Membrane-tethered mucin-like polypeptides sterically inhibit binding and slow fusion kinetics of influenza A virus

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Edited by William F. DeGrado, University of California, San Francisco, CA, and approved April 22, 2020 (received for review December 13, 2019)

The mechanism(s) by which cell-tethered mucins modulate infection by influenza A viruses (IAVs) remain an open question. Mucins form both a protective barrier that can block virus binding and recruit IAVs to bind cells via the sialic acids of cell-tethered mucins. To elucidate the molecular role of mucins in flu pathogenesis, we constructed a synthetic glycocalyx to investigate membrane-tethered mucins in the context of IAV binding and fusion. We designed and synthesized lipid-tethered glycopoly peptide mimics of mucins and added them to lipid bilayers, allowing chemical control of length, glycosylation, and surface density of a model glycocalyx. We observed that the mucin mimics undergo a conformational transition at high surface densities from a compact to an extended architecture. At high surface densities, asialo mucin mimics inhibited IAV binding to underlying glycolipid receptors, and this density correlated to the mucin mimic’s conformational transition. Using a single virus fusion assay, we observed that while fusion of virions bound to vesicles coated with sialylated mucin mimics was possible, the kinetics of fusion was slowed in a mucin density-dependent manner. These data provide a molecular model for a protective mechanism by mucins in IAV infection, and therefore this synthetic glycocalyx provides a useful reductionist model for studying the complex interface of host–pathogen interactions.

Significance

Influenza A viruses cause tens of millions of cases of seasonal flu each year. Before a virus infects a host cell, it must first pass through a dense forest of sugar-bearing proteins called mucins. The basic mechanisms of how the virus navigates and chops down this forest of mucins remain up for debate, and many flu drugs target the interactions of the virus with mucin-like proteins. In this work, we make synthetic versions of mucins to create an artificial forest that can be tuned and modified at a molecular level to study how mucins impact influenza A virus binding and fusion.


The authors declare no competing interest.

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This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1921962117/-/DCSupplemental.
compact at low surface densities to extended structures at high surface densities, consistent with polymer brush theory. Using our synthetic glyocalyx, we discovered that IAV binding to underlying glycolipid receptors is inhibited in the presence of asialo mucin mimics only at surface densities at which the mimics were extended. We further observed that IAVs directly interacting with sialylated mucin mimics are able to undergo fusion, and that the kinetics of fusion is also modulated in a surface density-dependent manner congruent with the conformational transition. These data suggest a host-protective mechanism of cell-tethered mucins in influenza infection.

Results Design and Synthesis of Glycopolyptide Mucin Mimics. In order to construct a synthetic glyocalyx, we required chemically defined mucins that could be tethered to a bilayer. To mimic the structural elements of native mucins, we chose to synthesize glycopolyptides by N-carboxyanhydride (NCA) polymerization. Importantly, NCA-derived glycopolymers have been shown to possess secondary structures found in natural mucins (16, 25).

The glycopolymers were initiated from lipid-bearing organometallic species based on previous studies using nickel(II) leucine amides as polymerization initiators (16, 25–27). The lipid tail identities were chosen based on examples of anchors of metallic species based on previous studies using nickel(II) leucine amides as polymerization initiators (16, 25–27). The lipid tail identities were chosen based on examples of anchors of metallic species based on previous studies using nickel(II) leucine amides as polymerization initiators (16, 25–27). The lipid tail identities were chosen based on examples of anchors of metallic species based on previous studies using nickel(II) leucine amides as polymerization initiators (16, 25–27). The lipid tail identities were chosen based on examples of anchors of metallic species based on previous studies using nickel(II) leucine amides as polymerization initiators (16, 25–27). The lipid tail identities were chosen based on examples of anchors of metallic species based on previous studies using nickel(II) leucine amides as polymerization initiators (16, 25–27). The lipid tail identities were chosen based on examples of anchors of metallic species based on previous studies using nickel(II) leucine amides as polymerization initiators (16, 25–27). The lipid tail identities were chosen based on examples of anchors of metallic species based on previous studies using nickel(II) leucine amides as polymerization initiators (16, 25–27). The lipid tail identities were chosen based on examples of anchors of metallic species based on previous studies using nickel(II) leucine amides as polymerization initiators (16, 25–27).

We introduced terminal sialic acids by enzymatic methods directly onto the glycopolymer scaffolds. Small-scale reactions showed that the promiscuous α-2,6-sialyltransferase Pd26ST using CMP-Neu5Ac as a donor sugar was able to sialylate the MM6 scaffold (SI Appendix, Fig. S6) (29, 30). To scale the reaction, a CMP sialic acid synthetase (NmsCSS) was added along with CTP and Neu5Ac to generate CMP-Neu5Ac in situ. This one-pot multienzyme system was used to sialylate poly-(lactosyl)serine-co-alanine scaffolds to afford sialylated mucin mimics MM7–9 (Fig. 2B). Regiospecificity of this transferase has been previously demonstrated (30, 31), and we observed only α-2,6-sialosides, as determined by NMR spectroscopy analyzing the shift of the 3'-axial proton (SI Appendix, NMR spectral data for MM7–9). We found that using a scaffold containing a 1:1 mixture of Alas-Ser(Lac) gave ~50% sialylation of lactose glycans, independent of polymer length (DP [degree of polymerization]) or lipid tail (Fig. 2 A and B). Several α-2,3-sialyltransferases were tested (e.g., PmST1Δ24, PmST3, CstI) (29, 31–33), but none were able to functionalize the lactose units under literature conditions.

Finally, the free N termini of the glycopoly peptides were acylated with commercially available Alexa Fluor 488 (AF488) NHS ester, and size exclusion chromatography was used to separate free dye. With this modular and tunable workflow, we synthesized a panel of dual-end functionalized glycopoly peptides bearing terminal glycan structures found on native mucins (Fig. 2A).

We analyzed secondary structure of our mucin mimics by circular dichroism (CD). CD has previously been used to determine secondary structures adopted by NCA-derived polypeptides. Previous work has shown that glycopoly peptides can adopt α-helices, random coils, or mucin folds depending on glycan identity, glycosylation density, and copolymer composition (16, 25). We observed that both the asialo and sialylated (MM2 and MM7, respectively) mimics adopted a random-coil-type structure in solution (SI Appendix, Fig. S7). Mucin mimics of varying lengths (MM2 and MM5, respectively) also had similar structures (SI Appendix, Fig. S8).

C14 Anchors Afford Mucin Mimics with Efficient and Stable Insertion into Fluid Membranes. We compared incorporation and partitioning of each mucin mimic into a membrane via insertion into small unilamellar vesicles (SUVs) and SLBs with quantification by fluorescence microscopy. SLBs were created in a microfluidic flow cell by rupturing SUVs formed through extrusion (34). An equimolar amount (0.1 nmol) of each mucin mimic labeled with AF488 (MM1–4) bearing a different lipid anchor was added above separate SLBs and allowed to incorporate (t = 1 h) before rinsing with citrate buffer (pH 7.2). The AF488 intensity from polymer insertion was then quantified using fluorescence microscopy.
relative to a standard curve of a known concentration of AF488-labeled DOPE in a bilayer (SI Appendix, Fig. S9). As seen in Fig. 3A, MM1 bearing a cholesterylamine inserted to the greatest extent. For the bisalkyl glyceryl lipids, we observed that as the carbon chain length of the lipid decreased, insertion increased (MM4 < MM3 < MM2).

We examined membrane partitioning of mucin mimics bearing different anchors to ensure stability of insertion when challenged with another membrane, which is critical for studying interactions with enveloped viruses such as IAV. SLBs were formed and mucin mimics were incorporated and washed thoroughly as described above. SUVs containing a Texas Red (TR) fluorophore (0.1 mol% TR-DHPE) were then added to the microfluidic device and incubated with the SLBs for 3 to 5 min. The incubated SUVs were extracted from the sample chamber, diluted 1:100, deposited on glass, and imaged for AF488/TR colocalization (35). Vesicles that have colocalization of TR and AF488 indicate that the anchored mucin mimic is able to partition from the SLB into the incoming vesicles. Only the cholesterylamine anchor displayed colocalization above background (Fig. 3C). The partitioning of the cholesterylamine anchor makes it unsuitable to study phenomena that rely on stable membrane tethers. As shown in Fig. 3A and B, the C14 anchored mucin mimics had the highest insertion efficiency among the membrane-stable anchors. Thus, it was exclusively employed as the anchor for all influenza binding and fusion experiments (MM2, 5, 7, and 8).

We assessed diffusion of the mucin mimics using fluorescence recovery after photobleaching (FRAP). SLBs were created and mucin mimics were inserted as previously described. All targets were found to have lateral diffusion coefficients, \( D \), within error of AF488-labeled DOPE, indicating that the mucin mimics of all anchors tested are comparably fluid in the SLBs between 0.005 and 0.1 mol% loading (SI Appendix, Table S1).

### Mucin Mimics Undergo a Surface Density-Dependent Conformational Change

We assessed the extension length of the mucin mimics anchored in a membrane as a function of mimic concentration using fluorescence interference contrast microscopy (FLIC) and dynamic light scattering (DLS) as complementary methods. FLIC is an interferometric method where the interference between the direct and reflected light of a fluorophore in close proximity to a flat mirrored surface (Si) modulates the fluorescence intensity as a function of the distance between the fluorophore and the mirror. This technique was developed by Lambacher and Fromherz and has since been used to calculate extension heights (36–39). Using a fabricated chip of varying silicon dioxide heights on a silicon support, we were able to measure the fluorescence intensity as a function of silicon dioxide height (Fig. 4A). As the distance of the fluorophore from the surface increases, the peak fluorescence intensity shifts to lower silicon dioxide heights. We incorporated MM2 at low and high surface concentrations into a bilayer to determine the FLIC-derived heights of the longest mucin mimic. At low surface concentrations near 0.01 mol%, MM2, the AF488-labeled N terminus of MM2 resided on average within error of AF488-labeled DOPE (Fig. 4B). At estimated surface concentrations >>0.01 mol%, we observed a shift of peak fluorescence intensity to lower silicon dioxide heights, indicating an extension of AF488 away from the bilayer (Fig. 4A). At estimated surface concentrations >>0.01 mol%, the AF488-labeled N terminus of...
MM2 was best fit by a calculated intensity curve for AF488 extended 10.6 ± 3.7 nm from the SLB (Fig. 4A).

To independently assess the FLIC extension length, DLS was used to examine the polymer length in SUVs. When high concentrations of MM2 (>0.01 mol%) were loaded into vesicles, an increase in the effective diameter of 23.2 ± 3.2 nm (SD) was measured, which predicts the extension length of the polymer at high loading capacity to be just over 10 nm (SI Appendix, Fig. S12). We found MM5 had one-third the effective diameter increase of MM2 (6.1 ± 3.5 nm [SD]), which is consistent with MM5 having one-third of the DP of MM2.

