlying M3 glycan structure may be dispensable for certain functions and that matriglycan binding is length-dependent.

The importance of glycan structure and the underlying polysaccharide for binding and biological activity is difficult to study because glycan biosynthesis is a non-template mediated process, and therefore conventional genetic approaches do not allow modulation of glycan structures in a systematic manner. Glycan array technology, in which hundreds of well-defined compounds are printed on a surface, have been instrumental in establishing binding partners of glycan-binding proteins. The primer was reacted with NHS-activated propargyl moiety. The primer

Results

Matriglycan synthesis and glycan microarray screening.

Matriglycan oligosaccharides with a defined number of repeating units were prepared chemoenzymatically to develop a glycan microarray to establish structure-binding relationships for LG-domain binding proteins. First, xyloside 1 (Fig. 2a) was synthesized, which has an anomerich annonemopentyl-linker for immobilization of the glycans to a carboxy reactive surface for microarray fabrication. We opted for a strategy in which 1 was primed by the enzyme β-1,4-glucuronyltransferase (B4GAT1) to provide the disaccharide GlcA-β1,4-Xyl (2), which is an appropriate substrate for the glycosyltransferase LARGE. It was anticipated that exposing 2 to LARGE1 in the presence of excess UDP-Xyl and UDP-GlcA would result in the formation of oligosaccharides having different numbers of repeating units. Fractionation of the mixture would then give a range of oligosaccharides of different chain length.

Thus, compound 1 was primed by B4GAT1 in the presence of UDP-GlcA to provide 2 in a near quantitative yield, which was exposed to LARGE1 in the presence of UDP-GlcA (21 eq) and UDP-Xyl (20 eq). A slight excess of UDP-GlcA relative to UDP-Xyl was employed to ensure that each structure terminated with the same monosaccharide unit. Analysis of the reaction mixture by electrospray ionization mass spectrometry (ESI-MS) revealed the presence of oligosaccharides having 2–14 repeating units. Matriglycans with 2–8 disaccharide repeating units (Figs. 2a, 3a–g) could readily be fractionated by semi-preparative HPLC using a Waters XBridge BEH Amide hydrophilic interaction liquid chromatography (HILIC) column and ESI-MS for detection (Supplementary Fig. 1, Supplementary Table 1, Supplementary Information). Separation by HILIC-HPLC was more challenging for compounds with nine or more repeating disaccharide units and these matriglycans were isolated as mixtures of 9–11 (3h) and 12–14 (3i) repeating units. Each compound terminated in a glucuronic acid moiety which may be due to the slight excess of UDP-GlcA, but possibly also due to the higher catalytic activity for GlcA transfer at the reaction used conditions, most notably the pH (MES buffered solution, pH 6.0).

Matriglycans 3a–i were printed on N-hydroxysuccinimide (NHS) activated glass slides. The resulting slides were exposed to different concentrations of the anti-α-DG antibody IIH6, the α-DG binding protein laminin LG4/5, and LAV glycoprotein 1 (LASV GP1). The antibody IIH6 is widely employed to detect functional glycans on α-DG, however, its ligand requirements regarding matriglycan length have not been established. Co-crystallization and NMR binding studies have demonstrated that a matriglycan pentasaccharide can bind to the laminin globular (LG) 4-domain, but laminin has not been examined in microarray binding studies with defined matriglycans. No or weak binding to the antibody IIH6 was observed for matriglycans with less than 4 repeating units (2 and 3a–b, 2–8 monosaccharide units). Interestingly, a compound with 4 repeating units (3c, 10 monosaccharide units) was well recognized by the antibody IIH6 and binding gradually increased as the matriglycans were elongated (3d–i), despite the absence of the α-DG polypeptide or the underlying O-Man M3 core (Fig. 2b). Both laminin LG4/5 and GP1 LASV GP1 also displayed length-dependent binding (Fig. 2c, d) to the printed matriglycans. No binding was observed for secondary antibody alone.

Cell-surface glyco-engineering with well-defined matriglycans.

The microarray studies indicated that the binding of various proteins to matriglycan is length-dependent requiring at least ~4 repeating units (n = 4, decasaccharide). To establish whether the structure-binding data correlates with biological function, we sought to modify the plasma membrane extracellular surface of human HAP1-DAGI cells with well-defined matriglycans for functional studies. These cells have a mutation in the DAGI gene, which encodes α-DG, and therefore do not present matriglycan on α-DG at the cell membrane surface. We opted for a cell-surface glycan engineering strategy that utilizes recombinant ST6GAL1 and CMP-Neu5Ac derivatives modified at C-5 with a bi-functional entity composed of a matriglycan of defined length and biotin. The approach exploits the finding that ST6GAL1 tolerates modification at C-5 of CMP-Neu5Ac and can readily transfer a modified sialic acid to N-linked glycoprotein acceptors of living cells. The biotin moiety provides a handle for monitoring the cell-surface labeling with concurrent structure-function analysis.

