

I am currently an undergraduate researcher in Dr. Kathleen Gallo's laboratory. I am working on finding the binding constant and heat of reaction of the intramolecular interaction between the SH3 domain of the protein mixed lineage kinase 3 (mlk3) and a sequence of approximately ten peptides that exists near the CRIB domain of the protein. My first priority is to purify the SH3 domain of MLK3. This is done by overexpressing the gene encoding the protein and then purifying the protein which has a GST tag attached to it via a thrombin cleavage site. As of now I have purified the protein and my next step is to cleave the GST tag from the SH3 domain which I have purified. This is to be done using thrombin, a protease. However, I have had a lot of difficulty with this procedure as it appears that thrombin has been digesting my protein as well as the cleavage site. I have ran four different experiments testing the conditions of the digestion however none have yielded favorable results. Thus, I am currently attempting to insert the gene encoding the SH3 domain into a new vector which will have the same GST tag however a different, more specific proteolysis site. After this, purification and digestion should be accomplished easily.

After I have my purified protein I will run experiments using iso-thermal titration calorimetry so as to find the binding constant between these two domains and the heat of reaction ( $\Delta H$ ). This information is very important as SH3 domains are known to bind to proline rich sequences however in mlk3, SH3 binds to a domain which has only one proline. This is uncommon amongst SH3 domains found in other proteins and is a novel interaction. Through varying the peptide sequence of SH3's ligand, and through mutating certain residues on SH3, we will be able to figure out what amino acids are essential for this reaction and be able to construct an accurate model for this interaction.

Understanding the strength of this interaction is very important as mlk3 plays a role in the MAP Kinase pathway which is involved in cell growth and cell death and is seen to play a role in Parkinsons disease and cancer.

I have also been a part of two other research projects in other laboratories. During my freshman and sophomore year I worked in Dr. Joan Broderick's laboratory and was calculating the binding constant between S-adenosylmethionine (SAM) and spore photoproduct lyase. I was also researching the role that iron-sulfur clusters played in this interaction.

In Dr. Alex Chen's laboratory I was researching the effect of diabetes on wound healing. I injected the mice with a cytotoxin which rendered the mice diabetic and then wounded them and compared their wound healing times to those of non-diabetic mice. I was also researching how insulin treatment effected wound healing.