**Tethered Mucin Mimics Inhibits Influenza Binding to Glycolipids in a Surface Density-Dependent Manner.** Single influenza virus-binding assays were performed in a microfluidic device to measure the impact of tethered mucin mimics on IAV binding to underlying ganglioside receptors. GD1α was used as a model sialic acid receptor because its interaction with IAV has been rigorously quantified, GD1α can be directly reconstituted into the bilayer at a quantified constant amount relative to increasing mucin density, and the terminal sialic acids reside close to the surface of the bilayer (40–42). Without GD1α present, there is no significant binding of IAV to SLBs with MM2 or MM5 (SI Appendix, Fig. S13). SLBs were formed from SUVs containing a fixed 2 mol% GD1α (70POPC/20DOPE/10chol/2GD1α), and either MM2 or MM5 was incorporated at increasing concentration as previously described (Fig. 5C). Influenza virions, fluorescently labeled with TR-DHPE, were incubated with SLBs displaying tethered mucin mimics, and binding was quantified using spot detection of fluorescence micrographs (SI Appendix, Image Analysis). These binding assays were carried out using two lengths of C14-tethered mucin mimics (MM2 and MM5; average DP of 93 and 33 residues, respectively). For both lengths, increasing mucin mimic concentration in the SLB decreased the number of virions bound; however, the longer mimic MM2 ablated binding at ~10-fold lower concentration than the short MM5 (Fig. 5A vs.
The concentration dependence of increasing MM2 and MM5 on IAV binding can be fit by a Langmuir–Hill equation indicating positive cooperativity (SI Appendix, section 4). Furthermore, this ablation of binding is specific to tethered mucins. There is no significant decrease in virus binding when up to 1,000× more soluble mucin (MM6 or MM10) is added to the flow cell in the same virus binding assay (SI Appendix, Fig. S15).
Virions Bound to Sialylated Mucins Undergo pH-Induced Fusion to Membranes with Slower Kinetics than Glycolipid-Bound IAV. While it is known that virions bound to GD1a can fuse with membranes in late-endosomal conditions, it has yet to be demonstrated whether or not IAV bound to sialylated mucins are able to undergo fusion. To investigate this outstanding question, we employed a tethered vesicle assay to collect single virus lipid-mixing events from single IAV particles bound to sialylated mucin in order to isolate the impact of binding from membrane fusion. By using sialylated mucins, we were able to ensure that each virion is only interacting with a target membrane through a mucin-mediated interaction. Vesicles (69POPC/20DOPE/10Chol/1BiotinDPPE) containing either a constant 2 mol% GD1a or a quantified amount of MM7 or MM8 were tethered using neutravidin to a biotin-PEG-pLL functionalized slide within a micro-fluidic device. Influenza virions labeled with a self-quenched concentration of TR-DHPE were bound to the vesicles through sialylated mucins or GD1a and unbound virions were rinsed from the system (Fig. 6A). Virus binding was found to be dependent on the presence of sialic acid and was ablated by neuraminidase pretreatment of the mucin mimics (SI Appendix, Fig. S16). Additionally, increasing the concentration of sialylated mucin increased binding of IAV until 0.01 mol% MM7 is reached. Above 0.01 mol% MM7, a critical binding transition similar to that observed for the nonsialylated mucins, occurs (SI Appendix, Fig. S17). IAV binding to sialylated mucin mimics could be titrated by introducing increasing amounts of nonsialylated mucin mimic, which reduced binding (SI Appendix, Fig. S18).

We examined the ability of virions bound via sialylated mucin mimics to undergo fusion upon the introduction of low pH comparable of that found in a maturing endosome. To induce fusion, low pH buffer (pH 5.1) was exchanged into the flow cell, and fluorescence microscopy was used to monitor single virus lipid-mixing events by fluorescence dequenching (Fig. 6B). Single virus lipid-mixing events were then compiled into a cumulative distribution function (CDF). Display of the kinetics as CDFs removes the necessity of binning, which is intrinsic to a histogram. The t_{1/2} of lipid mixing was obtained from each CDF to compare the time at which one-half of all fusion events occurred for each condition (SI Appendix, Table S2). Virions tethered via sialylated mucin mimics were able to undergo lipid mixing; however, the rate slowed and t_{1/2} of lipid mixing increased roughly twofold compared to GD1a (Fig. 6C). For comparison, previous work found no difference in fusion kinetics from virions bound to GD1a or DNA-lipid surrogate receptors (9). The fusion kinetics from IAV tethered to vesicles with a high surface density of MM7 (mean, 5.9 mucins per vesicle) or MM8 were markedly slower than IAV bound to a vesicle with a low surface density of MM7 (mean, 1.9 mucins per vesicle) (Fig. 6D and SI Appendix, Fig. S19). Assuming a PC headgroup size of ~60 Å², five mucin mimics corresponds to ~0.01 mol% of the outer leaflet of a 100-nm SUV (43). At high surface density of tethered sialylated mucin mimics (>0.01 mol %), virions displayed the same shift in fusion kinetics whether they were bound to MM7 or MM8 when compared to GD1a control (Fig. 6C).

Discussion
In order to construct a synthetic glyocalyx containing membrane-tethered mucin mimics, we developed a chemoenzymatic approach to afford lipid-linked glycopolypeptides bearing terminal mucin glycans. We developed NCA polymerization techniques to introduce C-terminal lipids by using lipid-functionalized initiators and demonstrated that glycopolyptide scaffolds can be substrates for enzymatic glycan elaboration. Given that 1) past glycopolymers...
for glycocalyx engineering have relied upon nonquantitative conjugation chemistries, 2) native mucins are incompletely sialylated, and 3) asialo lactosyl glycans should be inert to HA and neuraminidase, the nonquantitative sialylation by Pd26ST was sufficient and 3) asialo lactosyl glycans should be inert to HA and neuraminidase. Furthermore, while NCA polymerization affords relative uniform polypeptides (D = 1.1 to 1.2), dispersity increased after deprotection of the sugars (D = 1.2 to 1.4) (SI Appendix, Synthetic Procedures). The dispersity of polymers likely accounts for distribution of observed extension lengths. Nonetheless, the mucin mimics are still significantly more uniform and controlled than native mucins, which notoriously afford proteins of a wider variety of lengths from a single gene. Thus, our polypeptides provide improved control over glycocalyx height compared to native or recombinant mucins.

Membrane stability was a critical aspect in the design of our mucin mimics to ensure that surface densities remained consistent. We found that cholesterol anchors were readily inserted into the target membrane, but then transferred when challenged with addition of vesicles. While such anchors are useful for facile incorporation and may be useful for some cellular membrane platforms that are not reliant on quantitative addition and insertion, this work suggests that such an anchor may be ill-suited for applications that require membrane insertion stability. Prior work has circumvented this stability pitfall by creating constructs with multiple cholesterol moieties that lead to stable insertion (44, 45).

Cell-tethered mucins have been proposed to conform to polymer-brush theory, which states that mucins exist in two conformations on the cell surface, a compact “mushroom” structure at low densities and an extended “brush” architecture at high densities (46–48). The polymer brush model is consistent with our observations that 1) at high surface density of mucins, IAV is unable to bind to the underlying ganglioside receptors, 2) mucin mimics adopt a random coil in solution implying conformational flexibility, and 3) at high surface density of mucins, DLS and FLIC corroborate an extended polymer architecture. Tethered mucin mimics display a surface concentration-dependent inhibition of virus binding to GD1α glycolipids. MM5 has three times fewer residues than MM2, and its exclusion concentration roughly scales with the length squared. This is congruent with the polymer brush model that has been previously described for mucins (46–49). Thus, we hypothesize that, at low surface densities, mucins adopt a random coil structure, which then transitions to a more extended structure to accommodate high surface densities.

Put into the broader context of the role of mucins in IAV entry, these two behaviors of tethered mucins suggest that increasing asialo mucin expression level serves as a steric barrier to membrane access. The increase in mucin expression levels during influenza infection would likely increase the surface density of cell-tethered mucin. Our results suggest that increasing surface density of cell-tethered mucin would shift the surface mucin population toward an extended confirmation and the distance at which an extended confirmation was observed. If extension length were the only factor impacting membrane fusion, then one would expect to see a significant difference in fusion kinetics from IAV bound to the short and long polymer, but no difference was observed. This suggests that the extension length is not the main factor impacting the delay in rates in this assay; however, both polymer lengths used were shorter than biological mucins where the extension length could be prohibitive toward fusion. In the context of our assay, we can reasonably conclude that the delay in IAV lipid mixing is impacted more by tethered mucin density than the length of the tethered mucin. One explanation for this density-related rate delay is that extended mucin alters the interaction between the flu and the membrane.

The use of synthetic glycocalyces to study complex biological phenomena has provided key insights into the protective mechanism of tethered mucins in IAV entry. We show that asialo mucins serve as a steric barrier against IAV accessing membrane receptors in a density-dependent manner. Additionally, the single virus kinetics collected from IAV bound to sialylated mucin suggests that mucins can serve as surrogate receptors in viral membrane fusion when IAV is bound in an acidifying endosome. These results illustrate the utility of model systems in separating the roles of complex mucin components, (e.g., length and sialylation) in the biologically relevant phenomenon of up-regulation of mucin as part of host defense against IAV. While this work presents a basis for mechanistic interactions between mucin and IAV, further questions into the physiological behavior of mucin and its protective role in IAV entry remain. Anchor identity, mucin mimic length, and sialylation were explored as tunable parameters, and further opportunities are now available for other variables that are easily accessible using NCA polymerization, such as glycosylation density, comonomer composition, and glycan identity. These materials are readily integrated into existing SLB systems and could be used to study the impact of the glycocalyx (and specifically the conformational transition of mucin-like polypeptides) on membrane-localized phenomena, from receptor–ligand interactions to the effect(s) on receptor clustering, for which mucins are thought to be critically important.

**Materials and Data Availability**

Detailed procedures for synthesis, characterization, microscopy, and analysis can be found in SI Appendix. All analysis code is detailed in SI Appendix and available from previously published work. All other data discussed in the paper are available in the main text and SI Appendix.

**ACKNOWLEDGMENTS.** We thank Dr. Corianne Van der Akker for her invaluable support with FLIC-chip setup and analysis. We thank Raghveer Delaveris et al. PNAS Latest Articles | 7 of 8
Parthasarathy for sharing Matlab code that was useful for comparison with FLC analysis. We thank C. J. Cambier for helpful comments on the manuscript. This work was supported, in part, by National Cancer Institute Grant R01CA227942 (to C.R.B.) and NIH Grant GM118044 (to S.G.B.). E.R.W. was supported by Center for Molecular Analysis and Design and by a National Science Foundation Graduate Research Fellowship; C.S.D. was supported by a National Science Foundation Graduate Research Fellowship and a Stanford Interdisciplinary Graduate Fellowship affiliated with Chemistry, Engineering, and Medicine for Human Health. S.M.B. was supported by a National Institute of General Medical Sciences F32 Postdoctoral Fellowship. Part of this work was performed at the Stanford Nano Shared Facilities, supported by the National Science Foundation under Award ECCS-1542152.