CMP-Neu5Ac derivatives 10a–i (Fig. 3a) were prepared by a convergent strategy where the bifunctional entities 8a–i were assembled first, followed by conjugation to C5-azide functionalized CMP-Neu5NaZ (9) by copper-catalyzed alkyne–azide cycloaddition (CuAAC). The late-stage conjugation made it possible to preserve the labile sugar-nucleotide donor. Thus, xylose derivative 1 was reacted with NHS-activated propargyl glycone (4) in the presence of DIPEA to install an aryl ketone functionality (1a) and the resulting compound was immediately treated with Et3N to remove the Fmoc protecting group (1b). The amine of 1b was reacted with an NHS-activated biotin 5 to give bifunctional 6 having a biotin and alkyne moiety. The primer...
Fig. 2 Matriglycan microarrays of defined lengths for binding studies. a Chemoenzymatic synthesis scheme of defined lengths of matriglycan. b–d Microarray binding results with the matriglycan library at 100 μM utilizing b mAb IIH6 at 5 μg mL⁻¹, c recombinantly-tagged LG4-LG5 domains of mouse Laminin α1 (His8-GFP-Lama1) at 20 μg mL⁻¹, and d Recombinant LASV GP1 protein at 100 μg mL⁻¹. For b–d, measurements were taken at n = 4 technical replicates, where bars represent the mean and error bars represent SD. Source data of b–d are provided as a Source Data file.

Importantly, neither untreated or neuraminidase-treated fetuin cross-reacted with the IIH6 antibody nor was bound by laminin in the overlay experiment (Fig. 3b, c). Further experiments demonstrated that fetuin remodelled with matriglycans having 1 or 2 repeating units exhibits minimal reactivity in a Western Blot with the IIH6 antibody, while a mixture of 2–10 repeating units showed a strong signal (Supplementary Fig. 3). The strong signal observed with longer matriglycans was abolished by treatment with peptide N-glycosidase F (PNGase F), demonstrating that only N-glycans were modified (Supplementary Fig. 3). The latter is in agreement with previous studies that have shown that ST6GAL1 mainly modifies N-linked glycans.

To further demonstrate that matriglycan modified CMP-Neu5Ac can be transferred to N-glycan acceptors, a bis-galactosylated N-linked glycopeptide substrate (11, Supplementary Fig. 4) was isolated from egg yolk powder and enzymatically desialylated to provide a suitable substrate for reaction with ST6GAL1. The resulting N-glycan was treated with a mixture of CMP-Neu5Ac derivatives 10a-e in the presence of ST6GAL1 (Supplementary Figs. 4–6), and the product was analyzed by LC-MS using SeQuant ZIC-HILIC Amide column (Supplementary Fig. 7, Supplementary Table 3). We observed the formation of N-linked glycopeptides (compounds 12a-e, Supplementary Figs. 4, 7, Supplementary Table 3) modified by a sialoside bearing the expected 1–5 matriglycan repeating units. Only mono-sialylated products were observed, which is consistent with the branch selectivity of ST6GAL1, which prefers the 2-LacNAc over the α3-Man branch of N-glycans.

Next, attention was focused on engineering cells with well-defined lengths of matriglycan. Thus, HAP1-DAGI- cells were
Fig. 3 Synthesis and testing of CMP-Neu5Ac matriglycan compounds for labeling of N-linked glycoproteins. a Chemoenzymatic synthesis of bi-functional CMP-Neu5Ac compounds composed of defined matriglycan polymers and a biotin functionality for protein and cell-surface glyco-engineering. Analyses of the enzymatic transfers (ET) of the synthesized compound show positive labeling of fetuin via reactivity with the anti-glyco-α-DG antibody IIH6, introduces the ability to bind recombinant GFP-His-Laminin LG4/5 as determined by an overlay assay, and retards migration in SDS-PAGE as demonstrated by total protein Coomassie staining. +Neu +Neuraminidase, +ET Enzymatic Transfer by ST6GAL1. Three independent experiments were performed with similar results each time.
incubated with the matriglycan-modified CMP-Neu5Ac derivatives (10a-i, 100 μM) in the presence of ST6GAL1 and C. perfringens neuraminidase for 2 h at 37 °C (Fig. 4a). First, we confirmed that the matriglycan oligomers were displayed on the surface of HAP1-DAG1 cells by avidin staining followed by flow cytometry analysis. While the shorter oligomers gave somewhat more robust labeling suggesting more efficient transfer at equimolar concentrations (Fig. 4b), the results demonstrate that ST6GAL1 can also efficiently transfer the longer glycans including a compound having 6 disaccharide repeating units (10f; 14 monosaccharide units). Next, we examined whether the level of cell surface labeling can be controlled by varying the concentration of the CMP-Neu5Ac derivatives. Thus, different concentrations (1 to 100 μM) of matriglycan-CMP-Neu5Ac derivative 10d (n = 4; 10 monosaccharide units) was exposed to the HAP1-DAG1 cells (Fig. 4c). As anticipated, the level of labeling decreased as the concentration was reduced, but was still detectable at 1 μM.

