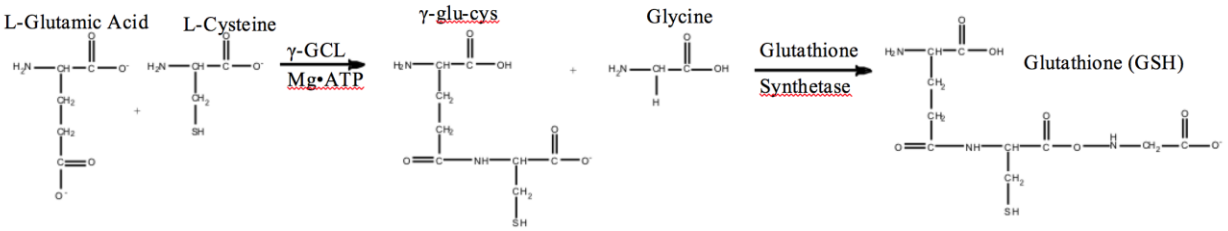
Sigma Xi Undergraduate Research Grant Proposal
Analysis of the Oligomerization of Ɣ-Glutamylcysteine Ligase

## Project Summary

Gamma-glutamylcysteine ligase (Ɣ-GCL) catalyzes the rate limiting step in the synthesis of glutathione, a compound essential in detoxifying cells. However, glutathione has been found to be upregulated in some bacterial cells, resulting in antibiotic resistance.[1,2] This project aims to investigate the molecular weight of the different oligomeric forms of Ɣ-GCL and the noncovalent interactions involved in the oligomerization process. The physiological relevant oligomer of *E. coli* Ɣ-GCL is currently unknown, thus studies characterizing the oligomeric forms and the interactions involved in the transitions between forms are valuable.

## Background

Glutathione is an essential compound found in most organisms due to its role in cell detoxification. Within a biological system, glutathione is conjugated to electrophilic molecules, including reactive oxygen species and reactive nitrogen species, targeting them for elimination from the cell.[1,2] The synthesis of glutathione involves the conjugation of L-glutamic acid and L-cysteine by Ɣ-glutamylcysteine ligase (Ɣ-GCL) to form Ɣ-glutamylcysteine which is then conjugated with glycine by glutathione synthetase to synthesize glutathione.[1]




While Ɣ-GCL is necessary for the survival of bacterial cells, overexpression can result in antibiotic resistance. Therefore, identification of inhibitors of Ɣ-GCL could be utilized in the

development of antimicrobial agents. An understanding of Ɣ-GCL structure and function is necessary for development of these therapeutic advancements.

In the Kelly laboratory, *E. coli* Ɣ-GCL has been observed in both the monomeric (*i.e.* one polypeptide chain) and dimeric (*i.e.* two interacting polypeptide chains) forms. Previous work suggests that purified *E. coli* Ɣ-GCL transitions between oligomeric states following storage at -20˚C and 4˚C, as well as with the addition of oxidizing and reducing agents.[3] The addition of these agents has served as a method to study whether covalent interactions, specifically disulfide bonds, are involved in the transition between these forms. The addition of oxidizing agents, hydrogen peroxide and ascorbic acid, to the monomeric form of Ɣ-GCL resulted in a shift to a higher order oligomer of unknown molecular weight. The biologically relevant form of Ɣ-GCL has yet to be determined, and therefore experiments characterizing each of the oligomeric forms are beneficial.

The molecular weight for the monomer and dimer were previously found to be 45.7 kDa and 88.2 kDa, respectively, using Ferguson analysis.[4,5] The higher order oligomer has yet to be characterized by molecular weight, and thus one of the foci of this research project will be to determine the molecular weight of the higher order oligomer. The role of noncovalent interactions in the oligomerization of Ɣ-GCL are also of interest and would advance our understanding of the oligomeric transition and stabilizing factors for the higher order oligomers.

<u>Proposed Project for Spring of 2014</u>

We plan to study the oligomerization of Ɣ-GCL and the intramolecular interactions involved in the oligomerization process. Specifically, our first goal of this project is to determine the molecular weight of the higher order oligomer induced by the addition of oxidizing agents hydrogen peroxide and ascorbic acid. This will be accomplished by inducing

the formation of the higher order oligomer and using native polyacrylamide gels to perform a Ferguson analysis for molecular weight determination. Liquid chromatography-mass spectrometry will be utilized as an additional method for determining the molecular weight of each oligomeric form, and these results will be compared to those of the Ferguson analysis.

The second goal of this project is to study the role of noncovalent interactions, such as hydrogen bonds, electrostatic interactions, and hydrophobic interactions, in the oligomerization of Ɣ-GCL using the denaturants guanidine hydrochloride and urea. Guanidine hydrochloride is proposed to impact hydrogen bonds and electrostatic interactions that may be stabilizing the dimer form of the protein. Urea is proposed to primarily disrupt hydrophobic interactions that may be stabilizing the dimeric form of the protein. We will add various concentrations of each denaturant to separated monomer and dimer forms and track the changes in migration patterns on native gel electrophoresis. Migration of a protein on a native gel is dependent upon molecular weight, shape, and charge. We hypothesize that as a given denaturant is added to a protein sample, we will observe a change in protein migration as the protein transitions between different oligomeric forms. This migration pattern will be compared to samples of separated monomer and dimer Ɣ-GCL as controls. However, because migration is dependent upon the three factors, molecular weight, shape and charge, in order to determine a change in molecular weight, we will perform a Ferguson analysis and monitor protein migration on gels that contain a different percentage of acrylamide. The Ferguson analysis will be necessary to determine if the change in migration patterns resulted from a transition between oligomers, a change in charge or shape, or denaturation.

Time Frame

In the first half of the semester we will focus on analyzing the noncovalent interactions

involved in the oligomerization. In March, we will continue studying the effects of guanidine hydrochloride and urea on the transition between oligomeric states. Starting in April, we will begin to investigate the molecular weight of the higher order oligomer through Ferguson analysis. By the end of April we hope to have the Ferguson analysis complete, leaving the month of May for mass spectrometry studies.

Literature Cited


1. Griffith O.W., Mulcahy, R.T. (1999). The Enzymes of Glutathione Synthesis: γ-Glutamylcysteine Synthetase. In D Purich (Ed.). Advances in Enzymology and Related Areas of Molecular Biology, Volume 73: Mechanism of Enzyme Action. John Wiley and Sons, Inc. 209-220.
2. Meister A, Griffith OW. (1979): Effects of methionine sulfoximine analogs on the synthesis of glutamine and glutathione: possible chemotherapeutic implications. *Cancer Treat Rep.* **63** (6): 1115-1121.
3. Boettcher, Koepsell, and Kelly (unpublished results).
4. Ferguson, KA. (1964): Starch-gel electrophoresis-application to the classification of pituitary proteins and polypeptides. *Metabolism* **13** (10): 985-1002.
5. Wiese and Kelly (unpublished results).

Proposed Budget

| Item | Use | Vendor | Cost |
|---|---|---|---|
| Urea | to determine the importance of non-covalent interactions in oligomerization | Sigma-aldrich U0631-500G | $62.00 |
| Guanidine hydrochloride | to determine the importance of non-covalent interactions in oligomerization | Sigma-aldrich 50935-50G | $262.00 |
| Any kD TGX gels | to monitor changes in oligomeric state | Biorad 456-9035 | $165.00 |
| **Total Budgeted** | | | **$ 489.00 .** |