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Three-Dimensional Structure of Glycolipids in Biological Membranes

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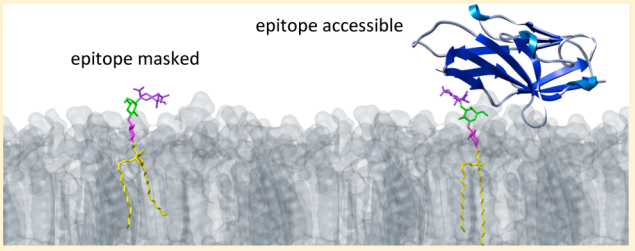
ABSTRACT: Characterizing the structure−function relationships of glycolipids in lipid membranes is a challenging endeavor. Glycolipid "structure" is rarely, if ever, a unique low-energy conformer, but an ensemble of dynamic states, which vary in their presentation of binding epitopes. The modulation of binding epitopes not only is an internal process but also is influenced by external factors such as glycolipid clustering and fluctuations in and composition of the fluid membrane environment. As with other glyco-conjugates, three-dimensional structural elucidation has relied heavily on nuclear magnetic resonance spectroscopy and computational modeling. Discrete conformational states can be discerned from motion-averaged experimental data by employing independent molecular dynamics simulations. Using model membranes such as micelles, bicelles, and bilayers, we can approximate the effect of their biological environment and quantify cell-surface presentation.



The term glycolipid defines any molecule containing one or more monosaccharides covalently bound via a glycosidic linkage to a hydrophobic group such as a ceramide, sphingoid, or prenyl phosphate (Figure 1). In vivo, glycolipid molecules [glycosphingolipids (cerebrosides, gangliosides, globosides, and glycophosphosphingolipids), glycosylphosphatidylinositols, lipopolysaccharides, lipooligosaccharide, etc.] are ubiquitously expressed on the outer leaflet of cell membranes and mediate diverse biological and pathological processes such as cell growth, fertility, immunity, metastasis, and viral and microbial invasion.[1−5] For multiple reasons, the structural and dynamic properties of glycolipids, and their interaction with protein ligands, are not well characterized. First, glycolipid molecules are not easily replicated, unlike nucleic acids and proteins for which amplification and recombinant methods can be employed. Glycolipids must be purified from biological sources or synthesized chemically or chemo-enzymatically. Second, both the carbohydrate and lipid domains may possess a considerable level of heterogeneity and structural flexibility, presenting challenges for analytical techniques requiring high-purity samples and structurally defined molecules, respectively. Third, glycolipid function is inherently tied to the local membrane environment, necessitating the inclusion of a membranelike environment when pursuing functional studies of glycolipids. Thus, structural and functional analysis of glycolipids often involves working with (and around) limited sample volumes, molecular heterogeneity, structural disorder, and membrane-containing systems.

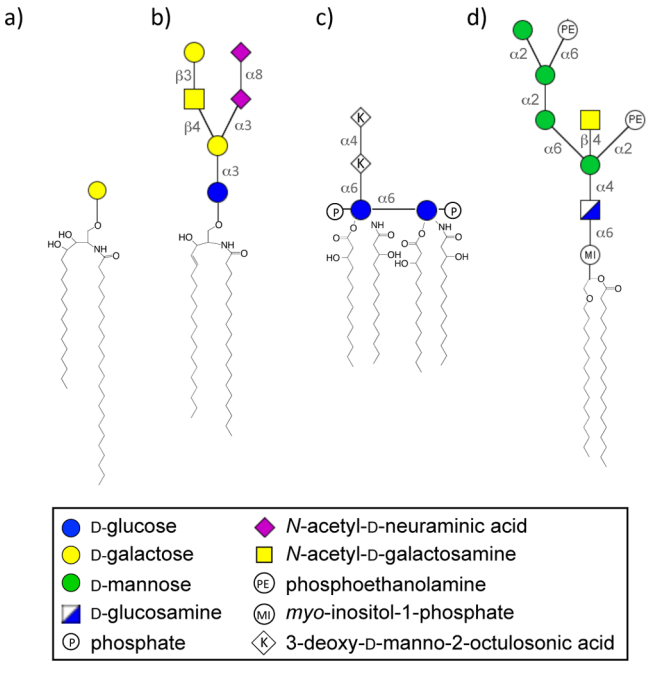


Figure 1. Glycolipids make up a diverse molecular class. Examples include (a) α-galactosylceramide, (b) ganglioside GD1b, (c) endotoxin KDO_2-lipid IVa, and (d) a glycophosphatidylinositol anchor.

