Proposal Narrative for Crown Award or Wise-Marcus 50-Year Friendship Award
"Defining the Role of Bok and Erlin2 in Cell Growth and Apoptosis"

Spinocerebellar ataxias and neurodegenerative diseases affect neurons (nerve cells) in the brain and spinal cord. Those who suffer from spinocerebellar ataxias experience a degeneration of the spinal cord and of the cerebellum (located at the base of the brain), which controls coordination and voluntary movement. Abnormal function of cells is followed by their demise. As these processes proceed, patients suffer from an array of symptoms, which include problems with coordination, vision, and memory loss. As more neurons die, symptoms worsen, and sadly, this results in an individual's inability to function in the world. In addition, millions of people suffer from Alzheimer's, Parkinson's, Huntington's, multiple sclerosis and amyotrophic lateral sclerosis (ALS), and as people continue to live longer, incidence rates are expected to rise. According to studies, in 2030, as many as 1 in 5 Americans will be over the age of 65. This is important because onset of these diseases is usually seen in mid-to-late life. If neurodegenerative diseases are left unchecked, more than 12 million Americans will suffer from them. However, some diseases occur at an earlier age such as intellectual disabilities and juvenile lateral sclerosis (medical relevance of a protein I am conducting experiments on – the erlin1/2 complex). Therefore, finding treatments and cures is an increasingly urgent goal. In due time, researchers and physicians will be able to treat and prevent many of these diseases, but major efforts must be made. In an effort to combat these challenges, it is our duty as a community to avoid the dramatic impact this can have on our loved ones and to do everything we can to avoid these diseases.

Autosomal dominant sensory ataxia (ADSA), a spinocerebellar ataxia with other neurological signs, is caused by degeneration of the posterior columns of the spinal cord. This disease is genetic in origin and like many others, is progressive. Furthermore, it is characterized by cerebellar atrophy linked to complications involving balance, posture, and voluntary muscle movements. One of the lab's main focuses is on RNF170, an enzyme that facilitates recognition and degradation of proteins, a process known as the ubiquitin proteasome pathway (UPP). A mutation in RNF170 causes ADSA. Studying RNF170 is important because it can revolutionize advances in trying to treat ADSA.

In the Wojcikiewicz lab, we study various aspects of IP_3 receptor biochemistry and molecular biology. Inositol 1,4,5-triphosphate (IP_3) is an intracellular messenger formed at the cell surface when activated by neurotransmitters, hormones or other drugs. The effects of IP_3 are mediated via IP_3 receptors (IP_3R), a protein located on the surface of the endoplasmic reticulum (ER), which governs the release of calcium ions

into the cell cytosol. The movement of calcium ions is of the utmost importance to many cellular functions, affecting processes such as fertilization, apoptosis (programmed cell death), neurotransmission (learning and memory), muscle contraction, synaptic activity, secretion, motility, membrane trafficking, excitability, gene expression, cell division, and cell-cell communication and adhesion. One of the lab's current focuses includes analyzing IP_3R down-regulation, when the IP_3 receptor is rapidly depleted from cells upon stimulation; it is considered an adaptive response that enables cells to adjust to their external environment. The mechanism by which this process occurs happens naturally via the ubiquitin proteasome pathway – the receptors are tagged with a protein, ubiquitin (long red oval chains), and then degraded by the proteasome (please see image on page 5).

IP_3 receptors, with their ability to release calcium (Ca^{2+}) stores from the endoplasmic reticulum to the cell cytosol, play a role in neuronal signaling, and this has greater implications when studied in SH-SY5Y human neuroblastoma cells. Studying IP_3R is important for examining neurodegenerative diseases because neurons are Ca^{2+}-regulated. Once IP_3R is activated, there is a dramatic decline in cellular IP_3R content, because the receptor is tagged with ubiquitin and degraded by the proteasome. Broader questions involve studying the UPP and elucidating exactly how this occurs. In addition to the receptor's being the site of Ca^{2+} release in the cells, there are two other major complexes (erlin1/2 and Bok) that play functional roles that have yet to be determined.