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Supporting Information: Membrane tethered mucin-like polypeptides sterically inhibit binding and slow fusion kinetics of Influenza A virus

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1. Biophysical Characterization: Materials and Methods

Materials

Dioleoyl phosphatidylethanolamine (DOPE), Palmitoyl oleoyl phosphatidylcholine (POPC), and cholesterol were purchased from Avanti Polar Lips (Alabaster, AL). Texas Red-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (TR-DHPE), Alexa Fluor 488 NHS ester (AF488), Chloroform, methanol, NeutrAvidin and buffer salts were purchased from Fisher Scientific (Pittsburgh, PA) and Sigma-Aldrich (St. Louis, MO). Polydimethylsiloxane (PDMS) was purchased from Ellsworth Adhesive (Hayward, CA). PLL-Peg and PLL-Peg-Biotin were purchased from SuSoS AG (Dübendorf, Switzerland). α2-3,6,8 Neuraminidase was purchased from New England BioLabs (Ipswich, MA). IAV was purchased (X-31, A/Aichi/68 H3N2) from Charles River Laboratories (Hollister, CA) and is obtained, stored, and used following an approved biosafety protocol. Glyceryl lipids were purchased either from Sigma Aldrich (St. Louis, MO) or BACHEM (Torrance, CA).

Buffers

The following buffers were used. Citrate Buffer = 10 mM NaH$_2$PO$_4$, 90 mM sodium citrate, 150 mM NaCl, pH 7.4. Fusion Buffer (FB) = 10 mM NaH$_2$PO$_4$, 90 mM sodium citrate, 150 mM NaCl, pH as indicated. HB buffer = 20 mM HEPES, 150 mM NaCl, pH 7.2.

Microscopy and Image Analysis

Epifluorescence micrographs and videos were obtained using a Nikon Ti-U microscope using either a 100x oil immersion objective, NA=1.49 (Nikon Instruments, Melville, NY), or 10x objective, NA=0.45 for FLIC; with a Spectra-X LED Light Engine (Lumencor, Beaverton, OR) as an excitation light source, and additional excitation/emission filter wheels (Texas Red images utilized a Texas Red filter cube (ex = 562/40 nm, bs = 593 nm, em = 624/40 nm), and additional excitation (ex = 560/55 nm) and emission (em = 645/75 nm) filters. AF488 images utilized a NBD filter cube (ex = 475/35 nm, bs = 509 nm, em = 528/38 nm), and additional excitation (ex = 460/50 nm) and emission (em = 535/50 nm) filters. Images and video micrographs were recorded with an Andor iXon 897 EMCCD camera (Andor Technologies, Belfast, UK) using 16-bit image settings, and were captured at 288 ms/ frame using Metamorph software (Molecular Devices, Sunnyvale, CA). Custom Matlab scripts (MathWorks, Inc.) were used to analyze the microscopy images and video micrographs as described previously (1).

Lipid-mixing assay

Lipid mixing assays were performed similar to previously published work (2) with the following differences. In brief, target membranes, ~100 nm diameter lipid vesicles containing 1% DPPE-biotin were tethered to glass slides functionalized with PLL-PEG-Biotin using NeutrAvidin inside of a microfluidic flow cell. Excess vesicles were rinsed from the flow cell. TR labeled IAV was added to the flow cell containing 100 nm vesicles with either 2% GD1a, MM7, or MM8, and the cell was then rinsed after 2-5 min to remove excess unbound virus. Fluorescence microscopy was used to collect a stream of images for 1000 frames at a frame rate of 3.47 frames/sec. After the start of the image stream, low pH buffer was immediately exchanged into the chamber and flow was started. The time between introduction of low pH to the field of view (FOV) and dequenching events was then analyzed using adopted inhouse Matlab code as previous reported (2).
**Vesicle preparation**

The desired lipid mixture was prepared in chloroform, dried down to a film under nitrogen gas, further dried under vacuum for several hours to remove any residual solvent, re-suspended by vortexing in Citrate Buffer and extruded through a track-etched polycarbonate membrane (Avanti Polar Lipids) using a mini extruder (Avanti Polar Lipids) to yield unilamellar vesicles. Mucin mimics were then added at the indicated mole percent and incubated overnight at 4 °C for DLS or incorporated at 24 °C for several hours in preformed SLBs. This procedure incorporates the mucin mimic into the outer leaflet of the vesicle or top leaflet of the SLB. Unincorporated mucin mimics were rinsed from SLBs using laminar flow.

**TR-DHPE labeling of IAV**

IAV was labeled as previously described in published work (1). Briefly, 3 µL of sonicated Texas Red-DHPE (0.75 mg/mL in ethanol) was added to 117 µL of HB and mixed. 72 µL of the TR-DHPE solution was added to 18 µL of IAV (2 mg total protein/mL), incubated at RT for 2 h, diluted by ~1.4 fold, centrifuged for 50 minutes at 21,000 x g to pellet the labeled virus, and resuspended in 100 µL of HB. Labeled virus was then stored on ice and used or frozen within 72 h. All IAV was labeled at a self-quenched amount for experimental ease. Binding images were taken at 5x the light intensity used for fusion experiments to ensure that all virions were imaged.

**Virus Binding Assay**

SLBs were formed by rupturing 100nm extruded LUVs (70POPC/20DOPE/10chol/2GD1a) onto a glass slide within a microfluidic flow cell. SLBs were wash liberally with water followed by citrate buffer. Mucin mimics were then added to the flow cell at increasing concentration and allowed to incorporate at room temperature. After incubation, SLBs were extensively rinsed with citrate buffer to remove any mucin mimic which was not stably inserted. 2 µL of TR labeled IAV was added to the flow cell and allowed to bind for a fixed time (3 min) then unbound virus was rinsed from the flow cell with citrate buffer. The remaining bound virions were quantified using fluorescence microscopy and analyzed with adapted Matlab scripts (2).

**Dynamic Light Scattering**

The extension length of densely packed mucin mimics in a liposome was characterized by DLS. To maximally incorporate mucin mimics, 20 µL of 50 nm extruded SUVs (70POPC,20DOPE,10Chol) (0.56 mM lipids) were incubated with 5 µM of the desired mucin mimic overnight on ice. Vesicle solutions were diluted 30x with citrate buffer and analyzed with a NanoBrook Omni DLS (Brookhaven Instruments Corp., Holtsville, NY) in the shared nano facilities at Stanford.

**Fluorescence interference contrast microscopy**

FLIC measurements were conducted following protocols detailed in previous work (3, 4). In brief, a flow cell was created by applying double sided tape to the edges of a prefabricated FLIC chip, with a series of 16 SiO₂ steps on Si, and binding it to a glass slide. A bilayer was formed on the chip by rupturing LUVs and thoroughly rinsing with DI water followed by citrate buffer. In the case of DOPE-AF488, SUVs were created with a known concentration of DOPE-AF488. In the case of mucin incorporation, LUVs were void of fluorophore. All bilayers displayed normal FRAP indicating uniform distribution of fluorophore across the SLB on the chip. For low mucin
incorporation, between 5-10 µL of 50 µM MM2-AF488 was incubated above the bilayer for 1-3 h before thoroughly rinsing with citrate buffer. All samples classified as low MM2 incorporation display fluorescence intensity at or below that of the standard 0.01 ± 0.02 mol% 488 bilayer used. For high mucin incorporation 12 µL of 2 mM MM2-NH2 + 4 µL of 100mM MM2-AF488 was incubated above the bilayer for 2-5 h before thoroughly rinsing with citrate buffer. Due to the mixture of labeled and unlabeled polymer, precise quantification of MM2 mol% could not be achieved, however given that 100x more MM2 was present, we reasonable conclude the surface concentration is >> 0.01 mol% MM2. Chips were imaged using a 10x objective, NA=0.45 in addition to the aforementioned widefield microscopy setup and Matlab analysis (3). Matlab codes from (3,4) were adapted to include the excitation and emission spectra of AF488 (thermofisher), and the Spectrum of Semrock FF509-FDi01 Optical Filter used for imaging. Given the extended regime of the MM2 can be crudely approximated by a ridge model, we fit our high surface concentration data assuming the orientation of the absorption and emission transition moment = 90° normal to the membrane (3). We cannot reliably make this assumption for the low concentration MM2 case therefore we only calculate the fluorophore distance from the bilayer at predicted surface concentration >>0.01 mol% MM2.
2. General Synthetic Materials and Methods

Unless stated otherwise, reactions were conducted in oven-dried glassware under an atmosphere of nitrogen using anhydrous solvents. Tetrahydrofuran (THF) and dichloromethane (DCM) were purified by first purging with dry nitrogen, followed by passage through columns of activated alumina. Deionized water was purified to 18 MΩ-cm using a Millipore Milli-Q Biocel A10 purification unit. All commercially obtained reagents were used as received without further purification unless otherwise stated. Flash chromatography was performed using Silicycle SiliaFlash P60 silica gel. Analytical thin layer chromatography was performed using glass-backed Analtech Uniplate silica gel plates containing a fluorescent indicator, and visualized using a combination of UV, anisaldehyde, KMnO₄, H₂SO₄, ninhydrin, and phosphomolybdic acid staining.

NMR spectra were obtained on Varian spectrometers at room temperature at the Stanford Department of Chemistry NMR Facility. NMR spectra are reported relative to deuterated solvent signals. Data for ¹H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz) and integration. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Data for ¹³C NMR spectra are reported in terms of chemical shift.

High-resolution mass spectrometry was performed at the Stanford University Mass Spectrometry core facility.

All attenuated total reflectance (ATR) Fourier Transform infrared (FTIR) samples were recorded on a Bruker Alpha spectrometer.

Organic gel permeation chromatography (GPC) was performed in THF using two sequential PolyPore columns (Agilent) and was detected with a 1260 Infinity variable wavelength detector (Agilent), a DAWN multi-angle laser light scattering (MALLS) detector (Wyatt Technology), and an Optilab T-rEX differential refractometer (Wyatt Technology).

Aqueous GPC was performed in PBS on a guard and analytical SEC column (Wyatt Technologies, product no. WTC-015N5) and was detected with a 1260 Infinity variable wavelength detector (Agilent), a miniDAWN TREOSII multi-angle laser light scattering (MALLS) detector (Wyatt Technology), and an Optilab T-rEX differential refractometer (Wyatt Technology). On-line dn/dc calculation was determined for aqueous GPC conditions for purified soluble polymers assuming 100% mass recovery.

Protein purification via fast protein liquid chromatography (FPLC) was performed on an ÄKTA pure protein purification system (GE Lifesciences) using HisTrap Ni²⁺ affinity column (GE Lifesciences) and degassed aqueous buffers.