Herein, we discuss methods and challenges in the characterization of the atomic-level structure and dynamics of glycolipids at membrane surfaces. Structural characterization of glycolipids involves determining the composition (both lipid and glycan), branching information, anomeric configuration, internal three-dimensional (3D) structure, internal dynamics, and insertion depth and orientation (also known as presentation) in a biological membrane. In vivo, presentation of course is also dependent on a multitude of other factors, including the

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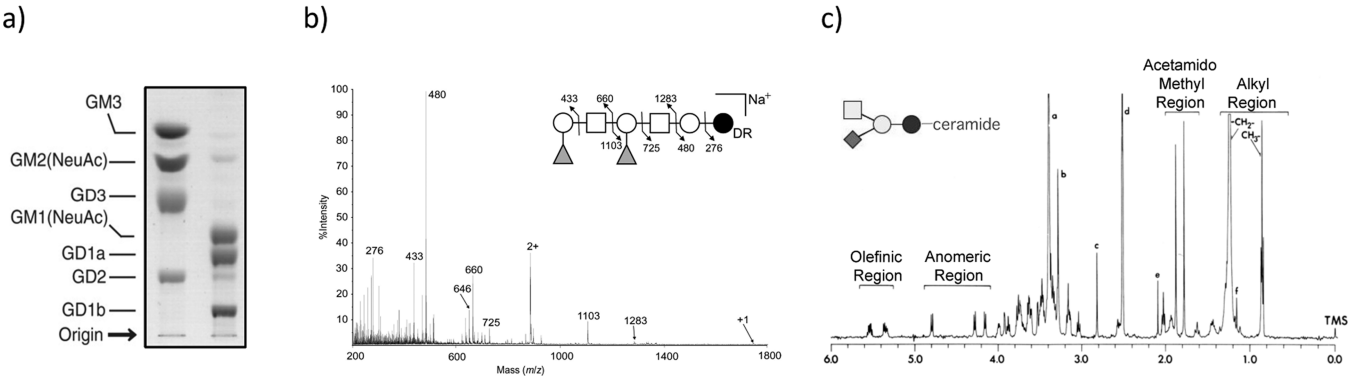

Figure 2. Determining the composition, branching, and linkage types for glycolipids typically includes several experimental techniques such as (a) TLC, (b) ESI-MS/MS, and (c) NMR spectroscopy. Image a) adapted with permission from ref 10. Copyright 2009 Oxford University Press. Image b) adapted with permission from 13. Copyright 2007 Oxford University Press. Image c) adapted with permission from ref 16. Copyright 1983 American Chemical Society.

characteristics and composition of the membrane in which it resides. Together, all these factors influence glycolipid presentation, and thus recognition and function.

GLYCOLIPID COMPOSITION, ANOMERIC CONFIGURATION, AND BRANCHING

The initial step in the structure determination of glycolipids involves deciphering their structural formula. In the setting of protein structure determination, the sequence would be determined and, assuming a linear polymer of L-amino acids, structure determination could ensue. Unfortunately with glycoconjugates, "sequence" or, more appropriately, composition provides limited information. With glycolipids, we can assume neither linearity nor a common linkage type; branching and anomeric configuration, along with composition, are required to fully define the structural formula. Historically, this involved releasing monosaccharide and lipid chains chemically or enzymatically, during which fractions were analyzed via gas chromatography (GC), GC−mass spectrometry (MS), thin-layer chromatography (TLC), high-pressure liquid chromatography, and nuclear magnetic resonance (NMR) spectroscopy.[6−10] More recently, tandem MS techniques have evolved to assist with these tasks.[7,11−13]

Because no equivalents to recombinant protein production or polymerase chain reaction amplification of nucleic acids for glycolipids exist, sample quantities are often limited. For this reason, the ability of MS to perform an analysis on subpicogram quantities of sample is very attractive.[12] MS glycoconjugate analysis relies heavily on soft ionization techniques, such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization (to ionize intact molecules), and tandem MS for the identification of isomeric compounds. Tandem MS fragmentation of carbohydrates occurs at the glycosidic linkage or via cross-ring cleavage, which is useful for determining monosaccharide composition or anomeric and branching information, respectively.[14] Although it requires more material than MS and a greater sample purity, NMR is particularly powerful in this arena as it can provide information about the composition, linkages, and anomeric configurations of glycans and glycolipids.[9,15,16] In summary, even the seemingly simple task of determining the structural formula of a glycolipid can require considerable time and multiple experiments (Figure 2).