The UPP has become increasingly relevant because this is the pathway by which important cellular proteins or misfolded proteins in the ER are degraded, and it is of great relevance to diseases such as cancer, neurodegeneration and diabetes. In recent years, the lab discovered that a novel complex composed of the proteins erlin1 and erlin2 mediates IP_3R ubiquitination, as well as other proteins coupled to activated IP_3R. Attachment of the erlin1/2 complex is critical for IP_3 receptor ubiquitination and proteasomal degradation by the UPP. Furthermore, the erlin1/2 complex is involved in autophagy, the intracellular degradation system by which cytoplasmic materials are delivered to the lysosome, the cell's "waste disposal system." When a cell lacks erlin2, the mutation can be associated with deficits such as intellectual disability, motor dysfunction, muscle shortening, and hereditary spastic paraplegia, including juvenile primary lateral sclerosis (a disorder with progressive weakness and stiffness of the muscles in the face, arms, and legs). Advances within this area of research can help explain the increasing number of diseases that are caused by erlin2 mutations. The lab has recently developed cells lines lacking erlin2, in which IP_3R down-regulation is blocked, to define the role of

IP_3R down-regulation and to define the role erlin1/2 plays in autophagy. My current experiments focus on analyzing the role of erlin2 in wild-type and knockout cells.

Another focus of my Capstone work includes characterizing the interaction of Bcl-2 family proteins that control apoptosis (programmed cell death) with IP_3 receptors. The lab recently discovered that Bok ("Bcl-2-related Ovarian Killer") binds constitutively to IP_3R and we want to define the significance of this interaction (left-hand side of image on p.5). Bok is a member of the Bcl-2 family of apoptosis regulatory proteins that controls the intrinsic apoptotic pathway. Interactions between these family proteins control and regulate apoptosis. Currently, Bok is considered pro-apoptotic (triggers apoptosis) and is related to Bax and Bak (other pro-apoptotic proteins). However, unlike Bax and Bak, little is known about Bok, and in order for apoptosis to occur, Bok needs either Bax or Bak to be present. Bok knockout mice (those lacking Bok) do not display any abnormalities, and apoptosis successfully occurs when Bax and Bak are present, which means the function of Bok is yet unknown. Our lab found that Bok binds constitutively to IP_3 receptors via the BH4 domain and protects them from proteolysis (breaking down into smaller proteins) during apoptosis, but does not appear to alter Ca^{2+} channel activity. Since IP_3Rs control Ca^{2+}, it was first hypothesized that the function of Bok binding may control Ca^{2+}, but the results were identical in wildtype and Bok knockout mouse embryonic fibroblast cells.

Clearly, Bok is a novel component of IP_3 receptor complexes. Bok is most similar to pro-apoptotic proteins Bax and Bak, but its cellular role remains challenging. It is expressed in all mammalian tissues and is one of the most highly conserved members of the Bcl-2 family. In addition, Bok is degraded by the UPP in parallel with the degradation of IP_3Rs, but Bok is not critical to the central function of IP_3Rs, which is to act as IP_3-gated Ca^{2+} channels. The Bcl-2 protein family is widely implicated in controlling cell viability, and knowing more about Bok will aid with ongoing efforts to develop therapeutic inhibitors. Moreover, the Bok gene is frequently deleted in cancer, suggesting that it might be a tumor suppressor, so the Bcl-2 protein family is a target for anti-cancer drugs. Because of the importance of IP_3 receptors, the ubiquitin/proteasome pathway, and the Bcl-2 protein family to cell biology, this work is significant to both our understanding of normal physiology, and also to diseases such as cancer and neurodegeneration.