Having confirmed that the CMP-Neu5Ac derivatives can efficiently install well-defined matriglycans on the surface of HAP1-DAG1 cells, binding of the IIH6 antibody was examined (Fig. 4d). Cells were labeled with 25 and 100 μM of the CMP-Neu5Ac derivatives and IIH6 binding was assessed by flow cytometry. Antibody binding was only observed for compounds having 5 or more repeating disaccharide units (10e; 12 monosaccharide units) and labeling became more robust when the length of the matriglycan increased (Fig. 4d). Even at 100 μM labelling concentration, IIH6 binding was not observed with matriglycan derivative 10d (4 repeats; 10 monosaccharide units), whereas similar IIH6 binding was observed with 10e (5 repeats; 12 monosaccharide units) at 25 and 100 μM (Fig. 4d). Cells modified with CMP-Neu5Ac derivative 10i having 9 repeating units (20 monosaccharide units) bound IIH6 only slightly weaker compared to wild type HAP1 cells that express endogenous α-DG (Fig. 4d). While there are likely more proteins harboring complex N-glycans than there are α-DG matriglycan sites, the chain length of matriglycan on α-DG may be longer thereby providing additional binding sites. The data was employed to choose lengths, concentrations, and labeling conditions for subsequent experiments that do not provide for more IIH6 reactive sites in the HAP1-DAG1 cells than present on wild-type HAP1 cells (Fig. 4d).

We enriched glycoproteins on HAP1-DAG1 cells that were labeled with matriglycan 10h having 8 repeating units by immunoprecipitation using an anti-biotin antibody and performed LC-MS/MS proteomic analysis. For comparison, HAP1-DAG1 cells were labeled in a similar fashion with biotinylated CMP-Neu5Ac that does not present a matriglycan moiety. Proteins were identified at a 1% false-discovery rate, and those identified in the negative controls were excluded from the final protein list. A similar subset of N-linked glycoproteins was labeled by ST6GAL1 using both modified CMP-Neu5Ac donors indicating that specificity of the enzyme is not substantially altered by using different modified donors (Fig. 4e). The spectral counts for CMP-Neu5Ac modified with biotin alone was higher which agrees with the finding that more complex derivatives transfer less efficiently.

**Cell-surface glyco-engineering rescues LASV infection.** Next, we sought to uncover the minimum number of repeating units required for matriglycan to elicit function. Towards this end, we employed a LASV-pseudovirus entry assay using a recombinant pseudotyped vesicular stomatitis virus (rVSV) in which the glycoprotein (GP) is replaced with that of LASV (rVSV-ΔG-LASV)11. The rVSV-ΔG-LASV contains a gene sequence for an enhanced green fluorescent protein (eGFP) that is utilized as a reporter and for quantification of infection. Using this assay, HAP1 wild type (WT) cells are readily infected by rVSV-ΔG-LASV in an α-DG-dependent manner, whereas HAP1-DAG1 cells resist infection. The infectivity of rVSV-ΔG-LASV (MOI 1) was assessed by fluorescence microscopy and quantifying the number of GFP-positive cells 24 h post infection using a Nexcelom Cellometer.

Matriglycans comprised of 2–9 disaccharide repeating units (10a-i) were displayed on HAP1-DAG1 cells at concentrations ranging from 0.1 to 100 μM using ST6GAL1 in the presence of C. perfringens neuraminidase. Remarkably, the cell surface glycan engineering could restore infectivity in a length- and concentration-dependent manner (Fig. 5a, b). At the lowest labeling concentration (0.1 μM), only the longest matriglycan assessed (10i; n = 9; 20 monosaccharides) restored infectivity, which was 80% of WT cells. At higher labeling concentrations, additional compounds rescued infectivity. At the highest labeling concentration assessed (100 μM), an oligosaccharide having 4 repeating units could rescue infectivity but not shorter ones. Although shorter matriglycans are more efficiently transferred (Fig. 4), they have reduced or no activity, highlighting the importance of matriglycan length for infectivity. To further evaluate these findings and to demonstrate that O-mannosylation is not substantially involved in the labeling process, we infected HAP1-POMT2 cells that are deficient in classical O-mannosylation. Infection was blocked in the HAP1-POMT2 cells compared to WT, but partially restored by labeling of the N-linked glycans with a matriglycan with 6 repeating disaccharide units (10f; 14 monosaccharide units; Fig. 5c).

**Defined soluble matriglycans can inhibit LASV infection in wildtype cells.** Previous studies have demonstrated that soluble, purified α-DG can inhibit LASV infection in vitro31. To determine if the synthetic matriglycans can act as a decoy receptor, we employed the matriglycans from Fig. 2a (3a-e) as inhibitors of α-DG-mediated viral infection of WT HAP1 cells. Thus, the cells were exposed to rVSV-ΔG-LASV (MOI 1) in the presence or absence of matriglycans having 0, 2, 4, and 6 repeating disaccharides at various concentrations (Fig. 6). Matriglycans with 0 or 2 repeats displayed no to little inhibition of infectivity. Matriglycans having 4 and 6 repeating units were potent inhibitors with IC50 values of 11.2 ± 0.7 and 3.2 ± 0.5 μM, respectively. Thus, substantial inhibition of infection was achieved with free matriglycans in the absence of the extended M3 glycan or the α-DG polypeptide.