INTERNAL 3D STRUCTURE AND DYNAMICS

Given the complete structural formula of a glycolipid, the next step is defining the internal structure of the molecule. For glycolipids, the internal structure includes the glycosidic torsion angles, hydroxyl group rotamers, intermolecular hydrogen bonds and salt bridges, ring puckering, etc., and determining the internal structure is analogous to defining the secondary and tertiary structure of proteins. At this stage, several challenges exist. (1) The carbohydrate domain may exist in multiple easily accessible conformational states. (2) The lipid domain also possesses a high degree of conformational flexibility. (3) The context in which one would like to gather structural information may include a biological membrane. One strategy is to reduce the complexity of the system by removing the lipid tail; the membrane is no longer necessary, and focus is placed on determining the internal structure of the glycan. The primary challenge of 3D structural determination of oligosaccharides then resides with the glycosidic linkage. Oligosaccharides are not structurally disordered but exist in multiple well-defined conformational states. Similar to the dihedral angles (ϕ and ψ) of a peptide bond describing conformations of a polypeptide, dihedral angles ϕ, ψ, and ω (in the case of 1−6 linkages, for instance) of the glycosidic linkage describe the internal structure of glycans.[17] Relative to the peptide bond, under physiological conditions the barrier to rotation about the glycosidic linkage is low, resulting in the characteristic high degree of conformational freedom observed in oligosaccharides. While not discussed in detail here, other structural factors of interest may include hydroxyl group rotamers, the orientation of substituents (sulfate, phosphate, *N*-acetyl, etc.), and ring puckering.[17−19]

Experimental methods that can capture conformational states of carbohydrates include X-ray crystallography and NMR spectroscopy. X-ray crystallography can capture a single snapshot of the conformational repertoire of a glycan. When oligosaccharides are bound to proteins and placed under cryo-cooled conditions, their degree of conformational freedom is reduced and high-resolution structure determination is possible.[20] This method is ideal for protein-bound systems, examining small carbohydrate fragments. A large database of 3D glycolipid structures would be an excellent resource for structural studies; however, there are only a handful of structures of glycolipids and glycolipid fragments bound to proteins in the Protein Data Bank (PDB). As a note of caution,

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one study identified that >30% of carbohydrate-containing structures in the PDB contained errors in the structural description of the glycan.[21] Error checking software pdb-care[22] is now available to alleviate these issues; however, the Research Collaboratory for Structural Bioinformatics currently does not require corrections to "ligand" errors in deposited structures, so it is ultimately up to the user to critically evaluate the structure and nomenclature of the glycan under consideration.

While crystallography can provide a representative high-resolution structure of a glycolipid or glycolipid fragment, there are many instances in which one would like to define the 3D conformational ensemble of a glycolipid. This can be accomplished by NMR spectroscopy, which can capture both structural and dynamic information of solution-phase biomolecules. For 3D structure determination, inter-residue nuclear Overhauser effects (NOEs) and ^3J coupling constants across the glycosidic linkage are particularly useful. NOEs occur "through space" when the nuclear resonance intensity of a nucleus is enhanced via dipolar interactions with another (radiofrequency-saturated) nucleus. Because the effect occurs through space, not through bonds, the change in intensity is related to the distance between the two nuclei in question. For glycans, via examination of two nuclei across the glycosidic linkage, the ensemble-averaged distance between those two atoms can be derived, providing information about allowed conformational populations. J couplings on the other hand involve spin−spin couplings between two nuclei connected via chemical bonds. For two nuclei connected via three bonds, the dihedral angle between these nuclei directly affects the magnitude of the coupling constant. Subsequently, the dihedral angle between two atoms can be estimated using the Karplus relationship.[23] For glycans, Karplus equations are not readily transferable among different linkage types as the composition of the linkage and neighboring substituents significantly affects the Karplus relationship.[24,25] For glycans, both ^3J couplings (vicinal couplings) and inter-residue NOEs can help define the ensemble-average glycosidic torsion angles.

