

**Title:** Investigating Protein Folding and Interactions through Electrostatics and Experiments

**Key Words:** Proteins, Electrostatics, Folding, Stability, Interactions, 3D Modeling,  $pK_a$ , Small Angle Neutron Scattering, NMR, *ab-initio* calculations, molecular dynamics

**Hypothesis:** The combination of experimental methods and computational modeling of the properties of proteins will enable a more fundamental understanding of biophysical principles and allow for innovative and practical solutions to problems in medicine, computational modeling, and bio-engineering.

### **Introduction:**

The role played by electrostatics on protein folding, stability, and interactions is a crucial element in our understanding of the cell and its functions. Particularly, charged amino acids influence the stability and interactions of proteins, but their charge state depends on their  $pK_a$ . Therefore, accurate predictions of  $pK_a$  values are critically important for successfully modeling the pH-dependence of protein folding, stability, and interactions. Accurately modeling these characteristics of proteins can improve the development of practical solutions to problems that arise such as when certain bacteria render an entire set of antibiotics useless (Norris 2007). However, calculating  $pK_a$  values of amino acids in proteins and modeling protein characteristics is a difficult task and a constant challenge in biophysics.

### **Research Plan:**

To calculate the  $pK_a$ s of proteins, one must calculate the energy difference between protein ensembles with ionized and neutral (non-ionized) amino acids to determine the  $pK_a$  shift, i.e. the change of  $pK_a$  induced by interactions within the protein. Common methods used in these calculations include the finite difference Poisson-Boltzmann (FDPB) method, the Generalized Born (GB) method, molecular dynamics (MD), empirical methods, and combinations of these techniques. However, with each of these methods there are strengths and weaknesses in predicting experimental data. Problems arise because protein structures are given by X-ray crystallography, and there are various structural artifacts due to the crystallization process. The calculations mentioned above must average over an ensemble of the multiple protein conformations. Since proteins do not have a static structure, the flexibility of proteins introduces more complexities as the charges will fluctuate due to this flexibility. Therefore, successful modeling of energies and  $pK_a$  values requires either (a) explicit modeling of these conformational ensembles or (b) the development of approaches that can mimic the effect of ensembles. My previous research focused on the second task, as described in my previous essay. My future research will focus on the explicit modeling of conformation ensembles.

The problem of modeling these complicated systems can utilize experimental methods as well. By complementing the computational modeling with experimental methods such as NMR spectroscopy and small angle neutron scattering, a stronger understanding of the properties of folding and conformation changes in protein interactions will be possible. At the University of Tennessee, there is continuous collaboration with Oak Ridge National Laboratory, and Oak Ridge has the premier Spallation Neutron Source (SNS). Neutron scattering allows for better spatial resolution of protein structures since neutrons interact with nuclei and the scattering can distinguish hydrogen from deuterium. Also, my work on the Palmetto cluster at Clemson enables me to work with larger supercomputers such as Jaguar and Kraken which are hosted at Oak

Ridge. With the resources of NMR spectroscopy at the University of Tennessee and the SNS at Oak Ridge, there is a great potential for more accurate models of proteins and a better understanding of protein interactions.

### **Anticipated Results:**

The use of *ab-initio* principles and molecular dynamics will accurately model changes made to ionization sites within proteins as I have demonstrated through the pKa cooperative initiative. With small angle neutron scattering, I will be able to use protein crystals to create more accurate 3D images of the protein. I will use this data to measure the accuracy of the proposed theoretical models and improve these models. Because of prior success, I am hopeful that the availability of new information from the scattering will further improve the accuracy of the models and aid in our understanding of protein folding, stability, and interactions.

During my research with Dr. Serpersu, I learned how important it is to utilize various experimental methods such as ITC and NMR to understand properties of proteins. These are not the only experimental tools available, and I will learn and use all of the necessary methods. The use of experimental methods will be used to further verify and understand protein characteristics. It is important to investigate whether or not the addition of *ab-initio* calculations correctly models pK<sub>a</sub>s. If the initial *ab-initio* calculations incorrectly model the pK<sub>a</sub>, I propose that *ab-initio* calculations are completed for all sites that are proximal to the charged amino acid. This will allow for more flexibility in the MD calculations. Using the method proposed above, it is expected that the results obtained will align closely with the experimental results. I have successfully modeled charged amino acids within alpha helices of proteins. In this case, *ab-initio* calculations rearrange the structure so that the side chain of the titratable group is modeled as facing the surrounding water or other medium. If the modeling of proteins using the aforementioned methods is indeed accurate, then the results of this research will allow scientists to predict the pK<sub>a</sub> of proteins and to model the 3D structure of proteins more accurately.

### **Broader Impacts:**

A more fundamental understanding of the properties of proteins will also allow us to take steps towards the development of methods to combat antibiotic resistant bacteria. The results of my research will be published in peer-reviewed scientific journals, and I will present my research to the public to cultivate an interest in science among the general public. My local presentations of research have struck many chords with young students. I mentor high school students throughout the year, and they consistently ask me how they can get involved with research. Many of these students have gone on to research in their undergraduate careers. Through encouraging and communicating with younger students, there is a great potential that they will also become interested in how they can help change the world through the progress of science.

### **Works Cited:**

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