Encoding biological recognition in a bicomponent cell-membrane mimic

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Self-assembling dendrimers have facilitated the discovery of periodic and quasiperiodic arrays of supramolecular architectures and the diverse functions derived from them. Examples are liquid quasicrystals and their approximants plus helical columns and spheres, including some that disregard chirality. The same periodic and quasiperiodic arrays were subsequently found in block copolymers, surfactants, lipids, glycolipids, and other complex molecules. Here we report the discovery of lamellar and hexagonal periodic arrays on the surface of vesicles generated from sequence-defined bicomponent monodisperse oligomers containing lipid and glycolipid mimics. These vesicles, known as glycodendrimersomes, act as cell-membrane mimics with hierarchical morphologies resembling bicomponent rafts. These nanosegregated morphologies diminish sugar–sugar interactions enabling stronger binding to sugar-binding proteins than densely packed arrangements of sugars. Importantly, this provides a mechanism to encode the reactivity of sugars via their interaction with sugar-binding proteins. The observed sugar-phase-separated hierarchical arrays with lamellar and hexagonal morphologies that encode biological recognition are among the most complex architectures yet discovered in soft matter. The enhanced reactivity of the sugar displays likely has applications in material science and nanomedicine, with potential to evolve into related technologies.

Janus glycodendrimers | lipid rafts | nanosegregation | atomic force microscopy | galectin

Soft matter self-organizes into a diversity of periodic and quasiperiodic arrays, including Frank–Kasper phases such as cubic Pm3n, known also as the A15 phase (1–4), tetragonal P43/mnm known the 2d (5), decagonal liquid quasicrystals (6–8), plus helical columns (9) and spheres (10), including some that disregard chirality. The aforementioned morphologies are responsible for a variety of functions in soft matter and biology (3, 11–15). These complex architectures, which were discovered and elucidated via structural and nanostructural analysis of libraries of self-assembling dendrons and dendrimers, employed diffraction methods analogous to those used to develop the field of structural biology (16). Later these same phases were found by the same methods (17) in block copolymers (18–22), surfactants (23–26), lipids (27–33), glycolipids (34), and in other systems (35–38). Recently, amphiphilic Janus dendrimers (JGDs) (39, 40), and their sugar-presenting analogs, Janus glycodendrimers (JGDs) (41), which provide synthetic alternatives to natural lipids and glycolipids that are readily functionalized, have been reported to self-assemble in either water or buffer (42) into vesicles, named dendrimersomes (DSs) and glycodendrimersomes (GDSs) (43), respectively. They can also be made into cell-like hybrids with either bacterial (44) or human cells (45). As with cell membranes, the bilayers of these supramolecular constructs act as a barrier between the inside and outside of DSs, GDSs, and cell-like hybrids (39, 40, 43, 44). Selective permeation can be provided, with comparable efficiency as with liposomes (13) and polymersomes (46), when either transmembrane proteins or their mimics are incorporated into the bilayer (44). Thus, DSs and GDSs can be used as biological-membrane mimics to elucidate concepts in cell biology and glycochemistry, such as compartmentalization, encapsulation and release, selective transport (39, 40, 43, 44), as well as fusion and fission (47–49). Intriguingly, while searching for the minimum concentration of sugar from JGDs that is able to aggregate proteins, we discovered that sequence-defined monodisperse JGDs containing β-lactose (Lac) or β-mannose exhibit higher activity toward sugar-binding proteins, known as lectins, on decreasing surface density of sugar (50–52). This inverse relationship between sugar density and aggregation of lectins is opposite to what is expected.
from classic kinetics (53, 54). Although the molecular structure of the JGDs exhibiting increased reactivity with decreasing sugar density is known (50–52), the three-dimensional structure providing this function is not understood. Indeed, elucidating the arrangement of glycoconjugates responsible for communication and recognition between cells, and cells with proteins (49, 55), remains a major challenge. Progress toward this goal, which will also enable the design of glycoconjugate-based biomaterials, is reported here.

