Title of Project: Heavy Metal Binding Capabilities with Riboflavin Binding Protein

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Project Description:

This summer, I will be working with Dr. [redacted] (Capital University) to dephosphorylate the riboflavin binding protein (RBP) and investigate what potentially harmful metals adhere to it, using a variety of methods and instruments in the chemistry laboratory on the campus of Capital University. The main methods used to complete this project will be ultraviolet-visible spectroscopy (UV-Vis) and Flame Atomic Absorption Spectroscopy. It is our hypothesis that the metals are binding to RBP and that harmful metals, like lead and cadmium, will bind similarly.

This project inspired multiple questions of interest that we hope to answer throughout this project. Those questions are:

- Where are the potentially toxic heavy metals binding to the riboflavin binding protein?
- What potential relevance could this have on humans consuming contaminated water while pregnant?
- How can this research ameliorate our understanding and guide new research into RBP?

The focus of this work is the study of the highly-stable and easily isolated RBP. RBP is a well-studied, stable protein that is purified from chicken eggs. The structure of RBP is characterized by a ligand-binding domain and a phosphorylated motif. (Monaco, 1997) Dephosphorylating this protein will allow us to determine whether the metal ions are binding to the negatively charged phosphorous group, or in the metal-binding site. Additionally, analyzing whether lead out-competes copper (II), which normally binds to RBP (Smith, 2006), will identify potential health-related applications. If lead is binding to chicken's RBP, then there is potential that a similar process could be happening inside humans. This is relevant to the Flint water crisis, where drinking water has a higher content of lead than is deemed acceptable. This experiment has been performed by previous students, with metal ions naturally found in chicken eggs, demonstrating its feasibility. I would like to add to this experiment by looking at lead and cadmium ions and potentially other heavy metals. I believe that by looking at the binding capabilities of these metals, one could apply the research to metal poisoning in humans. With RBP having a very similar role in both chickens and humans, there is potential that the metals would bind similarly in human RBP. This could be a possible source of embryonic metal poisoning.

Multiple methods will be used in this project. The first step of the project will be the purification of the RBP from the chicken egg whites. The RBP will be dephosphorylated using a phosphatase enzyme solution and tested with the metal ions. The lead ion will be tested under both the natural and dephosphorylated form of RBP. Both UV-vis and atomic absorption spectroscopy techniques will be used to determine the site of binding and what is binding.

At the end of this project, I will write a research paper detailing the findings and future directions. I will submit an abstract of this project at Capital University's Symposium and a regional or national chemistry conference (likely American Chemical Society).

Each member of this summer project has specific roles. I will be responsible for purifying the protein from egg whites, collecting and analyzing the data. I will also be responsible for keeping my lab space clean and taking care of the instruments that will be used for the project. I will be responsible for submitting the first draft of the paper and abstracts for the conferences. My faculty advisor, Dr. [redacted] will be responsible for helping me purify RBP. She will also have some responsibility in helping

me collect and analyze the data during the project and at the end, editing the paper and abstracts for the conferences

To help prepare me for this project, I am currently taking Chemical Analysis II (Chem 422) which incorporates training and theory on the instruments that will be used for this project. I have previously used the instrumentation needed for this research and am familiar with it. I also am in Biochemistry 2, which provides an understanding of the theory behind the research. In addition, I have also worked as a Teaching Assistant for Capital University Chemistry Department, so I have more in-depth, hands-on experiences with using laboratory instruments and leadership abilities. These experiences have given me knowledge of the equipment that will be used, knowledge of the theory behind the research, and leadership abilities to keep the project on track.

References:

Monaco, H.L. (1997) *Crystal structure of chicken riboflavin-binding protein*. **EMBO J.** 16: 1475-1483.

Smith, S. R., Pala, I., and Benore-Parsons, M. (2006) *Riboflavin binding protein contains a type II copper binding site*. Journal of Inorganic Biochemistry 100: 1730–1733.

Student Products and Project Assessment:

- This project will result in a paper summarizing the results of the research
- The project will result in abstract submissions to the Capital University Symposium on Undergraduate Scholarship and a regional or national chemistry conference (likely American Chemical Society). Assuming this is accepted, Nicholas will present posters at these meetings.
- The project will be evaluated for success based on the completion of the research and research project.

Timeline for Project:

- **Weeks 1–2:** Purifying RBP from eggs and dephosphorylating RBP
    - These weeks will include purchasing the egg whites and dephosphorylation solution, purifying RBP using a multistep method, and running the RBP through the dephosphorylation solution by a phosphatase enzyme solution.
- **Weeks 3–5:** Collect fluorescence data from metal ions
    - These weeks will include repeating experiments that have been previously performed, but with the dephosphorylated RBP, to see where the metal ion is binding.
- **Weeks 6–8:** Testing the lead and cadmium ions, and other heavy metals if time permits.
    - These weeks will consist of testing the lead ion, and cadmium and other heavy metal ions, if time permits, to view whether these ions bind to RBP or the dephosphorylated RBP
- **Weeks 9–10:** Writing and follow-up experiments
    - These weeks will consist of data analysis, finishing up all experiments, and writing the research paper.