**Title:** Role of glycosylation defects on rhodopsin trafficking & eye development in zebrafish

**Keywords:** glycosylation; rhodopsin; eye development; zebrafish

**Background:** Glycosylation is an important co-translational and post-translational modification required for protein folding and trafficking. N-glycosylation, glycans attached to asparagine side chains, is one of the two main forms of glycosylation and roughly half of all vertebrate proteins are N-glycosylated [1]. The process of N-glycosylation begins with precursor formation in the cytoplasm before being transferred onto a nascent polypeptide chain with consensus sequences in the ER lumen (Figure 1).

The importance of glycosylation for development is evident upon examining patients diagnosed with congenital disorder of glycosylation (CDG), a group of diseases characterized by N-glycosylation defects. CDG patients often exhibit multisystem symptoms, indicating proper glycosylation of proteins is essential for development of an organism[2]. Both pharmacologic and genetic approaches are used to elucidate the importance of N-glycosylation during development. Pharmacologically, tunicamycin inhibits dolichyl-phosphate N-acetylglucosamine phosphotransferase 1 (DPAGT1), the initial enzyme of the N-glycosylation pathway, resulting in decreased enzymatic activity and reduced glycolysis (Figure 1). Genetically, our laboratory identified a zebrafish line with an amino acid substitution in the zebrafish Dpagt1 protein. Histological analyses revealed that dpagt1 mutants have smaller misshapen eyes and exhibit a thinner photoreceptor cell layer compared to wild-type (WT) siblings. Rhodopsin (Rho), a G-protein coupled receptor found in disc membranes in outer segment of rod photoreceptors, is used to transduce visual signals and has two glycosylation consensus sites (N-2 and N-15) highly conserved in vertebrates. Regulation of Rho and its glycosylation sites are developmentally important as previous studies in mouse and Xenopus have shown that a decrease in Rho expression or glycosylation results in decreased outer segment size and defective disc membrane assembly, respectively[3]. Through the use of genetics and the ease at which eye development can be observed, zebrafish offer a unique model to examine both the morphologic and functional outcomes of a glycosylation defect on development.

**Hypothesis:** A mutation in zebrafish dpagt1 results in decreased glycosylation of Rho, preventing proper trafficking to the outer segments of rod photoreceptors. Lack of Rho trafficking causes stunted eye development and functional deficits in mutant embryos compared to WT.

**Aim 1:** Determine whether dpagt1 mutant embryos exhibit trafficking deficiency of Rho. Glycoproteins require proper glycosylation to signal for exit from the ER, and loss of glycosylation activates an ER stress response resulting in distended ER[1]. I will use transmission electron microscopy (TEM) to examine sections of retina of both dpagt1 mutants and WT siblings for distended ER in rod photoreceptor cells predicted to result from insufficient...
trafficking. Immunogold labeling of Rho during TEM will allow me to visualize cellular compartments in which the protein is trapped. I expect to find Rho in dpagt1 mutants trapped in the ER instead of trafficking to the disc membranes of outer segments due to a lack of proper glycosylation.

**Aim 2: Define morphologic changes in eye development using a nonglycosylated Rho genetic model.** By using a CRISPR/Cas9 system, I will be able to edit the zebrafish genome to evaluate how loss of conserved glycosylation sites (N-2 and N-15) on Rho contributes to eye development. Specifically, I will design a guide RNA to target glycosylation consensus sequences in the Rho coding sequence to mutate asparagine to cysteine, a nonglycosylated amino acid. Following verification of an established CRISPR knock-in zebrafish line, I will cross the CRISPR line with a rod photoreceptor-GFP transgenic line to image eye development over the course of embryo development using confocal microscopy. I expect that CRISPR knock-in embryos will recapitulate aberrant eye development observed in dpagt1 mutants, thus providing further evidence that proper glycosylation of Rho is necessary for eye development.

**Aim 3: Quantify visual impairment of mutants by measuring optokinetic response (OKR).** During an OKR assay, eye movements are stimulated by object movement and can be tracked in embryos as early as 80 hours post fertilization[4]. This assay will allow me to determine functional consequences of morphologic changes resulting from loss of glycosylation on Rho. With a simple rotating, striped drum apparatus, I will be able to track eye movements of WT, dpagt1, and CRISPR mutants to determine if a loss of glycosylation has an effect on retina function. This behavioral assay is a well-established tool to analyze vision performance, light adaptation, visual acuity, and contrast sensitivity. I expect that dpagt1 and CRISPR mutants will exhibit decreased OKR to the visual stimulus, indicating N-glycosylation of Rho contributes not only to eye morphology but also function.

**Study significance and broader impacts:** CDG patient symptoms suggest a correlation between disruptions in glycosylation pathways and developmental defects. However, the direct role of glycosylation in morphology and functional development remains a question in the field today. Glycosylation of Rho is used in this proposal as a way to address this question, but may apply to other proteins, such as BMP receptors participating in key signaling pathways essential for development [5]. Understanding more about glycosylation, a basic cellular mechanism, within the context of development can further elucidate development of organ systems and organ functions.

As a graduate student at ____________________________, I not only have the resources available to complete the aims listed above, but I am also surrounded by opportunities to present my data in multiple settings to people both in and outside of my field of study including at weekly research forums and annual department retreats as well as national meetings such as ____________________________. At community events, such as ____________________________, I will model for area school children how photoreceptors in the eye use Rho to transduce light signals to the brain to form images using retina models and coloring activities.