Seminal models of the structure of the cell membrane, including the lipid bilayer sea of the fluid mosaic model (56), and the microsegregated rafts model (57, 58), recognize that there are certain structures responsible for sugar (glycan) activity but have not fully elucidated the details of these structures. This is mostly since X-ray and other diffraction methods employed in the structural and retrostructural analysis of biological system and of dendrimer assemblies cannot be easily applied to the analysis of fluid natural and synthetic cell membranes (59–61).

Here, we employ an analysis of atomic force microscopy images and their associated fast Fourier transforms (FFTs), along with complementary molecular modeling, to address the question of what architectures program enhanced glycan function of JGDs vesicles. This diffraction-like methodology reveals that a complex (62, 63) hierarchical self-organization of sugar moieties arranged into lamellar and hexagonal nanophase-separated periodic arrays are responsible for the enhanced activity compared with GDS where sugars were densely packed and do not exhibit nanomorphologies. Such dense packing presumably results in stronger sugar–sugar interactions ultimately reducing their availability for binding to galectins. Furthermore, these bicomponent patterned hierarchical nanoarchitectures may provide a general mechanism to program and encode biological recognition and function of glycan, not only on bicomponent GDSs, but also on the surface of different vesicles containing glycoconjugates such as liposomes, polymersomes, synthetic cells, hybrid cells, and other globular assemblies.

Results and Discussion

Accelerated Modular Synthesis of JGDs and Sequence-Defined Oligomeric JGDs. GDSs were self-assembled from sequence-defined JGDs (Figs. 1 and 24). All JGDs in this work contain Lac, a sugar commonly found in human cells, and triethylene glycol (3EO) fragments (Figs. 1 and 2B) as their hydrophobic components. Lac substituents are diluted by increasing the number of 3EO substituents to create a library with systematic structural variation. The synthesis of JGD-4Lac is shown in SI Appendix, Figs. S1–S8, while that of JGD(5/1Lac) and JGD(5/1Lac) in SI Appendix, Figs. S9–S17.

Other JGDs (Fig. 1) were prepared as previously reported by accelerated iterative modular synthesis (39, 51), similar to JGD-4Lac. These JGDs share the same hydrophobic part containing 3,5-bis(dodecyloxy)benzoic esters. A library including high-sugar-density single–single JGD-1Lac, twin–twin JGD-2Lac, and tetra–tetr JGD-4Lac, and sequence-defined JGDs with relatively low-sugar density were involved in this study. The sugar densities decreased from 100% Lac (JGD-1Lac, JGD-2Lac, and JGD-4Lac) to 3/1 (3EO/Lac) for JGD(3/1Lac), 5/1 (3EO/Lac) for JGD(5/1Lac), and JGD(5/1Lac), 6/1 (3EO/Lac) for JGD(6/1Lac), and 8/1 (3EO/Lac) for JGD(8/1Lac, JGD(8/1Lac), JGD(8/1Lac), and JGD(8/1Lac).

The position of Lac on the hydrophilic 3EO dendrons was tuned in the 8/1 (3EO/Lac) sequence-defined JGDs. Lac was appended in the 2 position (counted from left to right) of the gallic amide for JGD(8/1Lac), JGD(8/1Lac), and in the 3 position for JGD(8/1Lac) and JGD(8/1Lac). The linker lengths of the sequence-defined JGDs were changed from a single 3EO unit (short linker, denoted as S) in JGD(8/1Lac) and JGD(8/1Lac) to two 3EO units (long linker, denoted as L) in JGD(8/1Lac) and JGD(8/1Lac).

