Cell Surface Remodeling with Mimetic Glycopolymers to Study the Effect of Sialic Acid 3D Presentation

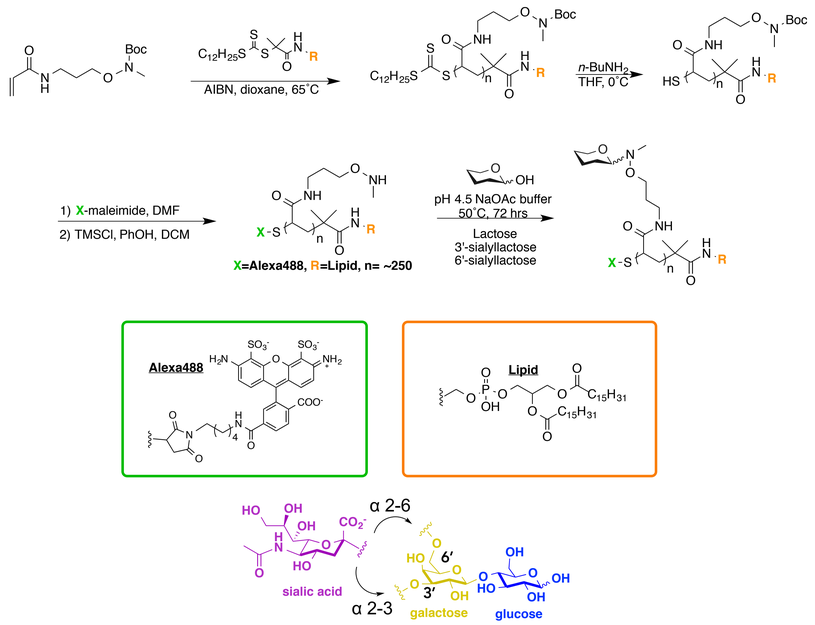
Rency Mallari, Chris Fisher, Kamil Godula
Department of Chemistry & Biochemistry, University of California San Diego, 9500 Gilman Drive, La Jolla, California

INTRODUCTION AND APPROACH

Carbohydrates are one of the four major classes of biomolecules. Although, carbohydrates are typically discussed as energy-storage and structural molecules for living cells, they also mediate a variety of biological processes at the cell surface through carbohydrate recognition. Some of these processes include stem cell differentiation, immune recognition, and host-pathogen interactions. Among the many monosaccharaides, the negatively charged sialic acid (Neu5Ac) is commonly found as a terminating unit for mammalian cell surface oligosaccharides (also known as glycans). The positioning of sialic acid, within this so called glycocalyx, makes it a common target for glycan-binding proteins, pathogens, and other cells; as an example, sialic acid is the primary receptor for the Influenza A Virus (IAV).

Although, sialic acid binding elements have been extensively studied, the effect of its 3D presentation on recognition by sialic acid-binding proteins, such as those found on IAV, remains poorly understood. In order to recreate the presentation of these glycans in a controlled fashion, we have synthesized biomimetic glycopolymers. These materials can be synthetically altered to change glycan presentation. Utilizing lipidated-glycopolymers, we have successfully remodeled cell surfaces with sialoglycans, which can allow for a detailed investigation of 3D presentation, and its relationship to the recognition of sialic acid on the surface of mammalian cells.

GLYCOPOLYMER SYNTHESIS



Scheme 1: Synthesis of Lipid Glycopolymer and structure of sialyllactose. Synthesis takes approximately 2 weeks.

DOUBLE SIALIDASE REMODELING

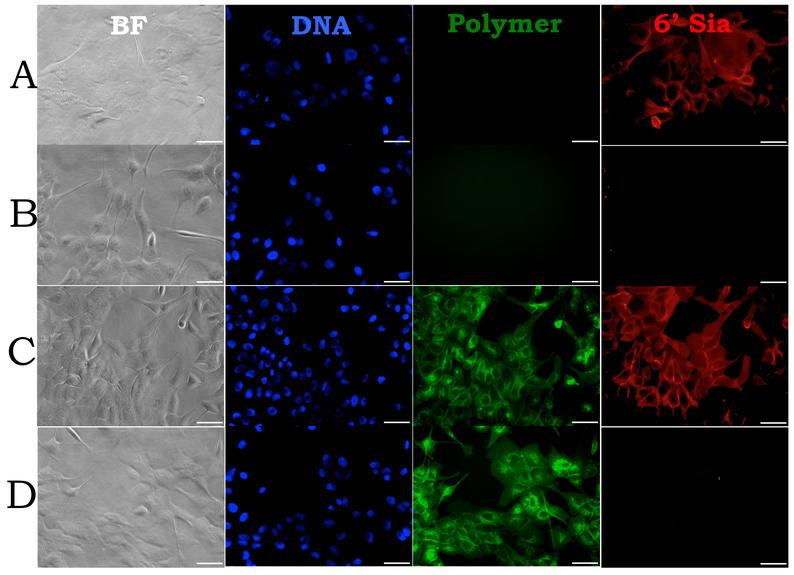


Figure 3: Fluorescence microscope images of double sialidase glycocalyx remodeling on Madin Darby Canine Kidney cells (MDCK). Scale bars: 50μm.