**Discussion**

Although most mammalian cells express α-DG core protein, its functional glycosylation is under strict tissue-specific control. There is data to support that the matriglycan component of α-DG is a tunable scaffold for LG domain-containing proteins, and by controlling matriglycan length, cells may regulate the recruitment and strength of interaction with such extracellular matrix proteins39. Furthermore, there are indications that the α-DG protein is not required for all of matriglycan’s functions61. Here, we demonstrate that matriglycan is both necessary and sufficient for binding to the clinically-useful IIH6 antibody and LG domain-containing proteins (Figs. 1 and 2). Furthermore, the binding of matriglycan to IIH6, laminin LG4/5, and LASV GP1 is length-dependent, requiring at least 4–5 repeating disaccharide units (10–12 monosaccharide residues, Fig. 2). In each case, the binding gradually increased when the oligosaccharide became longer. Importantly, similar structure-binding profiles were observed for the matriglycans presented on a microarray surface.
Fig. 4 Detection of matriglycan on HAP1-DAG1 cells by flow cytometry. a CMP-Neu5Ac’s modified with defined matriglycan polymeric repeats (100 μM) are engineered on HAP1-DAG1 cells using ST6GAL1 in the presence of C. perfringens neuraminidase. b Detection of matriglycan with 1, 4, 5, and 6 disaccharide repeats on HAP1-DAG1 cells by flow cytometry. Cells were stained with avidin-AF488 and co-stained with PI to exclude non-viable cells. c Detection of matriglycan with 4 repeats at various concentrations of modified donor. d Binding of IIH6 to HAP1-WT and HAP1-DAG1 matriglycan modified cells. e Shotgun proteomics analysis of proteins immunoprecipitated from HAP1-DAG1 cells labeled with Biotin or Biotin+8 disaccharide repeats. Proteins present in the negative control experiment (unlabelled cells), had fewer than 10 spectral counts in the CMP-Neu5Ac-(Biotin) labeling experiment, or known to be localized in intracellular compartments as assessed by UNIPROT annotations, were excluded. Proteins shown are all annotated in UNIPROT to contain sites of N-glycosylation or were manually validated to contain at least one N-X-(S/T) N-glycosylation sequon in the primary sequence. One representative run is shown. Three independent experiments were performed with similar results each time. Source data are provided as a Source Data file.
and thus it was surprising that IIH6 has a preference for much larger structures. A few examples have been described in which longer oligosaccharides are required for antibody binding. For example, the unusual antigenic properties of meningococcal serogroup B capsular polysaccharide, which is composed of α,2,8-linked N-acetyleneuraminic acid (Neu5Ac) residues, has been ascribed to a conformational epitope requiring at least a decasaccharide to adopt a local helical structure. Previous co-crystallization and NMR binding studies have shown that a matriglycan pentasaccharide is sufficient for binding to the laminin globular (LG) 4-domain. The binding data reported here demonstrate, however, that longer glycans are required for efficient binding by recombinant mouse laminin α1 LG4/5, and binding became more robust with increasing chain length. It is possible that the conformational properties of matriglycans are length-dependent and that a threshold length is required to adopt a recognition conformation. This possibility is supported by a recent cryo-EM structure of the LASV Spike protein that indicate it can accommodate a matriglycan of at least 13 monosaccharides in a defined spiral-like conformation. It is also conceivable that longer matriglycans can provide a scaffold for multivalent interactions with LG domain-containing proteins resulting in high avidity binding. Previous studies have indicated that in general, at least two sequential LG domains are required for high-affinity binding. Such an assembly of domains has been observed for the LG4/5 of laminins α1, α2, α4, and α5, agrin and pikachurin. Moderate to high-affinity binding by a three LG-domain containing elements has been observed for laminin-α2 and α4 and perlecian. Furthermore, the LASV Spike protein is a trimer with 3 available sites for binding matriglycan. The tandem LG domain found in laminin-α2 of the skeletal muscle isoform and perlecian expressed at the neuromuscular junction, exhibit the highest
affinity binding to dystroglycan, which is in agreement with the biological importance of the DG adhesion complex to stabilize skeletal muscle and the post-synaptic element of the peripheral nervous system. Thus, the presence of tandem arrays of LG domains and the length of matriglycan may provide a way to modulate binding avidity and biological activity. We also have demonstrated that glycan function can be decoded independently from glycoprotein identity in a cell-based environment as LASV-pseudovirus infection was rescued in α-DG-deficient cells modified with defined matriglycans (Fig. 5). LASV entry is mediated by a glycoprotein complex that is composed of a trimer of heterodimers, each containing a receptor-binding subunit GP1, a transmembrane fusion-mediating subunit GP2 and a signal peptide that has several functions and is retained in the virion as part of the complex. GP1 binds to matriglycan on an α-DG to enter the endocytic pathway, where it binds to lysosome-associated membrane protein 1 (LAMP1) before membrane fusion. It is not known whether the protein component of α-DG plays a role in infection but the recent structure of the LASV spike protein demonstrates that it binds matriglycan in the absence of the underlying scaffold. Our results indicate that matriglycan alone is sufficient for LASV-pseudovirus infection in the absence of the protein α-DG and emphasize the functional importance of post-translational glycosylation independent of protein identity. We have also demonstrated that defined soluble matriglycans can function as decoy receptors for LASV infection that is in agreement with recent data that undefined lengths of matriglycan inhibit infection, further emphasizing the importance of the terminal matriglycan independent of the underlying glycan or protein structure. Currently, there are limited treatment options for LASV, and antiviral drugs capable of limiting viral spread may provide the patient’s immune system a window of opportunity to develop a protective response. Targeting viral entry is a particularly promising strategy for therapeutic intervention, and matriglycan may offer such a lead compound.

