**Research Statement – Student B, PhD**

**Overarching Question – How do brains develop?**   
My research focuses on the cell signaling mechanisms that guide the development of the vertebrate nervous system. The human nervous system is composed of hundreds of billions of interconnected neurons that underlie our capacity for learning, memory, and behavior. These intricate networks are the result of precisely timed developmental events, including cell migration, axon guidance, dendrite branching, and synapse formation Understanding the complex molecular mechanisms that shape neuron morphology and connectivity will provide valuable insight into nervous system development and developmental disorders of the brain.

**Graduate Research – How do Slit and Robo help shape neurons?**   
My dissertation research at Prestigious University explored how extracellular cues shape the morphology of cells in the developing brain. Specifically, I examined how the secreted protein, Slit, communicates to neurons via receptors Robo1 and Robo2. When I began my research, we knew that Slit used Robo1 and Robo2 to guide migrating axons during development, but whether Slit used both receptors to promote the growth and branching of dendrites was not known. I cultured cortical neurons from mice that lack Robo1 or Robo2, and I found that both Robo1 and Robo2 mediate Slit-induced axon growth, but only Robo2 mediates Slit-induced dendrite branching (J. Round, 2009). *In vivo* analysis revealed no significant dendrite branching defects in the absence of either receptor, suggesting that other ligands and receptors compensate for the loss of Robos *in vivo*. In further research, I discovered that the adaptor protein, Nck, binds to Robo1 and Robo2 and is necessary for the Slit-induced growth of axons and dendrites (Round & Sun, 2011). My findings uncovered a central role for Nck in shaping the morphology of cortical neurons.

**Current Research – How do Slitrk genes contribute to brain development?**   
As a postdoc at Smaller College (SC), I launched an extramurally funded research program that provides many research opportunities for undergraduates. Building on my previous experience in a zebrafish laboratory (Julich *et al.*, 2005), I use zebrafish as a model organism to examine the role of a newly discovered family of proteins in the developing brain. These proteins, known as Slitrks (pronounced Slit-tracks), are integral membrane proteins that resemble the Slit family of guidance cues and the Trk family of neurotrophin receptors. Six Slitrk genes are expressed in the developing brain of mice and humans, and Slitrk mutations are linked to multiple neurological disorders, including Tourette’s syndrome, autism, and schizophrenia. However, the functional roles of Slitrks in the formation and maintenance of neural circuits are not well characterized.

In 2010, I taught SC students how to mine the zebrafish genome and EST databases for Slitrk genes. We also performed a series of RT-PCR experiments, which revealed that all six genes are expressed in zebrafish at developmental stages consistent with dendrite branching and synapse formation. In 2011, I worked with two SC students and two research technicians to generate spatial and temporal expression profiles for all six Slitrk genes using *in situ* hybridization. In 2012, we are generating full-length cDNA clones of each Slitrk gene to prepare for loss-of-function experiments. We presented our findings at a national conference in November 2011, and we anticipate the publication of our data in a peer- reviewed journal by Spring 2013.

**Future Research – What would Towson students do in my lab?**   
The zebrafish is an excellent model organism for the study of nervous system development. Zebrafish breed readily in captivity, develop rapidly *ex vivo*, and are inexpensive to maintain, making them highly amenable to student-centered research at Towson University. Fewer than ten peer-reviewed papers have been published on the contribution of Slitrks to nervous system development, which allows for an enormous variety of undergraduate research projects in this area. My future research, funded by an NSF Research at Undergraduate Institutions (RUI) Award through February 2014, is divided into two categories:

**Question 1**: **What happens when Slitrk is knocked down in zebrafish?**   
In close collaboration with Towson students, I will employ antisense oligonucleotide technology (morpholinos) to reduce Slitrk protein levels in developing zebrafish embryos. We will characterize defects in nervous system wiring using neuron-specific antibody staining and/or lipophilic dye injections. Examples of laboratory techniques that students will learn include:

* Microinjecting morpholino oligonucleotides into fertilized zebrafish embryos
* Performing dose-response assays to assess toxicity of Slitrk morpholinos
* Immunostaining with Slitrk antibodies to assess efficacy of Slitrk knock-down
* Performing lipophilic dye injections to assess defects in neural circuit formation

**Question 2**: **Which proteins interact with Slitrk proteins?**   
Slitrks are integral membrane receptors for which no ligand has been identified, and little is known about the intracellular signaling events that lie downstream of Slitrks. We will perform a variety of protein interaction assays in order to identify Slitrk ligands and signaling molecules. Examples of projects that students may undertake include:

• Conducting a yeast two-hybrid assay to screen for Slitrk-interacting proteins  
• Performing immunoprecipitation and western blotting to identify protein interactions   
• Performing column chromatography to capture Slitrk ligands from embryonic lysate  
• Using small molecule inhibitors to disrupt Slitrk-mediated intracellular signaling *in vitro*

Taken together, these and other experiments will increase our understanding of the functional roles of Slitrks in brain development. We will pinpoint specific populations of neurons that require Slitrks for proper wiring, and we will identify signaling molecules that cooperate with Slitrks to shape cell morphology. These research questions constitute an unexplored niche in Slitrk research and will provide valuable insight into the molecular mechanisms of neuronal signaling and connectivity.

**Learning Through Research**   
Meaningful research opportunities enhance the educational experience of undergraduates and encourage students to pursue careers in science (Lopatto, 2004). With six Slitrks genes to investigate, my research program will support many years of student-centered experiments that can be carried out in a laboratory course or independent research format. By characterizing the roles of Slitrks in nervous system wiring, Towson students will have the opportunity to make meaningful contributions to the fields of cellular and molecular neurobiology.

* Julich D, Lim CH, Student B, Nicolaije C, Schroeder J, Davies A, Tubingen 2000 Screen Consortium, Geisler R, Lewis J, Jiang Y, Holley SA. 2005. *beamter/deltaC* and the role of Notch ligands in the zebrafish somite segmentation, hindbrain neurogenesis and hypocord differentiation. *Developmental Biology*. 286: 391-404
* Lopatto D. 2004. Survey of undergraduate research experiences: first findings. *Cell Biology Education.* 3: 270-277
* Student B**.** 2009. The Role of Slit/Robo Signaling in Neuronal Morphogenesis. *Doctoral Dissertation*, Presitigious University, Somewhere, USA
* Student B, Colleague C. 2011. The Nck adaptor mediates Slit1-induced changes in cortical neuron morphology. *Molecular and Cellular Neuroscience.* 47: 265-273.