Observing Lectin Binding Specificity on Glycan Microarrays

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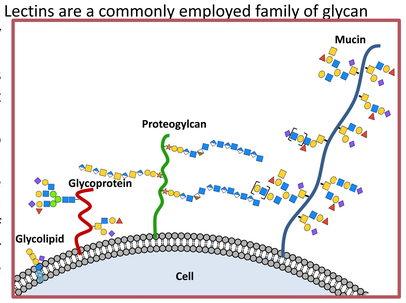
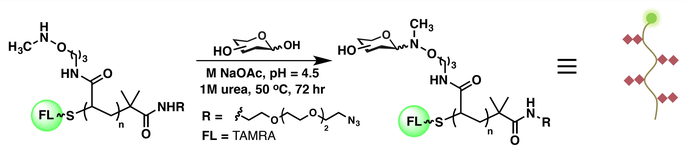
Introduction

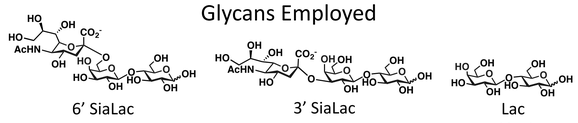
Cell surfaces are covered with glycans, or chains of sugar residues, that are connected to proteins and lipids to comprise what is known as the glycocalyx. There are many sugar residues that can be connected through numerous linkage types which makes the glycocalyx extremely hetergenous. The abundance of the different types of glycans allows them to function in many ways to control the development and function of organisms. For example, they can regulate interactions between cells, mediate host-pathogen interactions, and modulate cell signaling[1]. The biological function of glycans is facilitated through glycan binding proteins[2]. Lectins are a commonly employed family of glycan binding proteins in glycobiology. Lectins vary in binding specificity to certain glycan structures and their low affinity (μM range) is often increased through multivalent interactions[1]. Glycan microarrays have been developed as a high throughput platform to study the binding preferences of glycan binding proteins. However, when glycans are printed directly onto a functionalized surface[3], much of the natural presentation of glycans as multivalent structures is lost. Our approach more closely mimics native glycoproteins due to their multivalency while also affording us the advantage of systematically altering the presentation of known glycan structures which can then be correlated to the extent of protein binding.



Glycopolymer Synthesis: Sugar Ligation

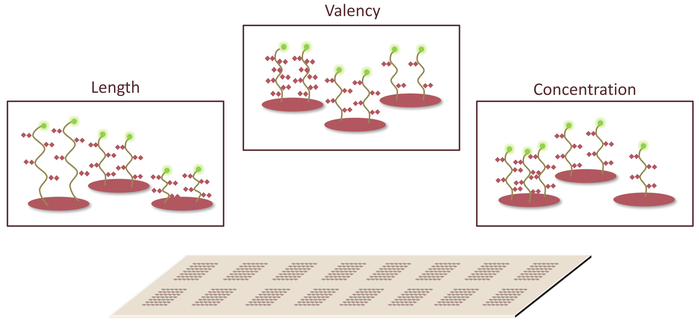


Glycans Employed



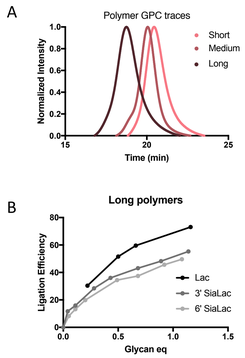
Three lengths of azide-terminated glycopolymers were synthesized as described previously[4] and appended with α2-6 sialyllactose (6′ SiaLac), α2-3 sialyllactose (3′ SiaLac), or lactose (Lac) at different valencies. The fluorophore TAMRA is incorporated as a means to determine polymer concentration.

Density Variant Glycan Microarrays



The tunable nature of our glycopolymer synthesis offers us control over the polymer length and glycan valency. The azide-functionalized polymers are then covalently attached to a cyclooctyne-funtionalized glass surface via micro-contact array printing at increasing concentration to provide arrays of glycan structures in various presentations.

Microarray Characterization



C

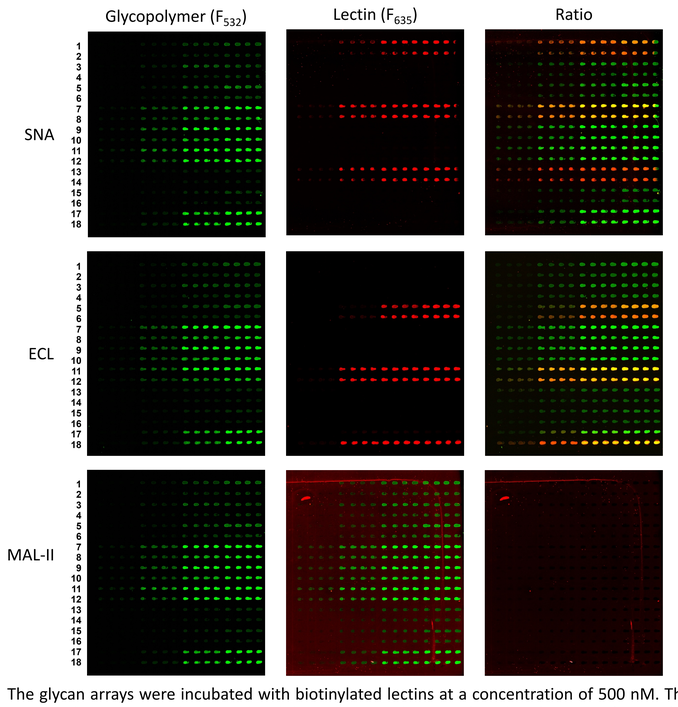
Microarray Format

Row	Glycan	Val	Length
1	6′ SiaLac	42	N = 150
2		56	
3	3′ SiaLac	48	
4		68	
5	Lac	49	
6		78	
7	6′ SiaLac	86	N = 212
8		108	
9	3′ SiaLac	86	
10		125	
11	Lac	66	
12		118	
13	6′ SiaLac	159	N = 424
14		210	
15	3′ SiaLac	183	
16		234	
17	Lac	117	
18		237	

A) GPC traces of polymer show a change in length. **B)** Glycan ligation, used to determine valency, is tunable to the equivalents of glycan added during synthesis. **C)** Table depicting a map of a microarray.

