Description of Projects in the Haltiwanger Lab:

The Haltiwanger laboratory investigates the structure, biosynthesis, and function of O-linked glycans on several cysteine-rich domains including Epidermal Growth Factor-like (EGF) repeats and Thrombospondin Type 1 Repeats (TSRs). We analyze the O-glycan structures and the proteins on which they occur using glycoproteomic mass spectral methods. We identify the enzymes which synthesize the glycans and examine their structures using X-ray crystallography. We also identify the genes encoding these enzymes and knock them out in mice to determine their biological roles. Often there are human diseases associated with mutations in these genes, and we characterize the effect of the mutations on function of the enzymes. We also evaluate the function of the O-glycans on specific proteins using *in vitro* and cell-based assays. There are many potential projects in the lab. Two of these are described below:

1. How does addition of O-glucose to EGF repeats affect the structure and function of fibrillin-1 microfibrils? Protein O-glucosyltransferase 2 (POGLUT2) and POGLUT3 add O-glucose to more than half of the 47 EGF repeats in fibrillin-1 (FBN1). Double knockout of *Poglut2* and *Poglut3* in mice results in neonatal lethality similar to *Fbn1*-null mice, suggesting the O-glucose is required for FBN1 function. We will examine how loss of O-glucose affects FBN1 secretion, incorporation into microfibrils, interactions of other extracellular matrix proteins known to bind FBN1, and the structure and stability of FBN1 microfibrils, using a variety of *in vitro* and cell-based assays.

2. How do CADASIL mutations affect O-glycans on NOTCH3 EGF repeats? CADASIL is an autosomal dominant arteriopathy that typically results in premature death or dementia. CADASIL is caused by missense mutations in the *NOTCH3* gene that most commonly delete or add a cysteine to one of its first five EGF repeats. This results in an unpaired cysteine which we predict would cause misfolding of the EGF repeat. The mechanism by which these mutations result in the disease is unknown. Since addition of O-glycans to EGF repeats requires proper folding (with 6 cysteines forming three disulfide bonds), we predict these mutations will cause alterations to the O-glycans on these EGF repeats. Using glycoproteomic mass spectral methods, we will analyze the structures of the O-glycans on NOTCH3 isolated from human vascular smooth muscle cells and determine how CADASIL mutations alter the glycosylation. This data will indicate whether the EGF repeats with the mutations are in fact misfolded, and whether the mutations affect folding of any of the other EGF repeats in NOTCH3.