Development of Glycopolymers Capable of Cell Surface Remodeling

Keaton Mertz, Chris Fisher, Greg Trieger, Mia Huang and Kamil Godula

Department of Chemistry & Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, California

Abstract

The Influenza A virus (IAV) subgroup has been responsible for all documented pandemic Influenza strains.[1] In order to infect cells, IAV binds to cell surface glycoproteins called mucins before it can be endocytosed and infect its host. Haemagglutinin (HA), the IAV protein responsible for its cell surface association, detects and binds to glycans on mucins which commonly terminate with specific 9-carbon negatively charged monosaccrides known as Sialic Acids (Sia).[2,3] Cell surface glycoproteins are complex and irregular, so the natural surface density of individual glycoproteins is difficult to control. In order to further study IAV's infection and cellular response, we aim to develop new chemical methods of remodeling the glycocalyx of mammalian cells. By identifying candidate glycans and synthesizing polymers capable of incorporating into cell membranes the number of sialylated glycans on a given cell may be manipulated to study mechanical properties of IAV infection.

Overview of Approach

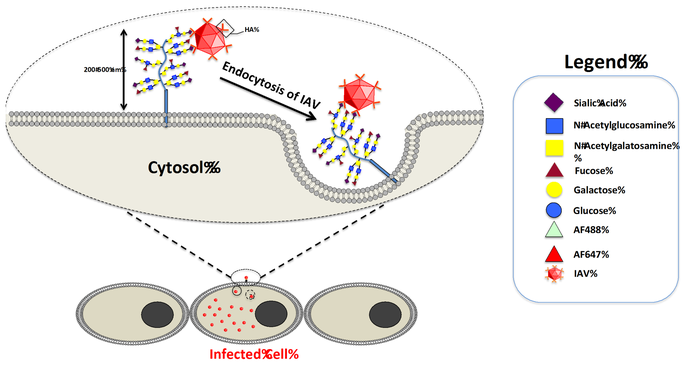
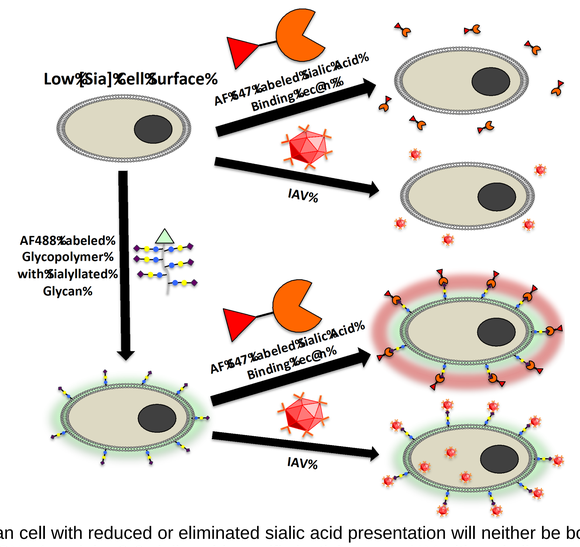
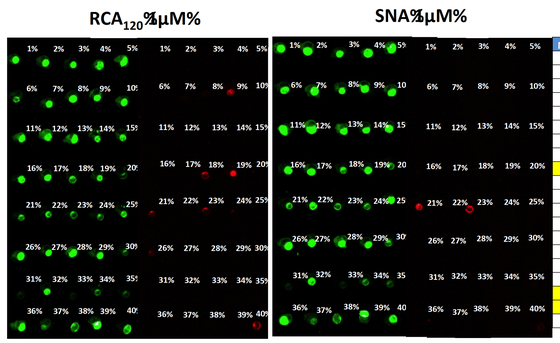


Figure 1 (A) IAV is binding to the natural sialic acid terminated glycans on cell surface mucins through its Haemagluttanin (HA) proteins. The interaction of HA and sialic acid act as the initial or "primary" binding event, thereby initiating IAV infection. After associating with the cell surface, the virus can be endocytosed through multiple mechanisms internalizing the virus on its way to other infective processes.



(B) A mammalian cell with reduced or eliminated sialic acid presentation will neither be bound by AF647 labeled SNA (an alpha 2-6 sialic acid specific lectin), nor an IAV with a similar specificity HA. After the synthetic glycopolymer is added, the sialic acid binding lectin should bind to the polymers which are incorporated on cell as they introduce sialic acid to the cell surface. If the cell is bound by this fluorophore labeled lectin, the resulting fluorescence acts as a stand in for IAV binding because the lectin and the HA should bind similarly. Although not currently known, this mimetic of the natural "primary" binding event may be capable of restoring IAV infection in these cells with limited sialic acid.

Microarray of Labeled SNA and RCA_{120} Lectins



Number	Mucin Glycan	Number	Mucin Glycan
1	Glucose	21	6'-sialylLac
2	GlcNAc	22	3'-sialylLacNAc
3	GlcNAc-6S	23	6'-sialylLacNAc
4	GlcN	24	2-Fuc-Lac
5	GlcN-2S	25	Antigen A
6	GlcN-6S	26	Antigen B
7	Galactose	27	Lewis A
8	GalNAc	28	Lewis B
9	GalNAc-6S	29	Lewis X
10	GalN-2S	30	Lewis Y
11	Mannose	31	3'-Sialyl Lewis A
12	Rhamnose	32	3'-Sialyl Lewis X
13	Fucose	33	Lacto-fucopentaose I
14	Xylose	34	Panose
15	Allose	35	Dextran
16	GlcA	36	Penta-N-acetylpentaose
17	GalA	37	Mannan
18	Lactose	38	Chitosan oligo. lactate
19	LacNAc	39	Talose
20	3'-sialylLac	40	Antigen H

Figure 2: A series of mucin glycans are presented on a mircoarray slide using covalently linked glycopolymers. The glycopolymers are labeled with AF488 producing the green spots indicating the presence of the polymer and its respective glycan. Red labeled lectins (AF647)RCA_{120} (A) and SNA (B) were added to the slides at a 1 µM concentration. Red spots indicate what glycans the lectins can bind.