Hierarchical Periodic Glycan Arrays Self-Organized by Self-Assembly of High-Density and Sequence-Defined JGDs. Lectins are proteins which recognize glycoconjugates on the cell membranes and thus mediate communication between cells with cell and with proteins. GDSs self-assembled from libraries of JGDs unveiled how the structures of lectins affect their bioactivity toward recognition by investigating the cross-linking of cell-like GDSs and the functional pairing between the cognate sugars and lectins (64–68). Lac-presenting JGDs were selected for the present study since they self-assemble into unilamellar vesicles comparatively simpler than those of onion-like multimamellar vesicles self-assembled from Man-presenting JGDs and since Lac is larger than Man and which is expected to facilitate analysis by atomic force microscopy (AFM) experiments.

Aggregation of GDSs with wild-type human galectin-1 (Gal-1) (Fig. 2C) reproduces the unexpected but general trend of an increase in activity of GDSs with decreasing density of sugars reported previously in both unilamellar (51) and onion-like (52) GDSs. There is a modest, yet statistically significant, increase in the activity from JGD-1Lac to JGD-2Lac and to JGD-4Lac (Fig. 2C and SI Appendix, Fig. S18), as expected by multivalency (53, 54). There is a marked increase in aggregation activity when the sugar density is decreased, in sequence-defined JGD(5/1Lac), JGD(6/1Lac), and JGD(6/1Lac) (Fig. 2E and SI Appendix, Fig. S19). Similarly, other complex galectins including heterodimERIC human galectin-8S (Gal-8S) (50, 51) and engineered galectin-1 tetramer (Gal-1)GG (67) presented trends in line with Gal-1 (Fig. 2C).

Structural Analysis of Lamellar Periodic Glycan Arrays of Giant GDSs Self-Organized from High-Density and Sequence-Defined JGDs. A structural analysis methodology using AFM, FFT, and molecular modeling was used to investigate the structures of GDSs (Fig. 3). Giant GDSs prepared by thin-film hydration were utilized, as the dimensions of giant GDSs (micrometer scale) rather than those of small GDSs (100–200 nm) obtained by injection (39) are required to access analysis of hierarchical nanodomains by AFM. FFT provides a diffraction-like pattern that is used for structural and retrostructural analysis. Giant GDSs prepared from JGD-1Lac and JGD-2Lac display a very smooth and compact surface (Fig. 4A) while those prepared from JGD-4Lac (Fig. 4B), JGD(3/1Lac), JGD(5/1Lac), JGD(6/1Lac), JGD(6/1Lac), JGD(8/1Lac), and JGD(8/1Lac) (Fig. 4C) unexpectedly exhibit hierarchical lamellar morphologies. Additional sequence-defined JGDs in which Lac is attached via a two-3EO linker JGD(8/1Lac), and JGD(8/1Lac) (Fig. 2C) generate GDSs which exhibit hierarchical periodic arrays of glycans with hexagonal patterns (Fig. 4D). Although the lamellar and hexagonal periodicities of these glycan arrays resemble those of supramolecular dendrimers (11, 16) and block copolymers (18–22), the three-dimensional structures of these GDSs represent an arrangement of building blocks on the surface of a vesicle. Detailed AFM images including phase and height profiles, and additional modeling of GDSs of self-assembled JGDs, are available in SI Appendix, Figs. S20–S25.

What is the mechanism via which hierarchical periodic glycan arrays with programmed activity are generated and how do they influence reactivity to galectin? Homogeneous and densely packed surfaces were observed only for single–single JGD-1Lac and twin–twin JGD-2Lac (Figs. 1 and 24) that can self-organize into GDSs with high-density bilayers (Fig. 4A). In contrast, tetra–tetra JGD-4Lac and all sequence-defined JGDs present more complex, hierarchically self-organized nanodomain patterns (Figs. 4B–D) with lamellar and hexagonal morphologies. The nanosegregation of sequence-defined JGDs may be anticipated since they are generated from dissimilar hydrophilic chemical fragments. However, the formation of nanosegregated domains by JGD-4Lac is unexpected and it is presumably driven by a different mechanism than the one acting in the case of sugar dilution. Molecular modeling (Fig. 5A and B) suggests that Lac moieties seek to maximize their interactions with each other. This can be achieved without nanosegregation in densely packed JGD-1Lac and JGD-2Lac (Fig. 4B). In contrast, the sugar moieties of JGD-4Lac must cluster
together to maximize interactions, resulting in alternating high- and low-density regions of Lac that exhibit a lamellar periodic array (Figs. 4B and 5A and B). The change from JGD-1Lac and JGD-2Lac to JGD-4Lac resembles the classic polymer effect that provides a transition from rigid to flexible backbone conformation (3).

