Principal Investigator: Catherine Leimkuhler Grimes
Grant Title: Regulation of Nod2 by O-GlcNAc glycosylation and its role in Crohn's Disease

(a) *Abstract*

The innate immune system is the human body's first line of defense against invading pathogens. This ancient system has evolved to properly respond to pathogens but also to live in the presence of the microbiome. When problems occur in the innate immune system, chronic inflammatory disorders such as Crohn's Disease can develop. Nod2 is an innate immune receptor that is responsible for sensing and responding to small fragments of bacterial cell wall. Nod2 has been shown to bind to a small fragment of bacterial cell wall, muramyl dipeptide (MDP). Mutations in Nod2 leave a person predisposed to Crohn's disease and unable to respond properly to the bacterial ligand MDP. In an effort to understand Nod2 signaling, the sequence of Nod2 was evaluated for consensus sites of post-translational modificaitons. As Nod2 is predicted to have many *O*-GlcNAc modification sites, we investigated the possibility that *O*-GlcNAc modifies and regulates Nod2. Recently, OGT has been reported to be essential for regulating the NF-kB transcription factor, the canonical transcription factor for the inflammatory response, reinforcing our hypothesis that *O*-GlcNAc may play a role in Nod2 dependent signaling.

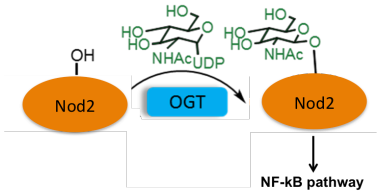

Figure 1: OGT post-translationally modifies Nod2 with a GlcNAc residue.

Using funds from the Mitutani foundation, we demonstrated that wild type Nod2 and a Nod2 Crohn's associated variant are *O*-GlcNAcylated and this modification affects Nod2's ability to signal via the NF-κB pathway (Figure 1)**.** Our research team consists of a glyco-biochemist (Zachara) with an intensive background in OGT biochemistry and a glyco-chemical biologist (Grimes) with experience in Nod2 signaling and synthesis of MDP-sugars/bacterial cell wall fragments (**Figure 2**). The collaboration reaches across universities: the University of Delaware and the Johns Hopkins University. This project was inspired at a glycobiology seminar series and the investigators are enthusiastic about bringing their diverse backgrounds in glycobiology to the field of innate immunity. We plan to attack this problem using tools from molecular biology, microbiology, immunology and synthetic organic chemistry. We aim to determine how and when Nod2 is modified by OGT and how exposure to bacteria and/or bacterial cell wall fragments alters this modification.

Figure 2: Investigators Zachara (left) and Leimkuhler Grimes lead a multidisciplinary team to investigate the role of *O*-GlyNAcylation on Nod2