**Methods**

**Materials.** All chemical reagents were purchased from Sigma-Aldrich (unless otherwise noted) and used without further purification. All biological reagents were purchased from Bio-Rad Laboratories, Inc., ThermoFisher Scientific unless otherwise noted. HILIC-HPLC purification of compounds was performed on a Shimadzu 20AD UFLC LCMS-TOF with a Waters XBridge BEH, Amide column, 5 μm, 10 × 250 mm or a SeQuant® ZIC®-HILIC column, 5 μm, 10 × 250 mm. β-Galactoside α-2,6-sialyltransferase 1 (ST6GAL1) and peptide-N-glycosidase F (PNGase F) were expressed with *Clostridium perfringens* α-NeuAc2-6-sialyltransferase 1 (ST6GAL1) and peptide-N-glycosidase F (PNGase F) were expressed as GFP-fusion constructs as previously described. All chemical reagents were purchased from Sigma. *Clostridium perfringens* (C. perfringens) neuraminidase was purchased from New England BioLabs. UDP-Glucuronic Acid was purchased from Sigma. UDP-Xylose was purchased from Carbosource (University of Georgia). HAP1 cells [Parental control (Catalog # C631), DAG1 (Catalog # HZGHC003205c001)] were used immediately for glycoengineering studies. Alkyne-matriglycan derivatives were stored as lyophilized solids at −20°C. After lyophilization, matriglycan modified CMP-Neu5Ac’s 1α-1 were used immediately for glycoengineering studies.

**Chemoenzymatic synthesis.** Experimental protocols for compounds 1, 4–6, and 9, and characterization data for all compounds are provided in the Supplementary Information.

**General procedure for the installation of β1,4-GlcA using B4GAT1.** Xylose acceptor 1 or 6 (10.6 μmol) and UDP-GlcA (15.9 μmol) were dissolved at a final xylose-derivative concentration of 10 mM in a MOPS buffered solution (100 mM, pH 7.0) containing MnCl2 (10 mM). CIAP (1% total volume) and B4GAT1 (43 μg/μmol acceptor) were added, and the reaction mixture was incubated overnight at 37°C with gentle shaking. Reaction progress was monitored by ESI-MS and if starting material remained after 18 h another portion of B4GAT1 was added until
no starting material could be detected. The reaction mixture was centrifuged using a Nanoprep® Omega ultrafiltration device (10 kDa MWCO) to remove enzymes and the filtrate was lyophilized. The residue was purified by HPLC using a SeQuant ZIC-HILIC Amide column (5 µm, 10 × 250 mm) with 1% of the flow diverted to the ESI-MS detector (See Supplementary Information). Following HPLC purification, fractions containing product were pooled and lyophilized to yield disaccharides 2 or 7.

**General procedure for disaccharide extension into matriglycans poly-saccharides using LARGEn.** Disaccharide acceptor 2 or 7 (2.0 mg, 1 equivalent) was dissolved at a concentration of 10 mM in a MES buffered solution (100 mM, pH 6.0) containing MnCl₂ (10 mM). For shorter matriglycan length (n = 4), 4 equivalents of UDP-Xyl (8.0 µmol) and 5 equivalents of UDP-GlcA (10.0 µmol) were added to the reaction mixture. For longer matriglycan length (n > 3), 17 equivalents of UDP-Xyl (34.0 µmol) and 18 equivalents of UDP-GlcA (36.0 µmol) were added to the reaction mixture. UDP-Xyl was used in excess to ensure conversion of matriglycans with GlcA. CIAP (1% total volume) and LARGE (200 mg/µmol acceptor) were added, and the reaction mixture was incubated overnight at 37 °C with gentle shaking. The reaction mixture was centrifuged using a Nanoprep® Omega ultrafiltration device (30 kDa MWCO) to remove enzymes and the filtrate was lyophilized. The residue for reactions yielding matriglycans 8 was purified by HPLC using a SeQuant ZIC-HILIC Amide column (5 µm, 10 × 250 mm) (See Supplementary Information). The residue for reactions yielding matriglycans 3 was purified by HPLC using Waters XBridge BEH Amide column (5 µm, 10 × 250 mm) (See Supplementary Information). Fractions were collected with a volume of approximately 250 µL (20 s intervals) and products were confirmed by ESI-MS before pooling and lyophilizing.