All DLS and Ellipsometry measurements were performed in the Stanford Shared Nano Facilities. Circular dichroism spectra were recorded on a JASCO CD spectrophotometer running in conventional scanning mode with samples in a quartz cuvette of 0.1 cm path length and prepared at 10 µM polymer in phosphate buffer saline.
3. Synthetic Procedures

N-Alllylcarboxy Leucine succinimidyl ester (S1)
The known N-allylcarboxy leucine (4.00 g, 18.6 mmol) (5) and N-hydroxysuccinimide (2.14 g, 18.6 mmol, 1.0 equiv.) were dissolved in anhydrous tetrahydrofuran (9 mL, 2 M) and cooled to 0 °C. To the vigorously stirred mixture was added a solution of dicyclohexylcarbodiimide (3.83 g, 18.6 mmol, 1.0 equiv.) in tetrahydrofuran (9 mL, 2 M). The reaction mixture was stirred vigorously for 12 h under nitrogen. The reaction mixture was filtered over glass wool and concentrated in vacuo. The oily residue was taken up in 50 mL ethyl acetate and washed 1 x 50 mL each of saturated NaHCO₃(aq), water, and brine. The organic phase was dried over MgSO₄, filtered, and concentrated in vacuo to afford S1 as an opaque viscous oil (5.31 g, 91%). Spectral data were consistent with the literature (6).

N-Alllylcarboxy Leucine 3-(N’-Boc-cholesterylamine)propylamide (1a)
A solution of N-allylcarboxy leucine (58 mg, 0.27 mmol, 1.1 equiv.), hydroxybenzotriazole monohydrate (36 mg, 0.27 mmol, 1.1 equiv.), and N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (42 mg, 0.27 mmol, 1.1 equiv.) in anhydrous dichloromethane (8 mL, 0.02 M final) was stirred at 0 °C for 30 min. To this reaction mixture was added a solution of N-(3-aminopropyl)-N-Boc cholesterylamine (132 mg, 0.24 mmol) (7) in anhydrous dichloromethane (4 mL). The reaction mixture was stirred overnight as the ice bath was allowed to expire over 12 h. The reaction mixture was diluted with 30 mL dichloromethane and washed with 30 mL 0.1 M aqueous NaOH. The aqueous layer was extracted again with 30 mL dichloromethane and the combined organic layers were washed with 30 mL brine, dried over Na₂SO₄, and concentrated in vacuo. The residue was purified by flash chromatography in 33% ethyl acetate in hexanes. Product-containing fractions were determined by TLC, collected, and concentrated to afford a yellow oil. The oil was then triturated with diethyl ether/hexanes to afford 1a as a pale yellow solid (47 mg, 26%). 1H NMR (400 MHz, Chloroform-d) δ 7.49 (s, 1H), 5.90 (ddt, J = 17.3, 10.8, 5.6 Hz, 1H), 5.37 – 5.13 (m, 4H), 4.56 (d, J = 5.3 Hz, 2H), 4.20 (d, J = 6.8 Hz, 1H), 3.48 – 3.10 (m, 6H), 2.53 (s, 1H), 2.07 – 1.75 (m, 7H), 1.75 – 1.22 (m, 16H), 1.19 – 1.04 (m, 4H), 1.01 – 0.89 (m, 15H), 0.86 (dd, J = 6.6, 1.9 Hz, 7H), 0.67 (s, 3H). 13C NMR (101 MHz, CDCl₃) δ 172.26, 121.56, 56.85, 56.25, 53.78, 50.19, 42.43, 39.86, 39.63, 38.54, 36.84, 36.30, 35.92, 31.99, 28.65, 28.37, 28.15, 24.89, 24.41, 23.95, 23.11, 22.97, 22.70, 22.13, 21.12, 19.59, 18.84, 11.99. HRMS (ESI) m/z: [M+H]⁺ calc. 740.5936, obs. 740.5920 [M+Na]⁺ calc. 762.5755, obs. 762.5734.

1,2-bis(tetradecyl)-3-benzyl-sn-glycerol (S2)
To a stirred suspension of 60% sodium hydride in mineral oil (840 mg, 21.9 mmol, 4.00 equiv.) in N,N-dimethylformamide (4 mL) at 0 °C was slowly added 3-benzyl-sn-glycerol (1.00 g, 5.49 mmol) as a solution in N,N-dimethylformamide (8 mL). This suspension was stirred on ice under nitrogen until all hydrogen evolution ceased. After 2 h, to the reaction mixture was slowly added tetradecylbromide (6.53 mL, 21.9 mmol, 4.00 equiv.). The reaction was allowed to gradually warm to room temperature, then heated to 70 °C and stirred for 16 h under nitrogen. The reaction was cooled to room temperature and then quenched with 2 mL cold water. The solution was diluted with 40 mL sat. NH₄Cl and 50 mL ethyl acetate. The organic phase was separated, washed 3 x 50 mL water, dried over Na₂SO₄, filtered, and concentrated in vacuo. Purification by flash chromatography from 0 to 10% ethyl acetate in hexanes afforded S2 as a colorless oil.
(840 mg, 27%). ¹H NMR (400 MHz, CDCl₃) δ 7.33 (d, J = 4.4 Hz, 4H), 4.55 (s, 2H), 3.65 – 3.48 (m, 7H), 3.43 (t, J = 6.7 Hz, 1H), 1.55 (m, 4H), 1.25 (m, 4H), 0.88 (t, J = 6.7 Hz, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 128.40, 127.71, 73.43, 71.85, 71.67, 71.41, 69.52, 63.03, 32.79, 31.91, 29.68, 29.66, 29.64, 29.61, 29.59, 29.46, 29.43, 29.34, 26.08, 25.73, 22.67, 14.09.

1,2-bis(tetradecyl)-sn-glycerol (S3)
To a solution of 3-benzyl S2 (840 mg, 1.46 mmol) in 4:1 ethyl acetate/methanol (7 mL, 0.2 M) was suspended 10% Pd/C (350 mg, 0.1 equiv.). Hydrogen was bubbled through the reaction mixture for 16 h. The reaction mixture was filtered through Celite, and the Celite bed washed with ethyl acetate. The filtrate was concentrated in vacuo to afford S3 as a white solid (600 mg, 85%). ¹H NMR (400 MHz, CDCl₃) δ 7.33 (d, J = 4.3 Hz, 1H), 3.64 – 3.57 (m, 2H), 3.55 – 3.40 (m, 4H), 1.60 – 1.52 (m, 4H), 1.26 (m, 4H), 0.88 (t, J = 6.78 Hz, 2H). ¹³C NMR (101 MHz, CDCl₃) δ 78.23, 71.85, 70.90, 70.39, 63.03, 31.92, 30.07, 29.70, 29.59, 29.46, 29.43, 29.34, 26.08, 25.73, 22.67, 14.09.

1,2-bis(tetradecyl)-3-tosyl-sn-glycerol (S4)
To a solution of S3 (600 mg, 1.24 mmol) in chloroform (12 mL, 0.1 M) and pyridine (1.2 mL) was added p-toluenesulfonylchloride (350 mg, 1.86 mmol, 1.5 equiv.), and the mixture was stirred at room temperature for 18 h. The reaction was concentrated in vacuo. The white residue was dissolved in minimal hot dichloromethane, precipitated with cold methanol, and collected by centrifugation (1000 rcf, 30 min, 4 °C). The precipitation was repeated twice more to afford S4 as a white solid (470 mg, 59%). ¹H NMR (400 MHz, CDCl₃) δ 7.79 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 4.22 – 3.94 (m, 2H), 3.68 – 3.52 (m, 1H), 3.50 – 3.25 (m, 6H), 2.44 (s, 3H), 1.56 – 1.40 (m, 4H), 1.25 (s, 46H), 0.97 – 0.74 (m, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 144.65, 133.00, 129.75, 127.98, 76.19, 71.76, 70.80, 69.66, 69.34, 31.92, 29.87, 29.70, 29.68, 29.65, 29.63, 29.61, 29.52, 29.47, 29.44, 29.36, 26.02, 25.95, 22.68, 21.61, 14.10.

1,2-bis(tetradecyl)-3-azido-sn-glycerol (S5)
To a solution of S4 (470 mg, 0.74 mmol) and sodium azide (290 mg, 4.4 mmol, 6.0 equiv.) in a 1:1 mixture of dimethylsulfoxide / N,N-dimethylformamide (6 mL, 0.1 M) was added 15-crown-5 (440 μL, 2.2 mmol, 3.0 equiv.). The mixture was stirred at 75 °C for 18 h. The reaction mixture was cooled to room temperature, diluted with 100 mL 1 M LiCl and extracted with 3 x 40 mL diethyl ether. The organic phase was washed 5 x 100 mL 1 M LiCl(aq) and 2 x 100 mL brine. The organic phase was then dried over anhydrous Na₂SO₄, filtered, and concentrated under vacuum to afford S5 as a white solid (366 mg, 98%). ¹H NMR (400 MHz, CDCl₃) δ 3.69 – 3.45 (m, 2H), 3.42 (t, J = 6.7 Hz, 3H), 3.36 – 3.20 (m, 2H), 1.63 – 1.49 (m, 4H), 1.25 (m, 4H), 0.87 (t, J = 6.8 Hz, 6H). ¹¹B NMR (101 MHz, CDCl₃) δ 144.65, 133.00, 129.75, 127.98, 76.19, 71.76, 70.80, 69.66, 69.34, 31.92, 29.87, 29.70, 29.68, 29.65, 29.63, 29.61, 29.52, 29.47, 29.44, 29.36, 26.02, 25.95, 22.68, 21.61, 14.10. FTIR (DCM, cm⁻¹) 2920.73, 2851.90, 2097.38, 1463.89, 1288.59, 116.42.

1,2-bis(tetradecyl)-3-amino-sn-glycerol (S6)
Hydrogen was bubbled through a stirred suspension S5 (0.366 mg, 0.72 mmol) and 10% Pd/C (80 mg, 0.10 equiv.) in 10% MeOH in ethyl acetate (8 mL, 0.1 M) for 3 h. The reaction was then stirred under positive pressure of hydrogen for 12 h. The hydrogen was removed and the reaction was diluted with 30 mL chloroform. The mixture was filtered through Celite. The filtrate was concentrated in vacuo to afford S6 as an off-white solid (339 mg, 98%). ¹H NMR (400 MHz,
solid was precipitated from methanol and collected by centrifugation.