Sequence-defined JGD(5/1Lac2), JGD(5/1Lac3), JGD(6/1Lac), JGD(8/1Lac2S), and JGD(8/1Lac3S) with programmed enhanced activity generate similar periodic lamellar structures to JGD-4Lac, except that both the sugar density and the steric interactions are lower than in JGD-4Lac (Fig. 4B and C and SI Appendix, Fig. S23). Consequently, the clusters of sugars on the surface of the GDSs self-assembled from sequence-defined JGDs are more accessible to lectin and their aggregation is therefore more efficient than for JGD-4Lac even though having the same lamellar morphology (Fig. 2C).

**Structural Analysis of Hexagonal Periodic Glycan Arrays of Giant GDSs Self-Organized from Sequence-Defined JGDs.** In contrast, JGD(8/1Lac2S) and JGD(8/1Lac3S) exhibit periodic hexagonal arrays (Fig. 4D), as demonstrated by indexing the FFT of their AFM phase images (shown for JGD(8/1Lac3S) in Fig. 5C). This hexagonal periodic array provides an alternative morphology to enhance sugar activity. AFM height analysis (Fig. 5D, Right) shows that the isolated domains are thinner (darker in Fig. 5D) compared with the continuous domain (lighter in Fig. 5D). This suggests that the Lac moieties form the continuous domain.

A model of hexagonal nanosegregation consistent with analysis of the FFT of AFM images (Fig. 5 E and F) comprises 19 molecules of JGD(8/1Lac3L): Lac moieties [red, space-filling Corey–Pauling–Koltun (CPK) view] interact at the edge of the domain, while the hydrophilic (blue) and hydrophobic (green) components of JGD(8/1Lac3L) occupy the remainder of the domain in three concentric layers: an outer layer (orange circles, I); an inner layer (purple circles, II); and a molecule at the center of the column (yellow circle, III). The hydrophilic and hydrophobic components of one molecule from each concentric layer are depicted in CPK view, while those of the other molecules are...
shown as faint line models for clarity. The linker of JGD(8/1Lac₃S) molecules contains two 3EO units (Figs. 1 and 2). In molecules forming the outer layer (I), this linker is disordered beneath Lac. For molecules in the inner layer (II), the linker is more extended to allow the Lac moiety to interact with sugars from molecules in the outer layer (I). Finally, the linker of the central molecule of JGD(8/1Lac₃S) (III) is completely extended to allow interaction between sugars. A molecule at the center of a larger domain would be unable to position its Lac moiety to allow for interaction with Lac moieties from the outer (I) and inner (II) layers, and hence the linker may restrict the size of the periodic hexagonal array. Conversely, the length of the two-3EO linker may be too long to allow sufficient confinement in periodic lamellar domains, consistent with the observation of lamellar phases only for sequence-defined JGDs with shorter single-3EO linkers, JGD(3/1Lac), JGD(6/1Lac), JGD(8/1Lac₂S), and JGD(8/1Lac₃S) (Fig. 4D and SI Appendix, Fig. S2A4). On the other hand, two sequence-defined JGDs JGD(5/1Lac₂S) and JGD(5/1Lac₃S) with two-3EO display only lamellar domains due to higher sugar density compared with JGD(8/1Lac₃S). These data, along with the generation of a hexagonal periodic array by a second molecule with a two-3EO linker, JGD(8/1Lac₂L) (SI Appendix, Fig. S2AB), suggest that both the linker length and sugar density determine the observed morphology. A columnar section of the bilayer comprising the repeating unit of the periodic hexagonal array (Fig. 5F) illustrates how hierarchical assembly of JGD(8/1Lac₃S) (Fig. 2A) containing a nonaggregating single Lac becomes a very efficient ligand for aggregation with lectins. Both the hexagonal and the nondenfine lamellar arrays from Figs. 4 and 5 indicate potential mechanisms, which by steric constrain–release combined with self-organization (Fig. 4B) and/or self-organization (Figs. 4 C and D and SF), generate more reactive glycan ligands than those obtained from highly packed assemblies (Fig. 4A).