**General protocol for conjugation of matriglycans to CMP-Neu5Az by CuAAC.** Stock solutions of 0.1 M CaSO₄, 0.2 M sodium lactate and 0.1 M TBT A in 0.1 M NH₄HCO₃ were freshly made before each CuAAC reaction. 2 equivalents of CuSO₄ per GlcA-carboxylate residue were used for each reaction. Sodium ascorbate and TBT A were adjusted to CuSO₄, quantities at a ratio of 5:1 for sodium ascorbate and CuSO₄. For TRIF/AcCO3, CuSO₄, sodium ascorbate and TBT A were pre-mixed by vortexing, and then added to a solution of alkyne-matriglycan with GlcA. CIAP (1% total volume) and LARGE (200 mg/µmol acceptor) were added, and the reaction mixture was incubated overnight at 37 °C with gentle shaking. The reaction mixture was centrifuged using a Nanoprep® Omega ultrafiltration device (30 kDa MWCO) to remove enzymes and the filtrate was lyophilized. The residue for reactions yielding matriglycans 8 was purified by HPLC using a SeQuant ZIC-HILIC Amide column (5 µm, 10 × 250 mm) (See Supplementary Information). The residue for reactions yielding matriglycans 3 was purified by HPLC using Waters XBridge BEH Amide column (5 µm, 10 × 250 mm) (See Supplementary Information). Fractions were collected with a volume of approximately 250 µL (20 s intervals) and products were confirmed by ESI-MS before pooling and lyophilizing.

**Microarray procedure.** All compounds were printed on NHS-activated Nexterion® slides purchased from Schott using a Scienion sciFLEXARRAYER S3 non-contact microarray printer equipped with a Scienion PDC80 nozzle (Scienion Inc.). Individual compounds were dissolved in a sodium phosphate buffer (pH 9.0, 250 mM) at a concentration of 100 µM and were replicates in triplicate at a spot volume ~400 µL at 20 °C and 50% humidity. Each slide contained 24 subarrays (3 x 8). Post-printing, slides were incubated in a humidity chamber for 24 h and then blocked for 1 h with a 5 mM ethanolamine in a Tris buffer (pH 9.0, 50 mM). Blocked slides were rinsed with DI water, spun dry, and kept in a humidity chamber for 24 h, arrays (3 × 8). Post printing, slides were incubated in a humidity chamber for 24 h. Following incubation, samples were stored at −80 °C until analyzed by Western blotting.

**Cell culture.** HAP1 cells were cultured in IMDM supplemented with 10% FBS and 1% penicillin/streptomycin. Cells were maintained in a humidified 5% CO₂ atmosphere at 37 °C and were passaged using 1X trypsin-EDTA or Non-enzymatic Cell Dissociation Buffer and were passaged approximately every 2–3 days (when cells reached 60–80% confluence).

**Flow cytometry analysis of matriglycan-engineered cells.** For detection of the biotin handle using avidin, matriglycan-engineered cells were stained with avidin-AlexaFluor-488 (Invitrogen A21370; 2.5 µg·mL⁻¹) in 1% FBS/DPBS for 20 min at room temperature, in the dark, for 1 h. After washing and drying (as described for IIIH6), the slide was then incubated with Streptavidin-AlexaFluor-635 (Invitrogen S32364; 5 µg·mL⁻¹ in PBSB) for 1 h in the dark to detect Laminin LG4/5. Following incubation, the plate was washed, dried, and visualized.

**Protein expression.** Recombinant expression of soluble, secreted versions of green fluorescent protein (GFP)- B4GAT1 and LARGE1 were expressed and purified as previously described. The laminin globular (LG) domains 4 and 5 of mouse Laminin alpha 1 (Gene symbol LAMA1, amino acid residues 2705-3083, UniProt P19137) was expressed as a soluble, secreted fusion protein (amino-terminal signal sequence, 8×His-tag, AviTag, and ‘superfolder’ GFP followed by a TEV-protease cleavage site, referred to as His-GFP-Lama1). GFP-B4GAT1, GFP-LARGE1, and His6-GFP-Lama1 were all expressed by transient transfection of HEK293F cells using 1 µg of plasmid DNA (Thermo Fisher Scientific). Laminin-C domain 4 (Laminin-421, UniProt P05086) was transfected as previously described. Six days post-transfection, the cell culture media was subjected to Ni-NTA chromatography (Millipore Sigma, St. Louis, MO). The respective proteins were pooled with 300 mM imidazole and concentrated to ~1 mg·mL⁻¹ using an Amicon centrifugal concentrator (Millipore Sigma, St. Louis, MO) with a 10 kDa molecular weight cutoff and buffer exchanged into PBS pH 7.2.