To a solution of 22.69, 21.61, 14.11. The residue was purified by flash chromatography on a gradient of 10 to 20% hexanes in ethyl acetate to afford 1b as a white waxy solid (229 mg, 48%). 1H NMR (400 MHz, CDCl3) δ 6.52 – 6.35 (m, 1H), 5.87 (ddt, J = 17.2, 10.8, 5.6 Hz, 1H), 5.34 (d, J = 8.4 Hz, 1H), 5.26 (dd, J = 17.2, 1.6 Hz, 1H), 5.17 (dd, J = 10.4, 1.4 Hz, 1H), 4.60 – 4.45 (m, 2H), 4.13 (d, J = 7.2 Hz, 1H), 3.53 (d, J = 9.3 Hz, 1H), 3.49 – 3.32 (m, 4H), 3.31 – 3.14 (m, 1H), 1.62 (dd, J = 12.7, 4.6 Hz, 1H), 1.57 – 1.43 (m, 4H), 1.23 (s, 48H), 0.91 (d, J = 6.4 Hz, 6H), 0.87 – 0.82 (m, 5H). 13C NMR (101 MHz, CDCl3) δ 172.10, 155.93, 132.61, 117.68, 76.51, 71.81, 71.46, 70.25, 65.74, 53.57, 41.92, 40.78, 31.89, 30.02, 29.67, 29.65, 29.62, 29.60, 29.50, 29.46, 29.33, 26.10, 26.08, 24.66, 22.85, 22.65, 22.04, 14.07. HRMS (ESI) m/z: [M+H]+ calc. 681.6140, obs. 681.6124 [M+Na]+ calc. 703.5959, obs. 703.5936.

N-Allylcarboxy Leucine 1’,2’-bis(tetradecyl)-sn-glycerol 3-amide (1b)

A solution of S7 (0.339 g, 0.70 mmol) and N-allylcarboxy leucine NHS ester (0.328 g, 1.05 mmol, 1.50 equiv.) in anhydrous tetrahydrofuran (7 mL, 0.1 M) was refluxed for 18 h. The reaction mixture was cooled to room temperature and the volume halved in vacuo and diluted with 100 mL dichloromethane. The organic phase was washed 1 x 100 mL each of NaHCO3, water, and brine. The organic phase was then dried over Na2SO4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on a gradient of 10 to 20% hexanes in ethyl acetate to afford S7 as a white solid (5.02 g, 82%). 1H NMR (400 MHz, CDCl3) δ 7.82 – 7.75 (m, 2H), 7.32 (d, J = 8.0 Hz, 2H), 4.14 (dd, J = 10.3, 4.1 Hz, 1H), 4.01 (dd, J = 10.3, 5.8 Hz, 1H), 3.59 (t, J = 5.0 Hz, 1H), 3.49 – 3.30 (m, 6H), 2.43 (s, 3H), 1.51 – 1.42 (m, 4H), 1.25 (m, 48H), 0.87 (t, J = 6.8 Hz, 6H). 13C NMR (101 MHz, CDCl3) δ 144.64, 132.98, 129.75, 127.98, 76.17, 71.74, 70.78, 69.64, 69.30, 31.93, 29.87, 29.71, 29.69, 29.67, 29.64, 29.62, 29.52, 29.48, 29.45, 29.37, 26.03, 25.95, 22.69, 21.61, 14.11.

1,2-bis(hexadecyl)-3-tosyl-sn-glycerol (S7)
To a solution of 1,2-bis(hexadecyl)-sn-glycerol (5.00 g, 9.24 mmol) in CHCl3 (80 mL, 0.1 M) and pyridine (10 mL) was added p-toluenesulfonylchloride (2.64 g, 13.8 mmol, 1.50 equiv.), and the mixture was stirred at room temperature for 18 h. The reaction mixture was washed with 3 x 100 mL 1 M HCl, 1 x 50 mL brine, and dried over anhydrous Na2SO4. The solution was filtered and concentrated to an oil. The crude product was dissolved in minimal diethyl ether and precipitated into cold methanol and collected by centrifugation at 1000 rcf to afford S7 as a white solid (5.02 g, 82%). 1H NMR (400 MHz, CDCl3) δ 7.82 – 7.75 (m, 2H), 7.32 (d, J = 8.0 Hz, 2H), 4.14 (dd, J = 10.3, 4.1 Hz, 1H), 4.01 (dd, J = 10.3, 5.8 Hz, 1H), 3.59 (t, J = 5.0 Hz, 1H), 3.49 – 3.30 (m, 6H), 2.43 (s, 3H), 1.51 – 1.42 (m, 4H), 1.25 (m, 48H), 0.87 (t, J = 6.8 Hz, 6H). 13C NMR (101 MHz, CDCl3) δ 144.64, 132.98, 129.75, 127.98, 76.17, 71.74, 70.78, 69.64, 69.30, 31.93, 29.87, 29.71, 29.69, 29.67, 29.64, 29.62, 29.52, 29.48, 29.45, 29.37, 26.03, 25.95, 22.69, 21.61, 14.11.

1,2-bis(hexadecyl)-3-azido-sn-glycerol (S8)
To a solution of S7 (5.03 g, 7.24 mmol) and sodium azide (2.82 g, 43.4 mmol, 6.00 equiv.) in a 1:1 mixture of dimethylsulfoxide / N,N-dimethylformamide (36 mL, 0.2 M) was added 15-crown-5 (4.31 mL, 21.7 mmol, 3.00 equiv.). The mixture was stirred at 75 °C for 18 h. The reaction mixture was cooled to room temperature, diluted with 100 mL a M LiCl and extracted with 3 x 50 mL diethyl ether. The organic phase was washed 5 x 100 mL 1 M LiCl(aq) and 2 x 100 mL brine. The organic phase was then dried over anhydrous MgSO4, filtered, and concentrated under vacuum to afford an oil. The residue was dissolved in minimal dichloromethane and a white solid was precipitated from methanol and collected by centrifugation and dried under vacuum affording S8 (3.63 g, 89%). 1H NMR (400 MHz, CDCl3) δ 3.60 – 3.49 (m, 4H), 3.46 – 3.40 (m,
**1,2-bis(hexadecyl)-3-amino-sn-glycerol (S9)**

Hydrogen was bubbled through a stirred suspension of S8 (3.63 g, 6.41 mmol) and 10% Pd/C (350 mg, 10% w/w) in 1:1 dichloromethane / ethyl acetate (36 mL, 0.1 M) for 2 h. The reaction was then stirred under positive pressure of hydrogen for 16 h. The hydrogen was removed and the reaction mixture was filtered through Celite. The filtrate was concentrated in vacuo to afford S9 as a white solid (1.37 g, 40%). 

1H NMR (400 MHz, CDCl₃) δ 3.64 – 3.54 (m, 1H), 3.51 – 3.36 (m, 5H), 3.36 – 3.30 (m, 2H), 1.61 – 1.51 (m, 4H), 1.35 – 1.24 (m, 53H), 0.92 – 0.84 (m, 6H).

**N-Allylcarboxy Leucine 1’,2’-bis(hexadecyl)-sn-glycerol 3-amide (1c)**

A solution of S11 (1.37 g, 2.53 mmol) and S1 (1.19 g, 3.80 mmol, 1.5 equiv.) in anhydrous tetrahydrofuran (40 mL) was refluxed for 16 h. The reaction mixture was cooled to room temperature and the volume halved in vacuo and diluted with 100 mL dichloromethane. The organic phase was then stirred at room temperature and the volume halved in vacuo. The organic phase was washed 1 x 100 mL NaHCO₃, 1 x 100 mL water, and 1 x 100 mL brine. The organic phase was then dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was purified by flash chromatography in 25% ethyl acetate in hexanes to afford 1c as a waxy solid (620 mg, 33%).

1H NMR (400 MHz, CDCl₃) δ 6.35 (t, J = 5.7 Hz, 1H), 5.89 (dd, J = 17.2, 10.7, 5.4 Hz, 1H), 5.30 (dd, J = 17.2, 1.6 Hz, 1H), 5.24 – 5.20 (m, 1H), 5.15 (d, J = 8.1 Hz, 1H), 4.56 (d, J = 5.6 Hz, 2H), 4.13 (s, 1H), 3.66 – 3.48 (m, 2H), 3.55 – 3.29 (m, 5H), 3.26 (dd, J = 12.0, 6.7 Hz, 1H), 1.67 (s, 2H), 1.57 – 1.47 (m, 3H), 1.25 (s, 56H), 0.94 (d, J = 6.2 Hz, 6H), 0.91 – 0.82 (m, 6H).

**1,2-bis(octadecyl)-3-tosyl-sn-glycerol (S10)**

To a solution of 1,2-bis(octadecyl)-sn-glycerol (1.00 g, 1.67 mmol) in CHCl₃ (15 mL, 0.1 M) and pyridine (1.5 mL) was added p-toluenesulfonylchloride (480 mg, 2.51 mmol, 1.5 equiv.), and the mixture was stirred at room temperature for 16 h. The reaction was concentrated in vacuo and the white residue was dissolved in minimal hot dichloromethane, precipitated with cold methanol, and collected by centrifugation (1000 rcf, 15 min, 4 °C). The precipitate was dried under high vacuum to afford S10 as a white solid (1.10 g, 100%).

1H NMR (400 MHz, CDCl₃) δ 7.79 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 8.1 Hz, 2H), 4.22 – 3.91 (m, 2H), 3.63 – 3.56 (m, 1H), 3.49 – 3.27 (m, 7H), 2.44 (s, 3H), 1.52 – 1.43 (m, 4H), 1.25 (s, 66H), 0.88 (t, J = 6.3 Hz, 6H).

**1,2-bis(octadecyl)-3-azido-sn-glycerol (S11)**

To a solution of S10 (1.10 g, 1.46 mmol) and sodium azide (570 mg, 8.79 mmol, 6.00 equiv.) in a 1:1 mixture of dimethylsulfoxide / N,N-dimethylformamide (16 mL, 0.1 M) was added 15-
crown-5 (870 µL, 4.39 mmol, 3.00 equiv.). The mixture was stirred at 75 °C for 18 h. The reaction mixture was cooled to room temperature, diluted with 1 M LiCl (100 mL) and extracted with 3 x 50 mL diethyl ether. The organic phase was washed 5 x 100 mL 1 M LiCl (aq) and 2 x 100 mL brine. The organic phase was then dried over anhydrous Na₂SO₄, filtered, and concentrated under vacuum to afford S11 as a white solid (852 mg, 94%). ¹H NMR (400 MHz, CDCl₃) δ 3.61 – 3.51 (m, 2H), 3.43 (dd, J = 7.4, 6.2 Hz, 3H), 3.36 – 3.28 (m, 2H), 1.64 – 1.48 (m, 4H), 1.26 (s, 66H), 0.88 (t, J = 6.2, 5.2 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 77.90, 71.76, 70.64, 70.11, 52.06, 31.93, 30.01, 29.70, 29.67, 29.64, 29.61, 29.51, 29.48, 29.34, 26.12, 26.10, 25.25, 24.69, 22.87, 22.67, 22.63, 22.05, 117.75, 76.48, 71.85, 71.47, 70.26, 65.79, 41.97, 40.82, 34.64, 31.91, 31.57, 30.04, 29.69, 29.67, 29.64, 29.61, 29.51, 29.48, 29.34, 26.12, 26.10, 25.25, 24.69, 22.87, 22.67, 22.63, 22.05, 14.09. HRMS (ESI) m/z: [M+H]⁺ calc. 793.7392, obs. 793.7376 [M+Na]⁺ calc. 815.7211, obs. 815.7188.