The clustering of sugars by self-organization into lamellar and hexagonal periodic arrays reported here suggests a mechanistic explanation for the enhanced reactivity observed with sequence-defined JGDs that was not predicted by the rafts model (57, 58).

Enclosing Biological Recognition by Hierarchical Nanomorphology. The goal of this paper was to report the discovery of nanoporous arrays on vesicles generated from sequence-defined bicomponent monodisperse oligomers containing lipid and glycolipid mimics and illustrate how they encode biological recognition and reactivity. Remarkably these nanosegregated morphologies with diminished sugar–sugar aggregation allowed the sugar to more readily bind to galectins than the densely packed arrangements of sugars, regardless of sugar dilution. The activity of these nanosegregated glycans depends on both morphology and sugar density (Fig. 2C). For lamellar morphology the binding to galectins increased with the decreasing density of sugar until a 1:6 dilution after which no further significant increase was observed (Fig. 2C and SI Appendix, Table S1). No statistically significant differences were observed for the binding between JGD(8/1Lac₂S) and JGD(8/1Lac₃S) displaying lamellar or between JGD(8/1Lac₂S) and JGD(8/1Lac₃S) displaying hexagonal morphology. However, a comparison between these two morphologies at the same dilution, with statistically significant results, evidenced higher activity for the lamellar, JGD(8/1Lac₂S) and JGD(8/1Lac₃S), rather than for the hexagonal, JGD(8/1Lac₂S) and JGD(8/1Lac₃S), organization (Fig. 2C and SI Appendix, Table S1) with Gal-1 and Gal-8S.

Conclusions The structures, morphologies, and mechanisms via which the bicomponent sequence-defined GDSs with decreasing sugar concentration provide enhanced glycan activity have been elucidated with a diffraction-like analysis methodology. The cell-membrane...
mimic, GDSs assembled from JGD-1Lac and JGD-2Lac (Fig. 2A), display a very compact arrangement of Lac units on their surface (Fig. 4A). GDSs programmed to exhibit higher activity by self-assembly of sequence-defined JGDs (Figs. 2A and 4B–D) self-organize hierarchical periodic arrays of glycans that reduce the dense packing of Lac. Favorable interactions with sugar-binding proteins are generated by these morphologies, resulting in an increased rate constant for aggregation, which overcomes the decrease in the concentration of the sugar on the GDS surface. This finding seemingly contradicts classic kinetic principles (53, 54), but is consistent with previous studies of self-assembled monolayers of sugars on gold surfaces (69), which demonstrated that lower sugar density provides higher affinity when applied as biosensors for lectins and viruses. However, in those studies (69), the arrangement of sugars within the monolayer was not elucidated. The hierarchical segregation of JGDs that have identical hydrophobic segments differ only in their hydrophilic parts for the self-assembly of vesicles. Neither the fluid mosaic model of the structure of cell membranes (56) nor the rafts membrane-organizing principles (57, 58) predict these glycan nanodomain morphologies on the structure of biological cell membranes or even on the structure of biological-membrane mimics. The observed hierarchical nanodomains represent an example of bicomponent rafts-like morphology that extends from a small rafts domain to the entire biological-membrane mimic and therefore could be elucidated at the molecular level. The hierarchical morphologies reported here are general for all unilamellar GDS generated by hydration and injection (41) regardless of the size of the carbohydrate from JGD and also for multilamellar “onion-like” (52) GDS. Quantitative correlations between hierarchical morphology and sugar reactivity requires the development of methodologies accessible below the limit of the diffraction of light. At the same time, these patterned nanoarchitectures are among the most complex structures yet discovered either on a planar or nonplanar surface (70–72). They provide a powerful example in which structure determines function (3, 10–14, 73), in this particular example how different supramolecular assemblies encode biological recognition. This supramolecular patterning offers a fundamental mechanism for programming enhanced activity via the surface morphology of globular glycoconjugate assemblies, such as liposomes, polymersomes, and cell-like hybrids (44, 45). Indeed, this approach infirms a general design and structural analysis principle for active complex soft matter (62, 63), which will enable technological applications ranging from materials science to nanomedicine (74). It remains to be seen if bottom-up approaches derived from FFT of AFM (75) images elaborated here can overcome the limits of other methods employed in the analysis of rafts (59–61, 76, 77).