The LASV GP1 subunit protein coding sequence (amino acids 1–257) was codon optimized for mammalian expression and cloned into a pcDNA3.1 intron vector as a protein fusion with mouse IgG Fc at the carboxy-terminus. The vector (pcDNA-Lintron-Lassa-GP1-mFc) includes a cytomegalovirus (CMV) promoter, and a β-globin intron was engineered into the 5′ untranslated region (UTR) to increase protein production. Suspension culture FreeStyle HEK293F cells (Thermo Fisher Scientific) were transfected as previously described. Six days post-transfection, Lassa-GP1-mFc secreted into the cell culture media was purified in batch format using a Protein G agarose (Cat. No. 20398) according to the manufacturer's protocol. One to two column volume glycan elution fractions were collected until A280 readings became negligible. The elution fractions were neutralized, pooled, and concentrated at 4 °C using Millipore Microcon-10kDa centrifugal filter units.

**Fetuin glyco-engineering with matriglycan-CMP-Neu5Ac’s.** Fetuin (25 µg) was suspended in 50 µL of culture medium without FBS containing the matriglycan-CMP-Neu5Ac derivative (10 equivalents), 10 µg·mL⁻¹ ST6GAL1, 50 µg·mL⁻¹ C. perfringens neuraminidase, 10 U·mL⁻¹ CIAP and 0.1% BSA for 2 h at 37 °C. Following incubation, samples were stored at −80 °C until analyzed by Western blotting.
4 °C in the dark. The cells were washed with DPBS without Ca/Mg, then detached using 150 μL of cell dissociation buffer for 2 min at 37 °C. The cells were suspended in 1% FBS/DPBS, centrifuged gently (500 rpm for 5 min), and resuspended in 500 μL of 1% FBS/DPBS and transferred to polystyrene tubes for flow cytometric analysis (Beckman Coulter HyperCyAn, CTEGD Cytometry Center, University of Georgia). Cell viability was determined by adding PI to cells suspensions 5 min prior to analysis. The live population of cells was gated based on forward and side scatter emission, and exclusion of PI positive cells on the FL3 (637/20 BP filter) emission channel. Avidin-AlexaFluor-488 binding was determined by fluorescence intensity on the FL1(530/30 BP) emission channel. Data points were collected in duplicates and are representative of two separate experiments (n = 4).

Immunoblotting and laminin overlay assay. Following SDS-PAGE, proteins were transferred to PVDF-FL (Millipore), blocked with Odyssey Blocking Buffer (Li-Cor), and probed with various antibodies as follows: The anti-α-DG core antibody (Goat 20 AP, 1:100 dilution) was detected by secondary antibody donkey anti-goat IgG IR800CW (1:4000, Li-Cor 926-32214). The anti-α-DG primary antibody (RSLC C18 Column [2 u particle size, 75 m ID, heated to 60 °C], following SDS-PAGE, proteins were reduced by incubation with 10 mM dithiothreitol by boiling for 10 min. Eluted proteins were transferred to PVDF-FL (Millipore), blocked with Odyssey Blocking Buffer (Li-Cor) and probed with various antibodies as follows: The anti-α-DG core antibody (Goat 20 AP, 1:100 dilution) was detected by secondary antibody donkey anti-goat IgG IR800CW (1:4000, Li-Cor 926-32214). The anti-core67 primary antibody (α-DG mAb 7D11 [1:10,000 dilution] for 30 min at 4 °C in the dark, the cells were washed with DPBS without Ca/Mg and were detached, resuspended and analyzed as described above. Data points were collected in duplicates and are representative of two separate experiments (n = 4).

