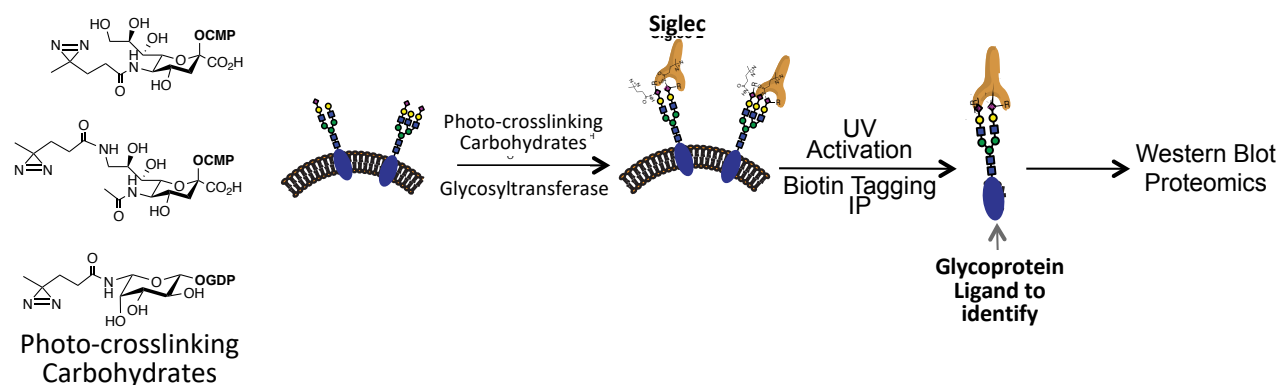


## BCHM 421/422 – 2020/2021

**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 the which carbohydrate structures are responsible for protein binding and the mechanisms by which glycans elicit cellular function is challenging. This multidisciplinary project will involve developing photo-crosslinking carbohydrate probes using enzymatic synthesis to capture glycan-protein binding partners. The probes will be installed on cells using glycosyltransferase enzymes to identify unknown glycoprotein ligands that bind to glycan binding proteins. A main focus will be on the Siglec (Sialic acid-binding Immunoglobulin-like Lectin) family of GBPs. Siglecs are important for cell-cell interactions, 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 identifying glycan biomarkers and functional targets for therapeutics as glycan mediated interactions feature prominently in disease such as cancer.



**Supervisor:** Chantelle Capicciotti

**Project Title:** Investigating Cellular Glycan-Protein Interactions with Photo-Crosslinking Probes

**Keywords:**

1. Glycobiology
2. Carbohydrate (Glycan)-Protein Interactions
3. Structure-Function Relationship
4. Functional Glycomics and Glycoproteomics

**Project Goals:**

1. Enzymatically prepare photo-crosslinking carbohydrate probes.
2. Install photo-crosslinking carbohydrate probes selectively on specific glycan classes on cell-surfaces using a cell-surface glyco-engineering methodology and interact with Siglecs or a second cell type.

3. Photo-crosslink binding partners, enrich and identify *cis* (same cell) and *trans* (adjacent cell) glycan-protein interactions.

#### **Experimental Approaches:**

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

#### **References:**

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2. M.R. Bond, H. Zhang, P.D. Vu, J.J. Kohler, Photocrosslinking of glycoconjugates using metabolically incorporated diazirine-containing sugars, *Nat. Protocols*, **2009**, 4, 1044-1063.