The critical goals of my Capstone project will be to (1) study the proliferation and sensitivity to apoptosis of αT3 cells replete with erlin2 or lacking erlin2, to define the biological role of IP_3 receptor down-regulation and (2) to study the viability and apoptotic activity of Bok cells replete with Bok or lacking Bok to define the biological role of the IP_3 receptor association with Bok. Preliminary experiments for my Capstone

include performing cell proliferation and apoptosis assays to assess cell growth, viability and apoptotic index using two of the following reagents: WST-1 and Apo-Live Glo. I will be working on IP_3 receptor association with Bok proteins studying wildtype (WT) and Bok-/- (knockout) mouse embryonic fibroblasts (MEF) cells and αT3-1 mouse pituitary cells. I will be using αT3 cells in which erlin2 has been knocked out, which is important because once the conformational change occurs and opens the Ca^{2+} channels, it triggers the association of the erlin1/2 complex, which recruits enzymes such as RNF170 that catalyze the ubiquitination of IP_3R. One of the main questions is to see if αT3 cells are more susceptible to apoptosis because of the lack of erlin2. If so, what role does this play in the endoplasmic reticulum? Another major aim of my project is to characterize the activity of Bok. Is it pro-apoptotic or anti-apoptotic? What is the biological significance of the novel Bok-IP_3R interaction? Does Bok binding regulate IP_3R function? Is binding to IP_3R required for Bok to influence apoptosis, and could the interaction site be a novel drug target?

I have previously done experiments to track cell growth, and to measure proliferation, in order to have a base point for starting cell concentrations to conduct future experiments with. In addition, I want to know the growth rate of the cells to be able to accurately detect when they reach maximal growth and I have been working on that. I am performing two types of assays that will show growth patterns, viability and apoptosis within the various cell types I am working with. An assay I am performing using the WST-1 reagent will detect cell proliferation and viability. Another assay I am performing using the Apo-Live Glo reagent is a two part assay that will enable me to test viability (number of living cells) and apoptosis (programmed cell death by detecting activation of a biomarker of apoptosis).

Throughout the rest of the semester I will be establishing the basic growth parameters of αT3 cells, HeLa cells, and Bok cells and establishing sensitivity to various ER stress agents in terms of viability and apoptosis. Some stress agents deplete ER Ca^{2+} stores, and induce autophagy by causing ER stress. Future experiments will include running western blot analysis and looking at interactions within the pathway. I will be performing time course assays from around January to May 15. I will start with Western blot analysis in May to account for the mechanisms for differences seen and continue with experiments throughout the summer, until the beginning of September. Meanwhile, I will be writing my introduction, methods and materials section, compiling all of my data and gathering my results. I will be done with all experiments and data collection by the end of August, so that I can select an Honors Reader and submit a first draft to my Capstone Advisor by September 20. I will submit a complete draft to my Honors Reader by October 4 and continue to revise this

draft until November 14. I will submit a “Capstone Summary” to my Honors reader on November 15 and will submit a final draft/iteration of my project to both my Honors Reader and Capstone Advisor by November 20 for any final revisions. I will turn in my Capstone on December 4 and present it on December 12. The reason I plan to submit and present my Capstone in December 2014 is because I will be going abroad to continue doing research and will volunteer with the National Israeli Ambulance (Magen David Adom) for six weeks during the spring of my senior year.

I plan to present my research at the National Statewide CSTEP conference in April 2014. Obtaining funding for my Capstone is essential for my personal growth within the realm of biomedical research and being able to further myself within my biology major by presenting a published project on work I find interesting. Being an Honors student has given me the privilege of being able to engage in remarkable opportunities at SU, and the Capstone will certainly help my achieve another facet of the wonderful things Honors offers its students. I hope I am able to obtain this funding to purchase the necessary materials to carry out time-coursed assays for the remainder of the semester and to continue with more advanced work in the summer. Receiving Capstone funding would be another great achievement for me, and it would certainly add to my experience as a Coronat Scholar at Syracuse University.