

BCHM 421/422 – 2019/2020

Project Outline: The surface of eukaryotic 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. It is well recognized that cell-surface glycans interact with glycan-binding proteins (GBPs) to initiate various cellular activities, and that these interactions are highly selective. However, understanding the carbohydrate structures responsible for protein binding and the mechanisms by which glycans elicit cellular function is challenging. Cell-surface glycans are complex and diverse in nature and, unlike DNA and proteins, glycan biosynthesis is not template-driven, which complicates understanding specific glycan function on a cellular level. Our group is working on understanding how glycans interact with GBPs and ultimately elicit biological function. We combine chemical and chemo-enzymatic carbohydrate synthesis to prepare glycan structures and carbohydrate-based chemical probes. We then employ chemical biology tools to install these molecules on cell-surfaces to study their function and how they interact with proteins in cells.

An important function of cell-surface glycans is facilitating inter-cellular interactions between glycoproteins and protein receptors on apposing (adjacent) cells. However currently there is a lack of methodologies to identify these binding partners. This multidisciplinary project will involve developing photo-crosslinking carbohydrate probes to capture protein binding partners. The probes will be installed on cells and used to identify unknown glycoprotein ligands that bind to known glycan binding proteins. GBPs such as Siglecs will be examined, which are important for cell-cell interactions, immune cell recognition and tumor-associated glycan recognition. A long-term goal is to explore native binding partners in cells for numerous glycan structures and GBPs. This knowledge will be critical for identifying glycan biomarkers and functional targets for therapeutics as glycan mediated interactions feature prominently in disease.

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Project Title: Investigating Glycan-Protein Interactions in Cells with Photo-Crosslinking Probes

Keywords:

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

Project Goals:

1. Chemically install photo-crosslinking functional groups on azido-carbohydrates.
2. Install photo-crosslinking carbohydrate probes selectively on specific glycan classes on cell-surfaces using a cell-surface glyco-engineering methodology and bind recombinant GBPs or other cells to modified cells with the carbohydrate probe.

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

Experimental Approaches:

- Chemical 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.