1,2-bis(octadecyl)-3-amino-sn-glycero-l (S12)
Hydrogen was bubbled through a stirred suspension of S11 (0.85 g, 1.37 mmol) and 10% Pd/C (150 mg, 0.10 equiv.) in 10% MeOH in ethyl acetate (14 mL, 0.1 M). The reaction was then stirred under positive pressure of hydrogen for 16 h. The hydrogen was removed and the reaction was diluted with chloroform. The mixture was filtered through Celite and then silica gel. The filtrate was concentrated in vacuo to afford S12 as an off-white solid (647 mg, 79%). ¹H NMR (400 MHz, CDCl₃) δ 3.69 – 3.34 (m, 7H), 1.62 – 1.44 (m, 4H), 1.25 (s, 64H), 0.88 (t, J = 6.8 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 31.92, 29.70, 29.65, 29.63, 29.35, 22.68, 14.11. FTIR (DCM, cm⁻¹) 2915.84, 2848.98, 2907.54. The precipitate was dried under high vacuum to afford S11 as a white solid (852 mg, 94%). ¹H NMR (400 MHz, CDCl₃) δ 3.61 – 3.51 (m, 2H), 3.43 (dd, J = 7.4, 6.2 Hz, 3H), 3.36 – 3.28 (m, 2H), 1.64 – 1.48 (m, 4H), 1.26 (s, 66H), 0.88 (t, J = 6.2, 5.2 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 77.90, 71.76, 70.64, 70.11, 52.06, 31.93, 30.01, 29.70, 29.67, 29.64, 29.61, 29.51, 29.48, 29.34, 26.12, 26.10, 25.25, 24.69, 22.87, 22.67, 22.63, 22.05, 14.09. HRMS (ESI) m/z: [M+H]⁺ calc. 793.7392, obs. 793.7376 [M+Na]⁺ calc. 815.7211, obs. 815.7188.

N-Allylcarboxy Leucine 1',2'-bis(octadecyl)-sn-glycero 3-amide (1d)
A solution of 3-amino-1,2-bis(octadecyl)-sn-glycero (0.647 g, 1.08 mmol) and N-allylcarboxy leucine NHS ester (0.508 g, 1.62 mmol, 1.5 equiv.) in anhydrous tetrahydrofuran (11 mL, 0.1 M) was refluxed at 85 °C for 18 h. The reaction mixture was cooled to room temperature and the volume halved in vacuo. The residue was diluted with 100 mL dichloromethane washed with 1 x 100 mL each successively of NaHCO₃, water, and brine. The organic phase was then dried over Na₂SO₄, filtered, and concentrated in vacuo. The residue was dissolved in minimal hexanes and purified via flash chromatography on silica gel on a gradient of 0 to 20% ethyl acetate in hexanes to afford 1d as a waxy solid (288 mg, 33%). ¹H NMR (400 MHz, CDCl₃) δ 6.38 (t, J = 5.7 Hz, 1H), 5.89 (ddt, J = 17.2, 10.8, 5.6 Hz, 1H), 5.28 (dd, J = 17.2, 1.6 Hz, 1H), 5.25 – 5.15 (m, 1H), 4.62 – 4.45 (m, 2H), 4.13 (td, J = 9.0, 5.1 Hz, 1H), 3.62 – 3.51 (m, 2H), 3.51 – 3.33 (m, 6H), 3.25 (dd, J = 11.9, 6.8 Hz, 1H), 1.73 – 1.58 (m, 2H), 1.58 – 1.43 (m, 6H), 1.24 (s, 66H), 0.93 (d, J = 6.1 Hz, 6H), 0.87 (t, J = 7.0 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 172.02, 132.59, 117.75, 76.48, 71.85, 71.47, 70.26, 65.79, 41.97, 40.82, 34.64, 31.91, 31.57, 30.04, 29.69, 29.67, 29.64, 29.61, 29.51, 29.48, 29.34, 26.12, 26.10, 25.25, 24.69, 22.87, 22.67, 22.63, 22.05, 14.09. HRMS (ESI) m/z: [M+H]⁺ calc. 793.7392, obs. 793.7376 [M+Na]⁺ calc. 815.7211, obs. 815.7188.

O-(heptaacetyl-β-lactosyl)serine N-carboxyanhydride
Synthesized as previously reported (8).

Alanine N-carboxyanhydride
Synthesized as previously reported (8). The product was further purified by repeated precipitation from anhydrous tetrahydrofuran / hexane.
AlexaFluor488 2,3-dioleyl-1-phospho(ethanolamine)glycerol
To a solution of 2,3-dioleyl-1-phospho(ethanolamine)glycerol (5.0 mg, 0.007 mmol, 4.0 equiv.) in chloroform (5 mL) and methanol (2.5 mL) was added AlexaFluor 488 NHS ester (1.0 mg, 0.002 mmol, 1.0 equiv.) and DIPEA (2 µL, 0.007 mmol, 4.0 equiv.). The reaction was stirred overnight. The reaction was then concentrated in vacuo in the dark and purified via HPLC.

General initiator preparation
Initiators were prepared in a dinitrogen-filled glovebox. To a homogenous yellow solution of Nickel(0) bis(cyclooctadiene) in (8.0 mg, 0.029 mmol) in anhydrous tetrahydrofuran (200 µL) was added 1,1'-bipyridine (4.5 mg, 0.029 mmol). The complex was allowed to at room temperature for 1.5 h, and it turned a deep purple. Then a solution of N'-allylcarboxyl Leucine amide 1 (0.029 mmol) in tetrahydrofuran (400 µL) was added and the solution turned a shade of orange, the precise color depending on the ligand. The reaction mixture was placed into a bomb tube, moved out of the glovebox to a heating bath, and heated at 80 ºC for 16 h. The catalyst complex was cooled to room temperature and moved back into the glovebox. This crude mixture was used directly in subsequent polymerizations.

General glycopolypeptide co-polymerization
All polymerization reactions were performed in a dinitrogen-filled glove box. To a solution of NCA in THF (100 mg/mL) was rapidly added a solution of crude initiator via syringe. The reaction was stirred at 20 ºC and polymerization progress was monitored by FTIR. Polymerizations were generally complete within 6 h. Aliquots were removed for GPC analysis immediately upon polymerization completion. Completion of polymerization was determined by FTIR, as measured by the consumption of characteristic N-carboxyanhydride peaks (~1850 cm^{-1} and ~1790 cm^{-1}) and appearance of the amide peak (~1650 cm^{-1}).

General glycopolypeptide deprotection procedure
To a solution of protected glycopolypeptide in 1:1 tetrahydrofuran / methanol (15 mg/mL) was added hydrazine monohydrate (4.0 equiv. per acetate) and the reaction was stirred for 1 h, at which point an additional volume of water was added to improve solubility. The deprotections were stirred for a further 16 h at room temperature. Reactions were quenched by the addition of a few drops of acetone. The solvent was removed in vacuo, resuspended at 20 mg/mL in water, and filtered through a 0.45 µm syringe filter. The clarified solutions were purified via dialysis in 3500 Da molecular weight cutoff (MWCO) dialysis cassettes and dialyzed against 4 L of deionized water for 3 days, with water changes twice per day. Dialyzed polymers were lyophilized to dryness to give glycopolypeptides as white fluffy solids. Degree of polymerization was determined by end-group integration for lipid-tethered polymer or aqueous GPC for polymers without lipid end groups.

MM1 – H₂N-poly[Ser(Lac)₀.₅-co-Ala₀.₅]₉₈-Leu-(cholesterylamine)-propylamide
Polymerization of 3 (112 mg, 0.15 mmol, 0.50 equiv.) and 4 (17 mg, 0.15 mmol, 0.50 equiv.) with 2a (0.03 equiv). and deprotection as described above afforded MM1 as a fluffy white solid (55 mg, 76%). ¹H NMR (500 MHz, D₂O) δ 4.69 – 3.22 (588 H), 1.65 – 0.68 (156 H), 0.92 – 0.75 (12 H). DP (ca. NMR) 98, Mn (ca. NMR) 28.9 kDa. GPC (THF) Đ 1.13.
MM2 – H₂N-polyser(Lac)₆5-co-Ala₃₉₃-Leu-dimyristyl-sn-glycerollamide
Polymerization of 3 (112 mg, 0.15 mmol, 0.50 equiv.) and 4 (17 mg, 0.15 mmol, 0.50 equiv.) with 2b (0.03 equiv.) and deprotection as described above afforded MM2 as a fluffy white solid (61 mg, 84%). ¹H NMR (500 MHz, D₂O) δ 4.72 – 3.21 (455H), 1.68 – 0.60 (187H), 1.33 – 1.11 (60H), 0.84 (12H). DP (ca. NMR) 93, Mn (ca. NMR), 23.6 kDa. GPC (THF) Đ 1.19.

MM3 – H₂N-polyser(Lac)₆5-co-Ala₃₁₉₇-Leu-dipalmityl-sn-glycerollamide
Polymerization of 3 (150 mg, 0.20 mmol, 0.50 equiv.) and 4 (23 mg, 0.20 mmol, 0.50 equiv.) with 2c (0.03 equiv.) and deprotection as described above afforded MM3 as a fluffy white solid (87 mg, 89%). ¹H NMR (500 MHz, D₂O) δ 4.72 – 3.15 (472H), 1.47 – 0.60 (217H), 1.33 – 1.11 (68H), 0.83 (12H). DP (ca. NMR) 101, Mn (ca. NMR), 24.7 kDa. GPC (THF) Đ 1.16.

MM4 – H₂N-polyser(Lac)₆5-co-Ala₃₁₉₇-Leu-distearyl-sn-glycerollamide
Polymerization of 3 (112 mg, 0.15 mmol, 0.50 equiv.) and 4 (17 mg, 0.15 mmol, 0.50 equiv.) with 2d (0.03 equiv.) and deprotection as described above afforded MM4 as a fluffy white solid (58 mg, 80%). ¹H NMR (500 MHz, D₂O) δ 4.54 – 3.26 (506H), 1.57 – 0.60 (227H), 1.33 – 1.11 (74H), 0.83 (12H). DP (ca. NMR) 107, Mn (ca. NMR), 26.5 kDa. GPC (THF) Đ 1.26.

MM5 – H₂N-polyser(Lac)₆5-co-Ala₃₃-Leu-dimyristyl-sn-glycerollamide
Polymerization of 3 (75 mg, 0.10 mmol, 0.50 equiv.) and 4 (12 mg, 0.10 mmol, 0.50 equiv.) with 2b (0.10 equiv.) and deprotection as described above afforded MM5 as a fluffy white solid (42 mg, 87%). ¹H NMR (500 MHz, D₂O) δ 4.65 – 3.26 (176H), 1.66 – 0.53 (63H), 1.30 – 1.11 (44H), 0.83 (12H). DP (ca. NMR) 33, Mn (ca. NMR), 8.7 kDa. GPC (THF) Đ 1.11.