Table 1: The particular glycans composing the library chosen are listed sequentially. RCA bound 9, 18, 19 and 40 and may bind 21, 23 and 26 whereas SNA exclusively bound 21 and 23 on the microarray.

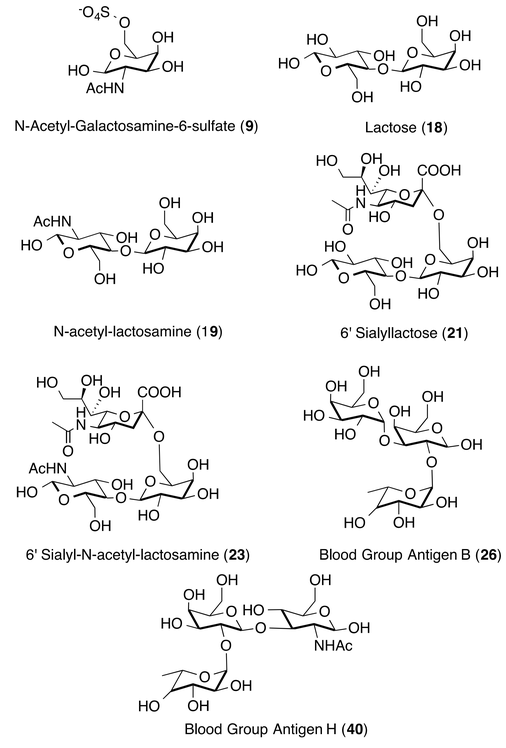


Figure 3. The sugar structures drawn are the sugars that the lectins (RCA and SNA) bound to in the micro array.

Synthesis and Application of Lipid Glycopolymers for Cell Surface Remodeling

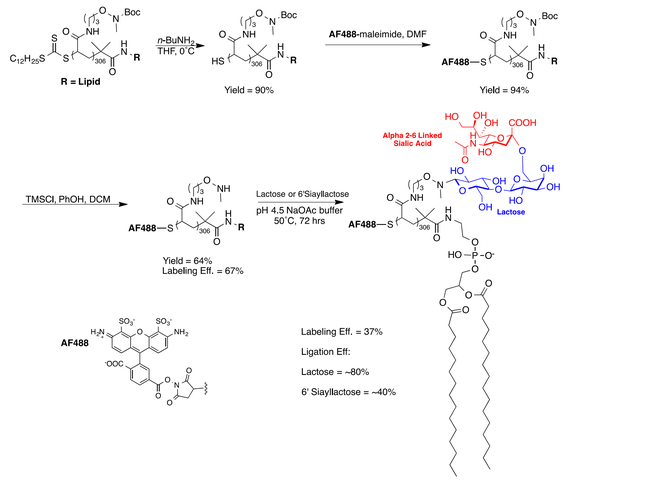


Figure 4. This is a schematic of the synthesis made to create lipid terminated glycopolymers based on the binding information of the microarray. The synthesis began with lipid polymer backbone created previously using RAFT polymerization. The final AF488 labeled polymer was synthesized with lactose and 6' sialyllactose. SNA, a lectin which binds sialic acid in a similar fashion to HA, should only bind to the sialylated sugar as seen in the Figure 2B. AF488 was attached to the polymer so as to indication where/if the polymer incorporates into the cell membrane independent of lectin binding.

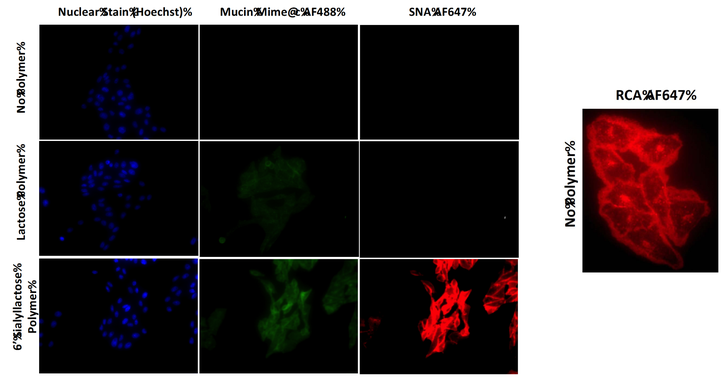


Figure 5: Remodeling of wt CHO cells using Glycopolymers. The drastic increase in SNA binding, indicated by AF647 only occurs on polymers with the proper sialic acid presentation. The brightness of the SNA stain appears even brighter than the RCA positive control done without polymer

CONCLUSIONS AND FUTURE DIRECTIONS

- Glycopolymers can be synthesized with specific sugars attached to the monomers with one lipid terminated side and another AF488 fluorophore terminated side to successfully remodel a cell surface.
- Determining if synthetic glycopolymers can stimulate infection in mammalian cells deficient in sialic acid.
- Determining if HA binding to sialic acid alone can lead to infection

References

Stevens, J.; Blixt, Ola.; Paulson, J.; Wilson, I. Nat. Rev. Microbio. 2006, 4, 857-864.
McGuckin, M.; Lindén, S.; Sutton, P.; Florin, T. Nat. Rev. Microbio. 2011, 9, 265-278.
de Vries, E.; de Vries, R.; Wienholts, M.; Floris, C.; Jacobs, M.; van den Heuvel, A.; Rottier, P.; de Hann, C. Prod. Natl. Acad. Sci. 2012, 19, 7457-7462.