1. Personal Statement

My goal is to be a major contributor to the highly interdisciplinary group of scientists who will move biology forward over the next few decades. To do so, I plan to combine my interests in complex questions and heavily documented, reproducible research with my skills in computer science, statistics, and molecular biology.

I began my research at Northeastern University in 2015 by analyzing the way in which patterns of redox potentials are organized across *Caenorhabditis elegans* feeding muscles using a genetically-encoded, ratiometric fluorescent sensor in the Apfeld Lab. Over time I completed many other projects, including investigating the role of transcription factor interactions and TGF-β signaling in organismal lifespan and oxidative stress resistance, respectively. I also completed projects in computational biology at Cygnal Therapeutics and Harvard Medical School, each of which strengthened my interest in the quantitative aspects of biological research.

Over the last six months, I have pursued a project with the Apfeld Lab that involves the mathematical modeling of certain properties of genetically-encoded sensors, the tools that first brought me to the Apfeld lab in 2015. I will use this summer to communicate the results of my project and promote its accessibility to the scientific community.

2. Previous SIRF Experience

I completed SIRFs in the summers of 2016 and 2017. In 2016, I conducted a broad inquiry into the cross-talk between transcription factors related to aging. In 2017. I successfully investigated the interactions between TGF-β dauer pathway genes in oxidative stress resistance, the results of which I presented at the 2017 International *C. elegans* conference in Los Angeles.

Professor Apfeld has previously emphasized that full-time research is vital to the success of undergraduate projects. My previous two SIRFs gave me the framework and funding to focus solely on research for two summers and they were immensely valuable learning experiences. During those projects I focused, with increasing independence, on the part of the scientific method involving iterative testing and revising of hypotheses. For my third and final SIRF, I am proposing to focus on the last steps of the scientific method: refining conclusions from data and communicating results.

3. Project Background and Objectives

Life relies on the compartmentalization of redox potentials, which allows for the building and breaking of carbon sources to take place concurrently in a single cell (Herrmann and Dick, 2012; Romero 2013). Further, redox-dependent modifications to proteins are used to integrate information about the metabolic state with biochemical signaling events (Fisher-Wellman and Neufer, 2012; Wang et al., 2012; Romero 2013).

Redox-sensitive green fluorescent proteins (roGFPs) have been developed to quantify dynamic redox processes in living cells (Cannon and Remington, 2006 and 2008; Hanson et al., 2004). Under Dr. Javier Apfeld, the use of roGFPs to measure redox in *Caenorhabditis elegans* led to novel insights about the redox couple glutathione and the redox patterns of long-lived animals (Romero-Aristizabal et al., 2014).

roGFPs have two states and are ratiometric, which reduces error and allows for concentration-independent measurements. Ratiometric measurements can be transformed into values such as the percentage of oxidized sensors and the redox potential (Meyer and Dick 2010). The non-linear nature of these transformations introduces errors and so certain variants of roGFP sensors are better suited to measure different ranges of redox potentials.

I have worked with Dr. Apfeld to build a model that describes the sensitivity and accuracy of any two-state ratiometric fluorescent sensor.[1] We believe this model is the first to enable a comparison of values that different sensors are well-suited to measure.

The theoretical framework developed by Dr. Apfeld and I is inaccessible in its current state. The objective of this project is to communicate our findings. I aim to (1) write a manuscript to submit to a peer-reviewed journal and (2) create a web application to help users to understand how to apply the framework to their own research.

4. Project Design and Methodology

Interactive notebook and web application

The codebase underlying the ratiometric sensor model is written in R, an open source programming language. To communicate the model to a larger audience, I will create an interactive notebook and a web application. I will use Jupyter to implement the interactive notebook. The completed notebook will be hosted for free on GitHub and on a server such as Google Co-laboratory. The web application will be built using R Shiny, a framework for building web applications entirely in R. The application will provide windows to upload custom data and interactively adjust relevant parameters.

Creating a Jupyter notebook walkthrough is straightforward and should not present any difficulty. However, I expect that building a Shiny App will be a challenging learning experience. I have experience building small Shiny Apps, but the proposed application will be significantly more complex than previous projects. Given the wealth of online resources, I expect that I will be able to overcome any challenges. However, if I fail to build the application on time, the Jupyter notebook will still provide sufficient documentation for publication and dissemination.

Academic paper

[1] I have included details on this framework in the attached Supplementary Materials.

The paper will consist of three sections. The first will describe propagation of error in the roGFP1-R12 sensor, which the Apfeld lab has used for most measurements. This will include a map of the redox potentials one may observe, given a certain 'true' redox potential. It will also contain a ratiometric sensor phase plot to describe the range of values a sensor is well-suited to measure. In the second section, I will expand my analysis to multiple sensors. I will present a wide range of ratiometric redox sensors and demonstrate the differences in the suitability of these sensors to measure different redox potentials. Then, I will include other ratiometric sensors, such as those that measure pH and ATP. In the third section, I will present the interactive notebook and web application, demonstrating the model's reproducibility and general applicability.

I expect that writing an academic paper will be a challenging and rewarding process. One difficult aspect of writing will be condensing my mathematical model into the limited page requirements of an academic journal. To do so, I will likely attempt to make the content of the paper simple and applications-focused but include a much more detailed and nuanced explanation of each concept in the supplementary materials.

5. Mentoring Plan

This project will be undertaken in close collaboration with Dr. Apfeld, who will provide substantial help and feedback while drafting a manuscript. I will have in-person meetings with Dr. Apfeld multiple times peer week and, at least once per week, we will discuss updates on the project's overall progress and goals. I will also participate in summer lab meetings with both the Apfeld and Cram research groups.

6. Outcomes Statement

I will give a short talk about my theoretical framework at the 2019 International *Caenorhabditis elegans* meeting in Los Angeles as part of a microscopy workshop, as well as present a project poster. This project also includes a written manuscript that will be made available to the public as a preprint while it undergoes formal peer review. I also plan to construct a public-facing web server to increase the accessibility of my framework and all derivations, graphs, and source code will be available on GitHub (https://github.com/julianstanley/Ratiometric_Microscopy) as soon as they are created.