MM6 – H₂N-polyser(Lac)₆5-co-Ala₃₁₂₅
A solution of Nickel(0) 1,1'-bipyridine cyclooctadiene, Ni(bpy)(COD), was prepared by complexing Nickel(0) bis(cyclooctadiene) in (8.0 mg, 0.029 mmol) with 1,1'-bipyridine (4.5 mg, 0.029 mmol) in anhydrous tetrahydrofuran (600 µL) for 1.5 h at room temperature in a dinitrogen filled glovebox. This initiator solution was used directly and immediately in the subsequent polymerization. Polymerization of 3 (112 mg, 0.15 mmol, 0.50 equiv.) and 4 (17 mg, 0.15 mmol, 0.50 equiv.) with a Ni(bpy)(COD) complex (0.03 equiv.) and deprotection as described above afforded MM6 as a fluffy white solid (47 mg, 65%). ¹H NMR (500 MHz, D₂O) δ 4.59 – 3.16 (11 rel. H), 1.50 – 1.05 (3 rel. H). GPC (THF) Đ 1.32. GPC (DPBS) Đ 1.41, dn/dc (ca. aq. GPC) 0.0796 mL/g, Mn (ca. aq. GPC) 30.4 kDa, DP (ca. aq. GPC) 125.

MM10 – H₂N-polyser(Lac)₆5-co-Ala₃₃₀
Prepared as for MM6 by polymerization of 3 (35 mg, 0.05 mmol, 0.50 equiv.) and 4 (5.7 mg, 0.05 mmol, 0.50 equiv.) with Ni(bpy)(COD) (0.09 equiv.). Afforded MM10 as a fluffy white solid (20 mg, 76%). ¹H NMR (500 MHz, D₂O) δ 4.61 – 3.11 (11 rel. H), 1.48 – 1.21 (3 rel. H). GPC (DPBS) Đ 1.20, Mn (est.) 7.8 kDa, DP (est.) 30.

General glycopolypeptide chemoenzymatic sialylation procedure
To a solution of glycopolypeptide in pH 8.5 100 mM Tris-buffer containing 2 mM MgCl₂ (2 mM with respect to lactose) was added cytidine triphosphate (10 equiv. per lactose) and N-acetyl neuraminic acid (10 equiv. per lactose). To this reaction mixture was added Pd26ST (final concentration 0.2 mg/mL) and NmCSS (final concentration 0.1 mg/mL). The sialylation was
incubated at 37 °C while being shaken at 180 rpm for 24 h. The reaction was quenched by the addition of an equal volume methanol and incubated at 4 °C for 30 min. The precipitates were pelleted by spinning at 3700 rcf for 30 min and the supernatants were filtered through a 0.8 µm syringe filter. The clarified supernatants were concentrated in vacuo to syrups and diluted with water. The clarified solutions were purified via dialysis in 3500 Da molecular weight cutoff (MWCO) dialysis cassettes and dialyzed against 4 L of deionized water for 3 days, with water changes twice per day. Dialyzed polymers were lyophilized to dryness and the lactosyl peak at 3.6 ppm (integrated to 1 H). Sialylation extent was reproducibly ~ 50% conversion. For the non-lipidated polymer, sialylation was also assessed by aqueous GPC.

**MM7 – H2N-poly[Ser(Sia-2,6-Lac)0.23-co-Ser(Lac)0.27-co-Ala0.5]93-Leu-dimyristyl-sn-glycerylamide**

Sialylation of MM2 (20 mg, 0.041 mmol lactose) as described above afforded MM7 as a fluffy white solid (22 mg, 84% mass recovery, 45% conversion by NMR). 1H NMR (500 MHz, D2O) δ 4.59 – 3.25 (703H), 2.71 – 2.61 (22H) 1.99 (65H), 1.78 – 1.61 (22H), 1.54 – 0.72 (215H), 1.29 – 1.13 (54H), 0.83 (12H). DP (ca. NMR) 93, Mn (ca. NMR), 23.6 kDa.

**MM8 – H2N-poly[Ser(Sia-2,6-Lac)0.25-co-Ser(Lac)0.25-co-Ala0.5]33-Leu-dimyristyl-sn-glycerylamide**

Sialylation of MM5 (15 mg, 0.029 mmol lactose) as described above afforded MM8 as a fluffy white solid (19 mg, 90% mass recovery, 50% conversion by NMR). 1H NMR (500 MHz, D2O) δ 4.60 – 3.29 (260H), 2.71 – 2.61 (8H), 2.05 (24H), 1.78 – 1.61 (8H), 1.54 – 0.72 (52H), 1.29 – 1.13 (54H), 0.83 (12H). DP (ca. NMR) 32, Mn (ca. NMR), 11.1 kDa.

**MM9 – H2N-poly[Ser(Sia-2,6-Lac)0.25-co-Ser(Lac)0.25-co-Ala0.5]125**

Sialylation of MM6 (12 mg, 0.022 mmol lactose) as described above afforded MM9 as a fluffy white solid (15 mg, 95% mass recovery, 50% conversion by NMR and GPC). 1H NMR (500 MHz, D2O) δ 4.59 – 3.45 (15 rel. H), 3.38 – 3.27 (1 rel. H), 2.71 – 2.62 (0.5 rel. H), 1.99 (1.5 rel. H), 1.73 – 1.65 (0.5 rel H), 1.50 – 1.05 (3 rel. H). GPC (DPBS) δ 1.6, dn/dc (ca. aq. GPC) 0.0796 mL/g, Mn (ca. aq. GPC) 40.0 kDa, DP (ca. aq. GPC) 125.

**General fluorophore labeling protocol**

To a solution of glycopolypeptide in DPBS (4 mg/mL) was added a stock solution of 5 mg/mL AlexaFluor 488 NHS ester in dimethylsulfoxide (5.0 equiv. per polymer chain end). The reaction was incubated at 37 °C while being shaken at 600 rpm for 24 h. The mixture was then diluted to 1 mg/mL with water and purified by NAP-25 size-exclusion purification column. The eluate was collected and lyophilized. Polymers were resuspended at 50 µM in DPBS, as determined by optical density at 490 nm.

**Expression and purification of recombinant enzymes**

Protein expression plasmids for Pd26ST and NmCSS were gifts from the lab of Xi Chen. The constructs were transformed into BL21(DE3) E. coli and expressed as previously described (9). In brief, 5 mL cultures were inoculated from a glycerol stock and grown out overnight. The confluent cultures were then diluted 1:500 into 1 L LB and grown for a few hours at 37 °C until OD ~ 0.8. At that point, protein expression was induced by the addition of 0.1 mM IPTG and the
cells were grown at 20 °C for 24 h with shaking at 210 rpm. The bacterial pellets were harvested
by centrifugation (3500 rcf, 15 min, 4 °C) and either immediately lysed or stored at -80 °C.

For purification of recombinant proteins, bacterial pellets (corresponding to 1 L of culture) were
lysed in 35 mL cold lysis buffer (250 mM Tris pH 7.5, 0.5 M NaCl, 0.1% TritonX100) with the
addition of a cOmplete Protease Inhibitor Cocktail tablet (Roche) and 20 µL DNAseI (Thermo).
Cells were lysed by homogenization and cellular debris was removed by centrifugation (16000
rcf, 1 h, 4 °C). The supernatant was directly loaded via FPLC onto a 1 mL HisTrap column (GE)
pre-equilibrated with washing buffer (100 mM Tris pH 7.5, 0.5 M NaCl, 20 mM imidazole). The
column was washed with 10 CV washing buffer and then the bound proteins were eluted over a
gradient over 12 CV to elution buffer (100 mM Tris pH 7.5, 0.5 M NaCl, 200 mM imidazole). The
eluted fractions were analyzed by PAGE and UV, and fractions containing the receombinant
enzyme were pooled and purified by dialysis against a storage buffer (50 mM Tris pH 7.5, 250
mM NaCl, 10% glycerol). Purified proteins were quantified by A280 using computationally
predicted molar extinction coefficients, aliquoted, and flash frozen in liquid nitrogen. Aliquots
were stored at -80 °C and only thawed immediately before use.
4. Polymer Brush Theory Calculations

Calculation of extension length using polymer brush theory and exclusion concentration

To examine the hypothesis that the mucin mimics follow polymer brush theory, the exclusion concentration of MM2 was used to calculate the predicted distance between polymers and related to the measured extension length of MM2. Here we denote average height of polymer bulk from surface as \( H \), distance between polymer anchors as \( D \), and \( R_g \) as the radius of gyration of the polymer.

Following seminal work by de Gennes, Alexander, and Milner (10–12), at the dilute limit, \( H \) is independent of polymer size. In this dilute regime, \( R_g < D \). As \( R_g \sim D \), this dilute limit assumption no longer holds. The transition from the mushroom to brush state is expected to occur when \( R_g \sim D \). We will use our measured exclusion concentration of MM2 to calculate the transition regime where \( R_g \sim D \) and compare this value to the measured extension height of MM2.

Using the measured exclusion value of MM2 (0.01 mol%) at the critical binding transition shown as a blue line on figure 5A, we can calculate the distance between polymer anchors (\( D \)) using some simple approximations of the bilayer. A PC head group is assumed to take up 0.6 nm\(^2\) (13) and no curvature is assumed to be induced within the SLB. Based on the exclusion concentration and PC head group size, we calculate approximately 200 polymers/micron\(^2\). Assuming equal spacing of the polymer within the bilayer, the max distance possible between the polymer anchors can be calculated \( \sim 30 \) nm at this critical point. Assuming the anchor is located at the center of this distance, \( D = 2 \ R_g \), therefore \( R_g \sim 15 \) nm. This \( R_g \) represents the radius of gyration of the polymer calculated from the critical binding transition in figure 5A for MM2. From this simple calculation, the polymer would be assumed to have a mushroom height on average \( < 15 \) nm and an extension height \( \geq 15 \) nm. We then compared this calculated extension value to our results. We found using FLIC, that below 0.01 mol% MM2 the polymer’s distal fluorophore resided on average at the SLB interface with a large variance indicating a heterogenous extension consistent with mushroom behavior. As the amount of loaded polymer increases, distance between polymer anchors decreases; therefore, \( D < 2 \ R_g \) which leads to extension of MM2 as shown schematically in figure 5C. At concentrations of MM2 above 0.01 mol% we measured the extension height of the polymer to be 10.6 nm ± 3.7 nm from the top leaflet of the SLB. While the measured and calculated values are not identical, the calculated extension height reasonably agrees with the measured DLS and FLIC values of MM2 extension height. Given the simplistic model used, our data are consistent with a polymer brush model.

To further support the assertion that the mucin mimics behave as polymer brushes we compared the measured extension height of MM5 (\( DP = 1/3 \) MM2) with its measured exclusion value of MM5 (0.1 mol%) at the critical binding transition. Again, we use the same approximations and assumptions listed above to calculated MM5 \( R_g = \sim 6 \) nm. This calculated value is approximately in agreement with our experimental determined extension height of MM5 from DLS.
5. Supplementary Figures

Fig. S1. Synthesis of polymerization initiators 2a-d from precatalysts 1a-d. Precatalysts were added to a solution of pre-complexed Nickel (II) (1,1'-bipyridine) (cyclooctadiene) and heated to 80 °C for 16 h.