At this stage, an "average" 3D structure of the glycan could be constructed from the NMR-determined ensemble-average glycosidic torsions and distance constraints; however, this could yield a structurally and/or physiologically improbable conformer. Take, for instance, the conformational space populated by the αNeu5Ac-(2−3)-βGal linkage of ganglioside GM3 [αNeu5Ac-(2−3)-βGal-(1−4)-βGlc-ceramide] in a phospholipid bilayer (Figure 3). In this case, the average glycosidic bond conformation is not a conformation populated by the glycan; moreover, it is not representative of any of the discrete conformational populations. Such an "average structure" may be considered a low-energy state if one uses ensemble-average NMR data as constraints in its derivation, but that does not necessarily translate to a biologically relevant conformer.

Dynamic molecular systems that populate multiple conformational states are not realistically represented by their average structure. The structural data must be deconvoluted into discrete conformational states to be of more value. For simple systems, this can be accomplished via a priori knowledge of the possible conformers. For example, given the βGal-(1−4)-βGlc linkage from GM3, *anti* and *−gauche* conformers are possible, in contrast to the *+gauche* conformer that is unlikely because of violations of the exoanomeric effect.[26,27] The NMR ensemble-average data can be fit to the known possible conformers (*anti* and *−gauche*), and novel data in this case would include the percent of time the disaccharide spends in each of the conformational states. For more complex oligosaccharides, or in cases where possible conformational states are unknown, we can turn to molecular dynamics (MD) simulations for help in deconvoluting ensemble-average data.

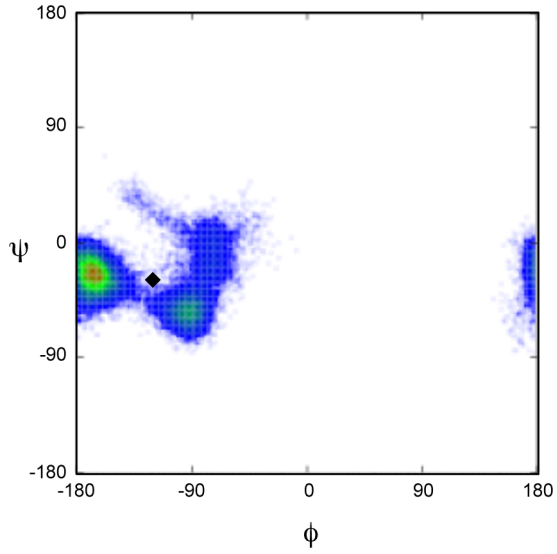

Figure 3. Glycans often populate multiple conformational states because of the low barrier to rotation about the glycosidic linkage (ϕ and ψ), as demonstrated by the αNeu5Ac-(2−3)-βGal linkage of GM3. The Ramachandran-style plot depicts experimentally verified data from a MD simulation of GM3 in a phospholipid bilayer and is colored by population density (blue for low and red for high).[28] The average glycosidic torsion angle can be determined from these data (ϕ = −120.9°, and ψ = −28.23°) (◆). In the case of molecules such as GM3 that populate multiple conformational states, averaging structural data can result in data that poorly represent the actual conformational properties of the molecule.

MD utilizes the time-dependent numerical integration of the classical equations of motion to simulate the behavior of biomolecular systems at atomic resolution. At the heart of MD is the molecular mechanics force field, which utilizes the basic properties of the atoms being simulated (atomic masses, partial charges, average bond distances and angles, etc.) to calculate the potential energy of the system and, ultimately, move the system in time. For structural biologists, MD simulations, used in conjunction with experiment, are an indispensible tool for exploring the structure and dynamics of conformationally heterogeneous biomolecules. For glycolipids in particular, MD simulations can resolve the various populated conformational states (see Figure 3) and, as has been shown with certain carbohydrate and lipid force fields, populate these states in biologically relevant ratios.[28,29] There are a few nuances to setting up a glycolipid MD simulation, including 1−4 scaling terms and force field selection, that need to be addressed before beginning an investigation of glycolipid structure using MD (details for the proper design and implementation of glycolipid simulations can be found in ref 30). For more information about the development and validation of popular carbohydrate force fields, the reader is directed to ref 31.

