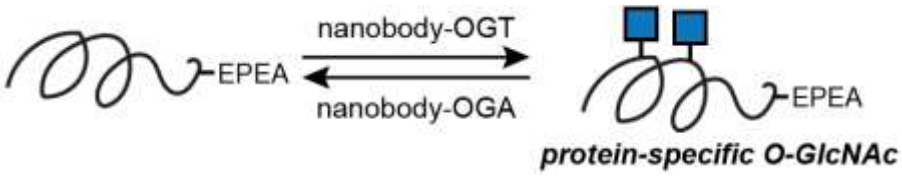
Principal Investigator: Christina Woo

Grant Title: A proximity-directed glycosyltransferase to edit O-GlcNAc on specific signaling proteins

1. Objectives



We proposed to develop a general method to edit O-GlcNAc occupancy on key T cell relevant proteins in order to systematically evaluate glycan function within a biological context. In particular, we will use recent advances in nanobodies and glycoproteomics to produce proximity-directed enzymes that can upregulate or downregulate O-GlcNAc occupancy on specific proteins of interest. These reagents will be applied to study the function of key glycoproteins that we identified as significantly upregulated during T cell activation by quantitative chemical glycoproteomics. Following characterization of specific functions for O-GlcNAcylated proteins, we will integrate our findings to broader cellular signaling pathways and the glycobiome. As O-GlcNAc signaling is found throughout biological systems, these studies will impact fields across biomedical sciences and establish a novel approach to study post-translational modifications that may be further applied to additional glycan modifications and proteins of interest.

2. Methods used

Our approach capitalizes on recent advances in nanobodies and glycoproteomics to produce proximity-directed enzymes that can edit O-GlcNAc occupancy on specific proteins of interest. These reagents will enable the direct control of a protein's O-GlcNAcylation state for the systematic evaluation of function within a biological context for the first time. We will apply these reagents to study the functional role of specific glycosites that we mapped to key glycoproteins involved in primary human T cell immunoactivation. Understanding how O-GlcNAc affects intracellular signaling has the potential to impact numerous biomedical science communities and transform methods to probe, perturb, and remediate cellular signaling.

3. Results

We successfully developed proximity-directed OGT and OGA fusion proteins and demonstrated the generality of the approach using two nanobodies against a series of co-transfected and endogenous target proteins in cells. We are now tuning the system for application to proteins involved in T cell activation. We are grateful for support from the Mizutani Foundation that enabled the development of this technology to elucidate the impact of O-GlcNAc in T cells.