Fig. S2. Amino acid derived monomers 3 and 4 are co-polymerized using 2a-d in tetrahydrofuran at ambient temperature. Glycopolypeptides are then deprotected using trifluoroacetic acid for 1 h (for 2a only) followed by hydrazine hydrate overnight.

Fig. S3. Synthesis of N-allylcarboxy leucine succinimidyl ester.

Fig. S4. Synthesis of cholesterylamine precatalyst 1a.
Fig. S5. Synthesis of bisalkyl glycerol precatalysts 1b-d.
Fig. S6. Sample aqueous GPC traces of MM6 and sialylated version MM9. The shift in the modal retention time is listed. Shift correlates to a molecular weight increase consistent with previous NMR and GPC data.

Fig. S7. Circular dichroism spectra for MM2 and MM7 reveal that both asialyl (MM2) and sialyl (MM7) mucin mimics adopt random coil conformations in solution.
Fig. S8. Circular dichroism spectra for MM2 and MM5 reveal that both long (MM2) and short (MM7) C14-tethered mucin mimics adopt random coil conformations in solution.
Fig. S9. Linear regression of increasing mol% of DOPE-AF488 in a bilayer against increasing fluorescence intensity. Error bars represent the standard deviation within sample replicates.

Table S1. Mucin mimics diffusion coefficients are not significantly different from labeled DOPE using a one-way ANOVA. Table below shows the diffusion coefficient calculated after fitting independent replicate FRAP curves for polymer loading between 0.005 and 0.1 mol%.

<table>
<thead>
<tr>
<th>construct</th>
<th>D (μm²/s)</th>
<th>stdev</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOPE</td>
<td>3.63</td>
<td>0.36</td>
</tr>
<tr>
<td>MM3</td>
<td>3.55</td>
<td>0.49</td>
</tr>
<tr>
<td>MM7</td>
<td>2.54</td>
<td>0.36</td>
</tr>
<tr>
<td>MM1</td>
<td>2.75</td>
<td>0.56</td>
</tr>
<tr>
<td>MM2</td>
<td>2.86</td>
<td>0.94</td>
</tr>
<tr>
<td>MM8</td>
<td>2.47</td>
<td>0.19</td>
</tr>
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</table>
Fig. S10. Fluorescence intensity as a function of SiO$_2$ height for AF488 conjugated to DOPE (grey) and [MM2] < 0.01mol% (black). Calculated intensity for 488AF located directly at the bilayer/bulk aqueous interface (0 nm including the water layer) is shown for reference in grey. 3 independent experiments are shown for each condition. Vertical error bars give the fluorescence intensity variation within a step. Horizontal error bars give the variation in ellipsometry measurement of SiO$_2$ height.

Fig. S11. Fluorescence intensity as a function of SiO$_2$ height for high surface concentration of MM2 overlaid with the best fit calculated intensity curve for AF488 extended 10.6 nm ± 3.7 nm from the SLB (red). Calculated intensity for 488AF located directly at the bilayer/bulk aqueous interface (0 nm including the water layer) is shown for reference in grey. Vertical error bars give the fluorescence intensity variation within a step. Horizontal error bars give the variation in ellipsometry measurements of SiO$_2$ heights. MM2 replicated from 3 independent preparations.
Fig. S12. Dynamic light scattering is consistent with FLIC measurements of extension length of mucin mimics. DLS measured effective change in radius between nominally (by extrusion) 50 nm SUVs (70POPC/20DOPE/10Chol) and SUVs with the addition of MM2 or MM5 after extrusion. Mean and standard error are shown from three independent experimental preparations. MM2 mean = 23.2 nm ± 3.7 nm, MM5 mean = 6.1 nm ± 3.5 nm, pval = 0.004.

Fig. S13. Non-sialylated mucin mimics do not bind IAV. Relative binding numbers of IAV are shown after incubation and wash with SLBs containing 2% GD1a, MM2 (0.02 mol%), MM5 (0.04 mol%), and phospholipid only SLB. Phospholipid SLB composition = (70POPC,20DOPE,10CHOL). Error bars display one standard deviation within sample replicates.
Fig. S14. Concentration Dependence of MM2 and MM5 on IAV binding display positive cooperativity. Relative binding of IAV as a function of increasing mol% of MM was fit using Langmuir-hill equation (dashed curve). Relative Binding = ([MM mol%])^n]/([IC50]^n + [MM mol%]). n yields the hill coefficient and IC50 yields the mol % at which IAV binding is 50%. A) n = 6.7; IC50 = 0.004 mol%; R^2 = 0.94. B) n = 19; IC50 = 0.03 mol%; R^2 = 0.86. Lower bound was constrained ≤ 0.1.

Fig. S15. Non lipidated polymer does not reduce IAV binding. Relative binding numbers of IAV are shown after incubation and wash with SLBs containing 2% GD1a, no GD1a, 2% GD1a + MM6 (50 pmol), 2% GD1a + MM10 (50 pmol) 2% GD1a + 0.01 mol% MM2 (10 fmol) , 2%GD1a+ 0.08mol% MM5 (60 fmol), Error bars display one standard deviation within sample replicates.
Fig. S16. IAV binds specifically via sialic acid moieties to sialylated mucin mimics. SLBs displaying MM7 was incubated with IAV with and without Neuraminidase (25 activity units). After NA treatment, no IAV binding occurs. Error bars display one standard deviation within sample replicates.
Fig S17. Increasing MM7 concentration increases IAV binding until a critical binding transition point. Increasing mol% of MM7 incorporated into SLB (70POPC,20DOPE,10Chol) led to an increase in IAV binding until 0.01 mol% at which point IAV binding decreased and remained constant. Error bars shown in x and y display standard deviation within sample replicates. The blue vertical dashed line demarcates 0.01 mol% transition consistent with 100mer extension.

Fig. S18. Increasing the ratio of MM2 to MM7 reduces binding of IAV. An increasing ratio of MM2:MM7 was added to SLBs (70POPC,20DOPE,10Chol). [MM2+MM7] = 10 µM in each condition. Error bars display one standard deviation within sample replicates.
Table S2. \( t_{1/2} \) of lipid mixing for IAV bound via GD1a at: high concentration \textbf{MM7}, high concentration \textbf{MM8}, low concentration \textbf{MM7}, and low concentration \textbf{MM8}. \( t_{1/2} \) of lipid mixing are obtained from corresponding CDF of single virus lipid mixing wait times and give the time when half of the total fusion events have occurred.

<table>
<thead>
<tr>
<th>IAV binding method</th>
<th>( t_{1/2} ) lipid mixing (s)</th>
<th>Number fused/total particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>GD1a</td>
<td>22.1</td>
<td>189/1111</td>
</tr>
<tr>
<td>High concentration \textbf{MM7}</td>
<td>43.2</td>
<td>426/1577</td>
</tr>
<tr>
<td>High concentration \textbf{MM8}</td>
<td>41.0</td>
<td>509/1817</td>
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<tr>
<td>Low concentration \textbf{MM7}</td>
<td>28.7</td>
<td>148/617</td>
</tr>
<tr>
<td>Low concentration \textbf{MM8}</td>
<td>25.2</td>
<td>47/235</td>
</tr>
</tbody>
</table>

Fig. S19. Characterization of low and high loading of \textbf{MM7} in Figure 6D. Mucin mimics were counted using single particle photobleaching of AF488. Representative histogram compiled from Low MM N = 212, High MM N= 209.
6. References


7. Spectroscopic data of novel compounds

N-\text{Allylcarboxy Leucine 3-}(\text{N'-Boc-cholesterylamine})\text{propylamide (1a)}
1,2-bis(tetradecyl)-3-benzyl-sn-glycerol (S2)
1,2-bis(tetradecyl)-sn-glycerol (S3)
1,2-bis(tetradecyl)-3-tosyl-sn-glycerol (S4)
1,2-bis(tetradecyl)-3-azido-sn-glycerol (S5)
1,2-bis(tetradecyl)-3-amino-sn-glycerol (S6)
N-Allylcarboxy Leucine 1',2'-bis(tetradecyl)-sn-glycerol 3-amide (1b)
1,2-bis(hexadecyl)-3-tosyl-sn-glycerol (S7)
1,2-bis(hexadecyl)-3-azido-sn-glycerol (S8)
1,2-bis(hexadecyl)-3-amino-sn-glycerol (S9)

\[
\text{H}_2\text{N} \xrightarrow{\text{O}(\text{CH}_2)_{16}\text{CH}_3} \text{O}(\text{CH}_2)_{16}\text{CH}_3
\]

\text{S9}
C:\Users\Public\FTIR\Data\c3-378-a-ir2.0  c3-378-a-ir2  6/20/2018
N- Allylcarboxy Leucine $1',2'$-bis(hexadecyl)-sn-glycerol 3-amide (1c)
1,2-bis(octadecyl)-3-tosyl-sn-glycerol (S10)
1,2-bis(octadecyl)-3-azido-sn-glycerol (S11)
1,2-bis(octadecyl)-3-amino-sn-glycerol (S12)
N-Allylcarboxy Leucine 1',2'-bis(octadecyl)-sn-glycerol 3-amide (1d)
MM1 – H₂N-poly[Ser(Lac)₀.₅-co-Ala₀.₅]₉₈-Leu-(cholesterylamine)-propylamide
MM2 – H$_2$N-poly[Ser(Lac)$_{0.5}$-co-Ala$_{0.5}$]$_{93}$-Leu-dimyristyl-sn-glycerollamide
MM3 – H₃N-poly[Ser(Lac)₀.₅-co-Ala₀.₅]₁₀₁-Leu-dipalmitoyl-sn-glyceroamide
MM4 – H₂N-poly[Ser(Lac)₀.₅-co-Ala₀.₅]₁₀⁷-Leu-distearyl-sn-glycercylamide
MM5 – H$_2$N-poly[Ser(Lac)$_{0.5}$-co-Ala$_{0.5}$]$_{33}$-Leu-dimyristyl-sn-glycerollamide
MM6 – H$_2$N-poly[Ser(Lac)$_{0.5}$-co-Ala$_{0.5}$]$_{125}$
MM7 – H₂N-poly[Ser(Sia-2,6-Lac)₉₀,₂₅-co-Ser(Lac)₅₀,₂₇-co-Ala₀,₅₉₃-Leu-dimyristyl-sn-glycerylamide
MM8 – H$_2$N-poly[Ser(Sia-2,6-Lac)$_{0.25}$-co-Ser(Lac)$_{0.25}$-co-Ala$_{0.5}$]$_{33}$-Leu-dimyristyl-sn-glycerylamide
MM9 – H$_2$N-poly[Ser(Sia-2,6-Lac)$_{0.25}$-co-Ser(Lac)$_{0.25}$-co-Ala$_{0.5}$]$_{125}$
MM10 – H₂N-poly[Ser(Lac)₀.₅-co-Ala₀.₅]₃₀