Whether by experiment or simulation, glycolipid structure determination can be simplified by using glycan fragments as models for the more complex molecule. What only a handful of studies have addressed, however, is whether the internal structure and dynamics of the glycolipid fragment in solution are an accurate representation of the intact membrane-bound

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glycolipid. In one study, it was found that the membrane presented a steric barrier to the population of a minor conformational isoform of GM3, relative to the glycan fragment in solution,[28] though overall, the internal structural properties of the glycan domain from both constructs were similar, as observed via MD and NMR.[28,32,33] While the glycan in solution was an adequate model of the intact membrane-bound glycolipid in this case, the applicability of this rule to other glycan–glycolipid pairs is unknown. Nevertheless, fragmenting the glycolipid is often a necessary starting point for increasingly complex structural studies.

PRESENTATION: INSERTION DEPTH AND ORIENTATION RELATIVE TO THE MEMBRANE SURFACE

For membrane-bound glycolipids, quantifying internal structure (as either an intact membrane-bound molecule or a fragment in solution) insufficiently describes the molecular system; ultimately, presentation (insertion depth and orientation relative to the membrane surface) determines biological activity. As a ligand, the orientation of the glycan portion (the glycon) with respect to the membrane surface and its insertion depth control the accessibility of binding epitopes.[28,34] Unfortunately, quantitating the presentation of dynamic binding epitopes is technically challenging. By utilizing methods that can accommodate membrane mimetics and dynamic molecular systems, namely, NMR and MD simulations, a multidisciplinary approach was developed to quantitate glycolipid presentation at atomic resolution.[29]

Fortunately, MD can easily accommodate the inclusion of membrane-mimicking environments (micelles, bicelles, bilayers, lipoproteins, etc.) for the simulation of membrane-bound systems.[35−38] To date, a small number of all-atom explicit solvent MD simulations of glycolipid–membrane systems (with production runs longer than 5 ns) have been reported.[28,29,34,37,39−49] Of these, only a couple of studies have experimentally validated the conformational properties of the glycolipid. Protocols for the design, execution, and validation of all-atom explicit solvent MD simulations of protein- and membrane-bound glycolipids have been recently described.[30] While MD provides the atomic-resolution structural properties in the context of a fluid membrane environment, experimental validation is required. To experimentally assess presentation, NMR spectroscopy with paramagnetic probes has been employed to generate a quantitative measure of ensemble-average presentation data, which can be used to validate observations from MD simulations (Figure 4).[29]

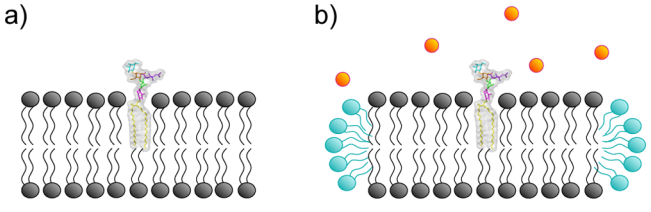

Figure 4. Combining MD simulations and NMR experiments with paramagnetic probes provides a reasonably quantitative way to assess glycolipid presentation. Cartoon representation of (a) a bilayer–GM1 MD simulation and the complementary (b) bicelle–GM1 NMR experiment. Long chain phospholipids (black), short chain phospholipids (cyan), and soluble paramagnetic probes (orange) are shown.

In these experiments, a mixture of GM1, long chain phospholipids, and short chain phospholipids (or detergents) was prepared to generate disk-shaped glycolipid-containing bicelles (Figure 4b). The rapid tumbling of the bicelles permits the collection of high-resolution NMR spectra, and their shape provides a planar surface that mimics the cell surface. Glycolipid presentation and insertion depth were assessed by monitoring the relaxation rates of GM1 protons upon the addition of an inert and water-soluble paramagnetic probe, Gd(III)-diethylenetriaminepentaacetic acid [Gd(DTPA)]. The paramagnetic relaxation rate enhancement (PRE) arises from the magnetic dipolar interactions between the unpaired electrons of the paramagnetic agent and an NMR active nucleus (such as a proton), resulting in increased relaxation rates. PREs are particularly well suited for structural studies of membrane-anchored glycolipids because the PRE effect is very large and can be detected at distances up to ∼25 Å; contrast this with NOEs where the effect is small, limiting distances to ∼6 Å. Additionally, the PRE effect is dependent on the distance (accessibility) from the paramagnetic probe to the NMR active nucleus and is thus related to the insertion depth and orientation (presentation) of the bicelle-bound glycolipid (Figure 5).