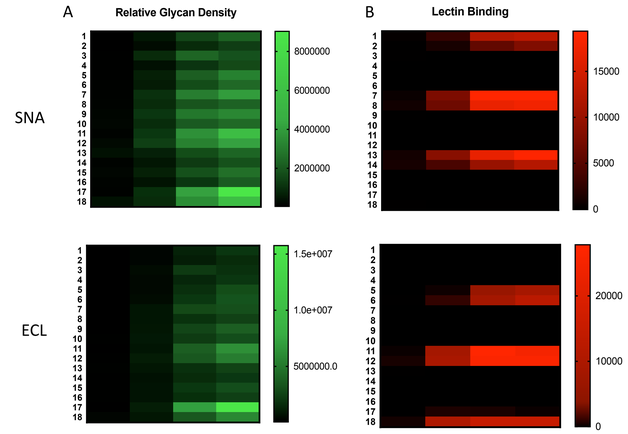
Lectin Screen

Biotinylated Lectins	Glycan Specificity[5]	Array Binding
Sambucus Nigra (SNA)	Sia2α6Gal/GalNAc	Siaα2-6Lac
Maackia Amurensis (MAL- II)	Sia2α3Galβ4GalNAc	None
Concanavalin A (ConA)	αMan, αGlc	None
Erythrina Cristagalli (ECL)	Galβ4GlcNAc	Lac
Succinilated Wheat Germ Agglutinin (SucWGA)	GlcNAc	None
Ulex Europaeus Agglutinin I (UEA-I)	αFuc	None
Vicia Villosa (VVA)	GalNAc	Lac
Peanut Agglutinin (PNA)	Galβ3GalNAc	Lac



The glycan arrays were incubated with biotinylated lectins at a concentration of 500 nM. They were visualized via streptavidin-Cy5. The slide was scanned at wavelengths of 532 nm (TAMRA) and 635 nm (Cy5). From these scans, it is evident that the SNA bound to polymers containing 6′ SiaLac. The lectins ECL, VVA, and PNA all bound Lac polymers. All other lectins tested showed no binding, similar to the images shown for MAL-II. All experiments were done in duplicate.

Data Analysis



A) Heat maps of relative glycan density were calculated based off of the polymer fluorescence seen at 532 nm. These values were multiplied by the valency of each polymer and divided by the TAMRA labeling efficiency. Lac polymers consistently showed greater relative glycan density, most likely due to the fact that it is an uncharged disaccharide. Interestingly, polymers of lower glycan valency seem to provide an increased glycan density compared to their higher valent counterparts. **B)** Heat maps depicting lectin binding. Both SNA and ECL showed the greatest binding to the mid-sized polymers. Binding also increased with increasing glycan density. It is unclear why ECL only bound the higher valent long polymers, but this was consistent for both replicates.

Discussion and Conclusions

Glycan microarrays were used to compare binding of lectins to glycopolymers that were printed at a range of concentrations. SNA bound polymers containing 6′ SiaLac and VVA, PNA, and ECL bound Lac polymers. ConA, SucWGA, UEA, and MAL-II did not bind any glycopolymers included on the array. These results are in agreement with the known glycan specificities of each lectin, except for MAL-II. This lectin exhibits strong binding to Sia2α3Galβ4GalNAc (3′ SiaLacNAc) which lead us to believe that it should bind 3′ SiaLac. Because no binding was seen, this may point to the importance of the GalNAc. In a future screen, WGA, a lectin that binds 3′ SiaLac, should be included.

We next sought to analyze binding of SNA and ECL in a microplate format. Cyclooctyne functionality was incorporated into the protein BSA and 6′ SiaLac containing an azide linker were clicked onto the protein to form a BSA-glycan conjugate. This conjugate was then immobilized onto a 96 well plate for an ELISA-type assay. Treatment of the conjugate with a neuraminidase enzyme should cleave the terminal sialic acid to reveal the underlying lactose. SNA should bind the untreated conjugates (containing 6′ SiaLac) in a dose dependent manner, while ECL should bind dose dependently the neuraminidase treated conjugates (containing Lac). This was then visualized using streptavidin HRP and TMB substrate. Quenching with sulfuric acid did not reveal the expected results. Every well displayed similar absorbance at 450 nm as the controls, leading us to believe that the immobilization of the BSA-glycan conjugate was unsuccessful.

References and Acknowledgements

[1] Varki, et al *Essentials of Glycobiology*. 2nd edition. (2009). **[2]** Grant et al *Glycobiology* (2014). **[3]** Blixt, et al *Proc Natl Acad Sci* (2004). **[4]** Huang, et al *Chem Commun.* (2015). **[5]** Vector Laboratories *Table of Lectin Properties* (2016)

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