THE GLYCOCALYX

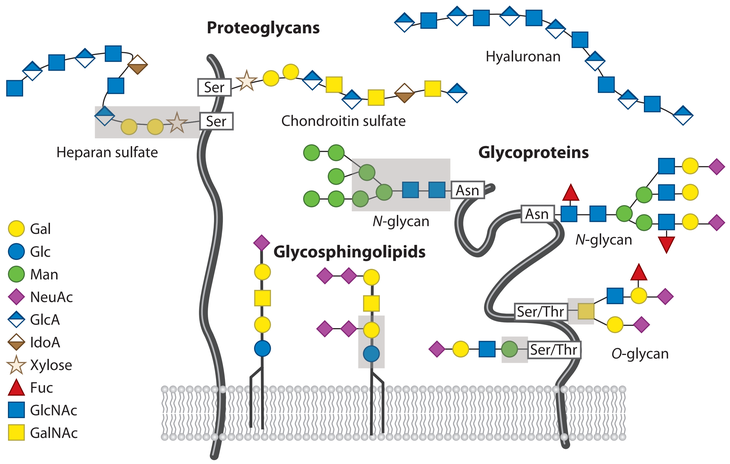


Figure 1: The above figure demonstrates the variety of glycan presentation platforms to help depict the glycocalyx (IE the cell surface) as a 3D environment. Figure credit: Rillahan and Paulson, 2011.

GLYCOCALYX REMODELING

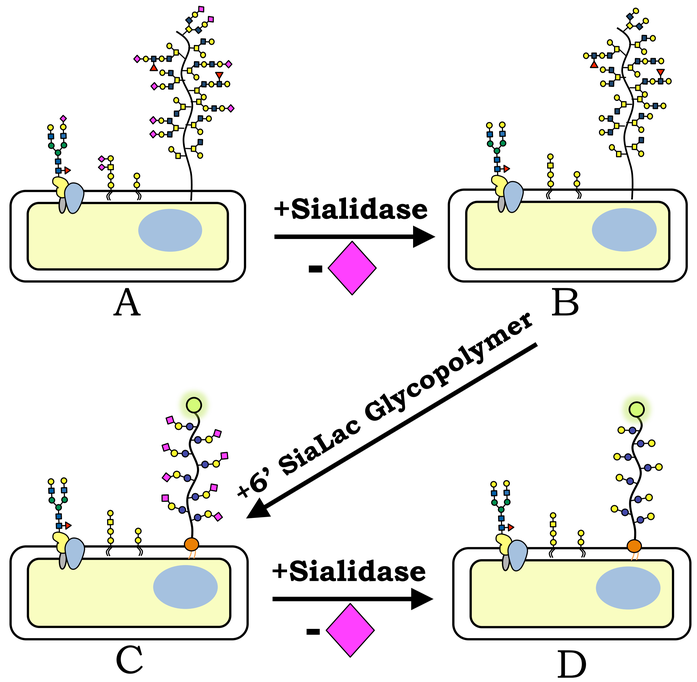


Figure 2: Depiction of the glycocalyx remodeling procedure.

CONCLUSIONS

- Successfully removed 6'-sialic acid from the cell surface by treating the cells with sialidase
- Lipidated-polymer successfully enters the cell membrane.
- As a result of the incorporation into the membrane, the glycocalyx was remodeled with 6' sialyllactose (6' SiaLac).
- The sialic acid found on the glycopolymer is susceptible to sialidase treatment: indicating polymer glycans are substrates for the enzyme in a similar fashion as the natural glycans.

FUTURE RESEARCH

- Quantify the amount of sialic acid added to the glycocalyx during remodeling using high-performance liquid chromatography (HPLC) and sialic acid derivitization (Construct a dose response graph).
- Test IAV infection of remodeled cells using a variety of different polymers to investigate the effect of 3D presentation.

REFERENCES

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Huang, M. L.; Smith, R. A.; Trieger, G. W.; Godula, K., Glycocalyx Remodeling with Proteoglycan Mimetics Promotes Neural Specification in Embryonic Stem Cells. *Journal of the American Chemical Society* **2014,** *136* (30), 10565-8.

Huang, M. L.; Cohen, M.; Fisher, C. J.; Schooley, R. T.; Gagneux, P.; Godula, K., Determination of Receptor Specificities for Whole Influenza Viruses using Multivalent Glycan Arrays. *Chem. Commun.* **2015,** *51*, 5326-5329.