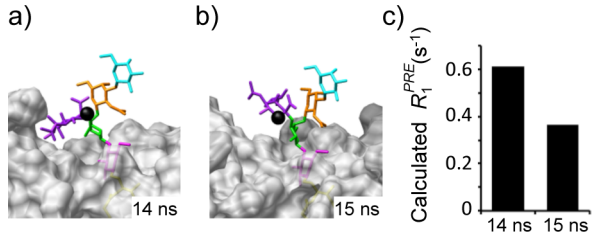

Figure 5. Paramagnetic relaxation enhancements (PREs) reflect the presentation of glycolipids because the PREs are dependent on the proximity of the paramagnetic probe to the protons of interest. (a and b) Using the example of a single proton (black sphere) from the sialic acid (purple) of GM1, as the conformation and presentation of GM1 changes relative to the fluid membrane surface it alters the accessibility of the water-soluble paramagnetic probe. The decreased solvent accessibility of the proton at 15 ns, relative to the snapshot at 14 ns, results in a diminished PRE effect. Observing (from experiment) and calculating (from simulation) the average PRE effect for multiple protons from GM1 defines the overall presentation of the molecule. (a and b) Structure of GM1 in a phospholipid bilayer (gray) from a MD simulation (c) and related PREs (R_1^{PRE}) calculated for the proton of interest.[29]

In addition to PREs observed in NMR experiments, PREs can be calculated from MD snapshots (Figure 4a), using a theoretical model of relaxation mechanisms and experimental variables such as correlation times, and salt concentrations (Figure 5).[29] Subsequently, the presentation of the glycolipid and 3D structures observed via MD simulation can be validated in a reasonably quantitative way by comparison of the calculated and experimentally observed data. Moreover, the sparse structural constraints from the NMR experiments can be translated into atomic-resolution structures using data from the MD simulations.[29]

Aspects of glycolipid presentation can be probed by other means, including glycan–glycolipid arrays,[50,51] surface plasmon resonance,[52] and flow cytometry.[53] These methods can provide a wealth of data about the specificities and affinities of glycolipid–protein interactions, but they are not designed to

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generate structural data. For instance, glycolipid arrays can be used to query the binding partners of a particular protein. From the results of the array, it may be possible to deduce a common epitope, thus providing a low-resolution understanding of the necessary presentation mode for binding.[54] MD simulations remain an attractive complementary approach to assist in interpreting low-resolution or sparse structural experimental data from dynamic membrane-bound systems.

FACTORS AFFECTING RECOGNITION

While the focus has been on characterizing the internal structure and dynamics of the glycan and its presentation in a membrane, a number of other factors directly and indirectly influence glycolipid recognition, including the chemical structure of the aglycon, the composition of the membrane, and glycolipid clustering. Along with glycan structure and presentation, these other factors should be considered when designing and/or interpreting an experiment exploring glycolipid recognition.

The Glycan. As an integral component of the outer leaflet of the plasma membrane, glycolipid molecules contribute to an interaction of the cell with its extracellular environment. Take, for instance, GM3, a known target of macrophages. Using cell-surface sialoadhesion, macrophages can adhere to cells expressing GM3. To develop an atomic-resolution understanding of the conformational properties of membrane-bound GM3 and the ability of GM3 to mediate protein binding, an MD simulation of GM3 in a lipid bilayer was performed.[28] Membrane-bound GM3 populated multiple, well-defined conformational states under physiological conditions [see, for example, the states populated by the terminal glycosidic linkage of GM3 (Figure 3)]. The MD simulations were validated by comparison with NMR data, and crystallographic data for relevant carbohydrate/ganglioside−protein complexes in the PDB. From the crystal structure of sialodhesin bound to the carbohydrate fragment of GM3, the binding conformation of the lectin to intact GM3 had been proposed,[55] but what conformation did this represent relative to the ensemble of structures populated by membrane-bound GM3? Via comparison of the conformation of the crystal structure with the conformational ensemble from the MD simulation, sialoadhesin was found to bind to a major conformational state populated by GM3. The lectin did not bind to an infrequently populated conformation, nor did it distort the solution-phase structure of the glycolipid to bind. Additionally, sialoadhesin interacted with one of the most accessible epitopes presented by membrane-bound GM3 (Figure 6). A similar study of ganglioside GM1 demonstrated that known protein receptors, including viral capsid protein VP1 and the bacterial cholera toxin, bound solution-dominant and membrane-accessible conformations of GM1.[29] In this study, the accessibility of each carbohydrate residue of GM1 was assessed via PREs calculated from MD simulations and measured from NMR experiments (Figures 4 and 5). It has been previously noted that proteins binding to gangliosides in trans tend to interact with only the terminal glycan portion of the glycolipid. The recent studies of membrane-bound GM3 and GM1 have provided a structural framework for these observations;[28,29] that is, certain glycolipid-binding proteins have evolved to recognize the most accessible and frequently presented glycolipid epitopes.