Methods

Preparation of Nanoscale GDSs by Injection. A stock solution was prepared by dissolving the required amount of amphiphilic JGDs in THF. GDSs were then generated by injection of 50 μL of the stock solution into 1.0 mL PBS, followed by 5-s vortexing.

Preparation of Giant GDSs by Hydration. A solution of JGDs in THF (100 μL) was deposited on the top surface of a roughened Teflon sheet (1 cm²), placed in a flat-bottom vial, followed by evaporation of the solvent for 2 h. The Teflon sheet was dried in vacuo for an additional 12 h. Milli-Q water (1.0 mL) was added to submerge the film on the Teflon sheet, and the vial was placed in a 60 °C oven for 12 h for hydration. The sample was then mixed using a vortex mixer for 30 s with a final concentration of 1 mg mL⁻¹.

Fig. 3. Illustration of combined AFM, FFT, and modeling methodology for structural analysis.

Fig. 4. Surface topology of GDS formed by self-organization of JGDs depends on glycan sequence and density. (A) JGD-2Lac; (B) JGD-4Lac; (C) JGD(6/1Lac); (D) JGD(8/1Lac). (B–D, Inset) FFT of AFM phase images. Color code: red, sugars; blue, hydrophilic 3EO; green, hydrophobic alkyl.

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AFM. The GDSs in water (−0.3 mg·mL⁻¹) were deposited and slowly dried on the freshly peeled mica. All images were acquired with a Multimode AFM NanoScope V (Digital Instruments) as topological scans in tapping mode in air, using silicon probes OTESPA-R3 (Bruker) with a nominal spring constant of 26 N·m⁻¹ and a tip radius of 7 nm. The phase images were obtained by monitoring the phase lag of the cantilever vibration compared with the z-piezo-drive voltage while the probe scans the surface with a preset constant amplitude of vibration. The phase data contain additional information about the tip-sample interactions resulting from adhesion, surface stiffness, and viscoelastic effects. The AFM scans including FFT were analyzed using Gwyddion software.

Cryogenic Transmission Electron Microscopy. Cryogenic transmission electron microscopy (cryo-TEM) micrographs were taken on a Carl Zeiss Libra 120 microscope. Cryo-TEM samples were prepared by plunge freezing of aqueous dispersion on plasma-treated lacey grids. The vitrified specimens were prepared before the agglutination assays and were maintained at 0 °C. The fresh JGD-2Lac solution was used as a reference. PBS solution of galectin (100 μL) was injected into PBS solution of GDSs (900 μL) and PBS solutions of galectin were prepared using Autodesk 3DS Max 2017. PBS solutions of galectin were prepared before the agglutination assays and were maintained at 0 °C (ice bath) before data collection.

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