Inhibitory activity assay. Following electrophoresis, gel slices were excised from the gel and the ladder bands were reduced by incubation with 10 mM dithiothreitol (Sigma-Aldrich) at 56 °C for 1 h, alkylated with 55 mM iodoacetamide (Sigma-Aldrich) for 45 min in the dark, and digested with trypsin digestion. Proteins in the destained gel slices were reduced by incubation with 10 mM dithiothreitol (Sigma-Aldrich) for 45 min in the dark, and digested with Trypsin (Promega) at 37 °C overnight. Tryptic peptides were extracted from the gel slice by incubating with increasing concentrations of acetonitrile (25%, 50%, 75% and 100%) respectively, centrifuged, and resuspended in 4% acetic acid in Solvent A (0.1% formic acid). The peptides were separated using a "Thermo Scientific" Ultimate" 3000 Rapid Separation Liquid Chromatography (RSLC) system equipped with a 15 cm Acclaim RSLC C18 Column [2 μm particle size, 75 μm ID, heated to 35 °C] using a 180 min linear gradient consisting of 1 – 100% B (80% acetonitrile, 0.1% formic acid) over 130 min at a flow rate of 200 nL/min. Separated peptides were directly eluted into a nanospray ion source of an Orbitrap Fusion Mass spectrometer. The stainless steel emitter spray voltage was set to 2200 V, and the temperature of the ion transfer tube was set to 280 °C. Full MS scans were acquired using Orbitrap Fusion from m/z 40 to 2000 to 60,000 resolution, and MS2 scans following fragmentation by collision-induced dissociation (38% collision energy) were acquired in the ion trap for the most intense ions in “Top Speed” mode within a 3 second cycle using Thermo Xcalibur Instrument Setup v3.0, Thermo Fisher Scientific). The raw spectra were searched against the Human (Homo sapiens) reference proteome database (UNIPROT, Version: "release-2018_08") using SEQUEST HT (Proteome Discoverer v1.4, Thermo Fisher Scientific) with a full MS precursor mass tolerance of 20 ppm and MS2 peptide fragment mass tolerance of 0.5 Da. Data are reported based on 2 missed cleavages by trypsin, which cleaves at the C-terminus of Lys and Arg residues, with a minimum peptide length set to at least 6 residues. The search parameters included Carbamidomethylation (on Cys residues) as a fixed modification, and Oxidation (at Met residues) as a variable modification. Protein identifications were filtered using ProteoIQ (v2.7, Premier Biosoft) at the protein level to generate a 5% false-discovery rate (FDR) for peptide assignments and 1% FDR for protein assignments. Proteins present in the negative control experiment (Unlabeled cells), had fewer than 10 spectral counts in the CMP-Neu5Ac-Biotin) labeling experiment, or known to be localized in intracellular compartments as assessed by UNIPROT annotations, were excluded. Proteins reported are all annotated in UNIPROT to contain sites of N-glycosylation or were manually validated to contain at least one N-X-(S/T) N-glycosylation sequence in the primary sequence. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository with the dataset identifier PXD024251.

LC-MS/MS proteome analysis. Following enzymatic cell-surface display of 10h on HAP1-DAG1 cells in 10 cm dishes (6.5 x 10^6 cells/plate), cells were washed with cold DPBS. Cells were lysed by scraping in RIPA buffer supplemented with protease inhibitor cocktail on ice. Lysates were centrifuged at 22,000 × g for 10 min and the supernatant was collected. The concentration of the supernatant was assessed using the BCA assay. Lysates were immunoprecipitated using protein G beads (Sigma-Aldrich) coconjugated with unconjugated anti-biotin antibody (Jackson ImmunoResearch Laboratories 200-002-211). Coated protein G beads were prepared by incubating the anti-biotin antibody with protein G beads in immunoprecipitation buffer (RIPA buffer without protease inhibitors) at a 3:2 volume ratio of protein G beads to antibody for 2 h at 4 °C. Lysates were precleared by incubating with protein G beads for 2 h at 4 °C. The precleared lysate was collected and then incubated with the antibody-coated protein G beads overnight at 4 °C at 1.0 mg of lysate per 50 μL of coated protein G beads. After overnight incubation, the beads were washed 5 times with RIPA buffer and then eluted with 2× sample loading buffer containing 10 mM dithiothreitol for boiling for 10 min. Eluted proteins were resolved by SDS-PAGE and the resulting gel was silver stained for in-gel tryptic digestion followed by proteomic MS analysis.

Each gel lane was excised into four sections above 50 kDa, followed by in-gel tryptic digestion. The digested gel sections were reduced by incubation with 10 μM dithiothreitol (Sigma-Aldrich) at 56 °C for 1 h, alkylated with 55 mM iodoacetamide (Sigma-Aldrich) for 45 min in the dark, and digested with Sequencing Grade Trypsin (Promega) at 37 °C overnight. Tryptic peptides were extracted from the gel sections by incubating with increasing concentrations of acetonitrile (25%, 50%, 75% and 100%) respectively, centrifuged, and resuspended in 4% acetic acid in Solvent A (0.1% formic acid). The peptides were separated using a "Thermo Scientific" Ultimate" 3000 Rapid Separation Liquid Chromatography (RSLC) system equipped with a 15 cm Acclaim RSLC C18 Column [2 μm particle size, 75 μm ID, heated to 35 °C] using a 180 min linear gradient consisting of 1 – 100% B (80% acetonitrile, 0.1% formic acid) over 130 min at a flow rate of 200 nL/min. Separated peptides were directly eluted into a nanospray ion source of an Orbitrap Fusion Mass spectrometer.
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Author contributions
All authors contributed significantly to the manuscript. M.O.S. and C.J.C. contributed equally as co-first authors and were intimately involved in the design of experiments, the generation of reagents, the execution of experiments, the interpretation of data, and the writing and editing of the manuscript. L.L., J.P., D.D., and T.W. were involved in the generation of reagents and execution of experiments. D.G.M., M.A.B., K.P.C., and K.W.M. were involved in the design of experiments, generation of reagents, and interpretation of data. Fiscal support was provided by D.G.M., M.A.B., K.P.C., K.W.M., L.W., and G.J.B. The co-corresponding authors, L.W. and G.J.B., oversaw the entirety of the project and were involved in developing the overall hypotheses being tested, the design of experiments, the interpretation of data, and the writing and editing of the manuscript and associated documents.

Competing interests
The authors declare no competing interests.

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