The Aglycon. Results from both experiment and theory demonstrate the importance of the lipid portion of glycolipids in directly and indirectly affecting the presentation of the

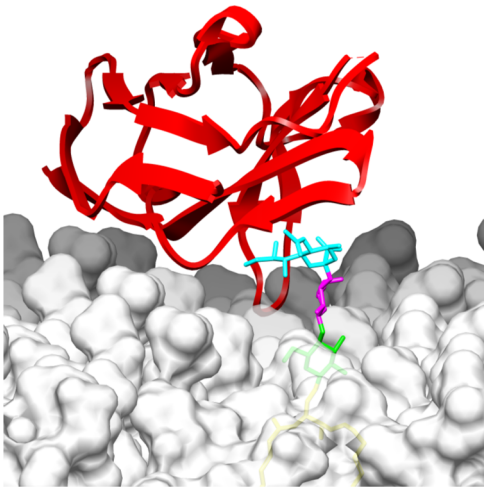

Figure 6. Protein receptors appear to bind to frequently populated and accessible conformations of membrane-bound glycolipids. For example, the N-terminal domain of sialoadhesin (red) binds through interactions with the terminal sialic acid (cyan) of ganglioside GM3. Sialoadhesin is docked to a snapshot from an MD simulation of GM3 in a phospholipid bilayer (gray), using a crystal structure of the glycan fragment of GM3 with sialoadhesin as a guide (PDB entry 1QFO).

glycolipid at membrane surfaces. From MD simulations, we have seen the importance of the conformation of the glycon−aglycon linkage in mediating the presentation of the glycolipid binding epitope.[28] As will be discussed, the lipid tail has significant control over the insertion depth of the glycolipid and the interaction of the glycolipid with the membrane. Subsequently, changes in the aglycon can affect the biological and pathogenic functions of glycolipids.

An example of the direct involvement of the aglycon in recognition involves the interaction between GM1 and Aβ peptides. Aβ (amyloid β) is the main component of amyloid plaques associated with Alzheimer's disease; as such, its interactions with other molecules, such as gangliosides, may lead to an improved understanding of its pathobiology. Experimental studies had demonstrated a potential interaction between Aβ and GM1[56] and, subsequently, that Aβ bound with high affinity and specificity to gangliosides, including GM1.[57] To interrogate the proposed interaction surface between the glycolipid and the Aβ peptide, PREs of protons from lyso-GM1 (GM1 with a single hydrocarbon chain) micelles were determined using spin-labeled Aβ peptides.[58] The NMR experiments identified the area about the glycon−aglycon junction as the primary interaction surface with the spin-labeled Aβ, demonstrating that both the carbohydrate and lipid portions can serve as recognition sites.

Indirectly, the aglycon mediates recognition of the glycolipid's carbohydrate epitopes. The hydrocarbon chain length of the glycolipid relative to the membrane phospholipids is implicated in the recognition of the carbohydrate epitope in glycolipids by protein receptors such as antibodies, lectins, and bacterial toxins. Increased glycolipid−phospholipid hydrocarbon chain length correlates with enhanced protein binding,[59−63] presumably by increasing the level of exposure of the glycan epitope above the membrane plane.

