

BCHM 421/422 – 2023/2024

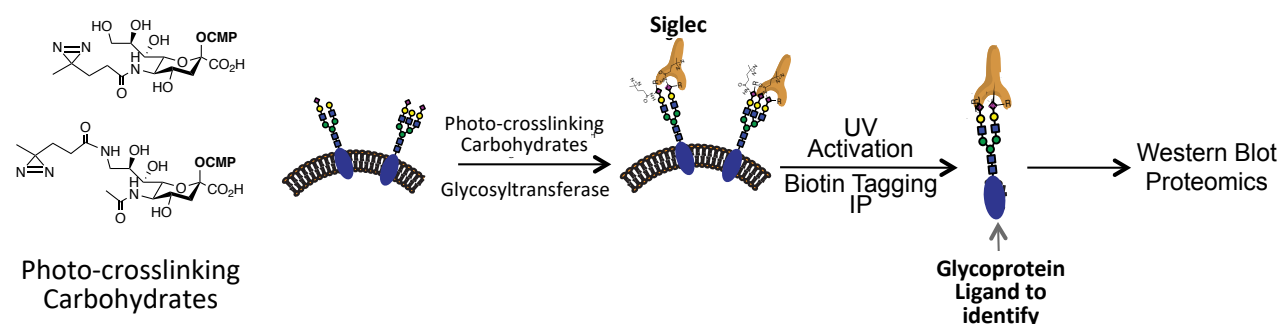
Project Title: Cellular Glyco-Engineering to Investigate Glycan-Siglec Binding Interactions

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Keywords:

1. **Glycobiology**
2. **Carbohydrate (Glycan)-Protein Interactions**
3. **Glyco-Engineering**

Project Outline: The surface of all cells is decorated with a diverse mixture of carbohydrate structures called glycans. These glycans play an essential role in many biological processes and have been implicated in almost every major human disease. Cell-surface glycans interact with glycan-binding proteins (GBPs) to initiate various cellular activities and these interactions are highly selective. However, understanding which carbohydrate structures are responsible for protein binding and the mechanisms by which glycans elicit cellular function is challenging. This multidisciplinary project will involve using carbohydrate probes (sugars with azides, biotin, or photo-crosslinkers) to interrogate and capture glycan-protein binding partners. The probes will be installed on cells using sialyltransferase enzymes to identify unknown glycoprotein ligands that bind to glycan binding proteins. A main focus will be on expanding enzymatic glyco-engineering using different sialyltransferases to examine how defined sialylated glyco-epitopes interact with the Siglec (Sialic acid-binding Immunoglobulin-like Lectin) family of GBPs. Siglecs are important for immune cell recognition, tumor-associated glycan recognition and play a major role in how cancer can evade detection and killing from immune cells. Understanding which glycans/glycoproteins are involved in interactions with Siglecs is critical for understanding the role of glycans in cancer and for identifying targets for novel cancer therapeutics.



Project Goals:

1. Install carbohydrate probes selectively on specific glycan classes on cell-surfaces using a cell-surface glyco-engineering methodology.
2. Assess Siglec binding of glyco-engineered cells
3. *Optional: Enzymatically prepare photo-crosslinking carbohydrate probes.*

Experimental Approaches:

- Mammalian and bacterial cell culture
- Mammalian and bacterial enzyme and protein expression
- Glycosyltransferase reactions and cell-surface modifications
- Biochemical assays, Western Blotting, Flow Cytometry, Immunoprecipitation, Microscopy
- Possible - enzymatic synthesis of carbohydrate probes, structure determination by NMR and Mass Spectroscopy

References:

J.L Babuilic, C.J. Capicciotti. Exo-Enzymatic Cell-Surface Glycan Labeling for Capturing Glycan–Protein Interactions through Photo-Cross-Linking *Bioconjugate Chem.* 2022, 33, 5, 773–780. DOI: <https://doi.org/10.1021/acs.bioconjchem.2c00043>