Along with modulating epitope exposure, the structure of the aglycon serves other important functions. Using a cell line devoid of glucose-based glycolipids, Ewers et al. revealed the dependence of endocytosis and infection of viral particles into

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host cells on the structure of the aglycon.[64] GM1 molecules with varying hydrocarbon chain lengths and levels of saturation were incorporated into mammalian cells and subsequently exposed to particles of simian virus 40 and its viral capsid protein, VP1. Acyl chain length was critical in inducing membrane invagination and tubules, where short acyl chains (relative to common GM1 chain lengths) failed to induce the membrane curvatures responsible for endocytosis and infection. In both bacterial toxin and viral protein recognition of host cells via glycolipids, the hydrocarbon length affects the mechanical ability of glycolipid receptors to induce membrane curvature.[64,65]

Membrane Composition. Just as the aglycon regulates glycolipid recognition and function, the local membrane environment has a considerable impact. In humans, glycolipids comprise between 5 and 20% of plasma cell membranes, depending on cell type, and are not uniformly distributed in the membrane.[17] In vivo, cell-surface glycolipids are localized in microdomains rich in sphingolipids, cholesterol, and select proteins (i.e., GPI-anchored proteins and signaling proteins), known as "lipid rafts". In vitro, the individual components of lipid rafts can be explored to determine the effect on glycolipid recognition. For instance, using liposome-embedded gangliosides, the role of cholesterol in trans recognition of glycolipids was assessed.[34] The level of binding of cholera toxin and verotoxin to GM1 and globotriaosylceramide (Gb3) was decreased in the presence of cholesterol. The proposed mechanism for the differential binding characteristics was a cholesterol-induced tilt of the glycon, which obscured the binding epitope. The ability of cholesterol to modify the orientation of glycolipids relative to the membrane surface was supported by independent MD simulations of Gal-ceramide in bilayers containing mixtures of phospholipid, sphingomyelin, and cholesterol.[39] Together, these studies highlight the dependence of the structure and function of glycolipids on their local membrane environment.

Glycolipid Clustering. Glycan-binding proteins often rely on multivalent interactions to boost avidity in lieu of the generally modest affinity carbohydrate−protein binding. A classic example is the pentavalent interaction of the B subunit of the cholera toxin with GM1 molecules. To determine the effect of glycolipid density and clustering on glycolipid recognition, Shi et al. varied the concentration of GM1 on microfluidic channels coated with phospholipid.[66] Interestingly, the binding of cholera toxin was negatively affected by increasing GM1 concentrations and was attributed to glycolipid clustering shielding GM1 epitopes. As studies have demonstrated the importance of lateral diffusion of glycolipids in clustering and optimal protein binding and the dependence of these properties on the membrane-support system,[53,66] similar ligand density experiments conducted with additional platforms would be of interest.

Aside from pathogenic roles, glycolipid clustering at membrane surfaces assists with normal biological functions, including lateral (cis) membrane interactions that control signaling cascades and trans interaction with endogenous protein.[67,68] The relationship between glycolipid clustering and function is exemplified by the adhesion of leukocytes to glycolipids on the surface on blood vessels.[69] By modeling molecular binding under conditions simulating blood flow, Schumacher et al.[70] showed the binding and rolling of cells correlated with the size, distance, and density of glycolipid clusters. This work reinforces the notion that glycolipid recognition events are optimized by the presentation and concentration of glycolipid binding epitopes.

CONCLUSIONS

Glycolipid binding epitopes are moving targets, constantly subject to the high internal plasticity of their glycosidic linkages, the rotatable hydroxyl groups, and the composition and dynamics of the fluid membrane environment. Because of these complexities, high-resolution structural and dynamic elucidation of membrane-bound glycolipids necessitates a multidisciplinary approach. Discrete conformational states can be discerned from motion-averaged NMR data with independent (unbiased) MD simulations. Model membranes such as bicelles and bilayers can be incorporated into experiment and simulation to approximate the biological environment and quantitate presentation. While these experiments and simulations represent a stripped down version of the true biological environment, they offer considerable insight into the cell-surface structure−function relationships of glycolipids.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

ESI, electrospray ionization; GM1, βGal-(1−3)-βGalNAc-(1−4)-[αNeu5Ac-(2−3)]-βGal-(1−4)-βGlc-(1−1)-ceramide; GM3, αNeu5Ac-(2−3)-βGal-(1−4)-βGlc-ceramide; MD, molecular dynamics; MS, mass spectrometry; NOE, nuclear Overhauser effect; PRE, paramagnetic relaxation enhancement; TLC, thin-layer chromatography.

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