Student Responses to RENEWAL Questions

The following apply only for renewal applications. Please consider these responses in making your assessment of the application.

1. **Please describe how successful you were in achieving the intended outcomes of and adhering to the plan/timeline of your original proposal.**

Several adjustments were made to my original proposal. Namely, the QUAS/QF system was investigated in place of the Gal4/GUAS system. This was largely due to several important discoveries with the QUAS/QF system that required further investigation. Moreover, unforeseen issues in this project required lengthier troubleshooting than would have been considered. In all, the nature of the project was very similar to the original proposal; a different genetic system was simply investigated.

This proposal focuses more on the mechanistic functions of the QUAS/QF system rather than gene expression dynamics. This direction is largely guided by the feedback of several well-respected figures in the field of synthetic biology. The mechanisms by which QUAS/QF functions in bacteria must be better understood if it is to be applied to more complicated applications. This proposal also grants me the opportunity to learn new techniques of protein molecular biology.

2. **Please describe how successful you think your relationship with your mentor was during your first semester of UROP.**

Dr. [redacted] was an important part of making my first semester of UROP enjoyable. She has provided excellent feedback and advice in the journey of troubleshooting experiments. We have talked on multiple occasions, both one-on-one and in group settings, about the progress of my project. Her advice and expertise have been an important aspect of making this work successful. While very busy, Dr. [redacted] has always been happy to discuss my project and offer advice. She has been especially supportive in helping me present my research. Several weeks ago, I had the opportunity to attend the national biomedical engineering society (BMES) conference to present my project, and Dr. [redacted] was instrumental in providing this opportunity. She even organized a lab meeting to let me practice presenting my poster! Perhaps the most useful aspect of our relationship is the perspective it provides as I prepare for a career in research.

3. **Please explain what you think the impact of an additional semester in UROP would be to your educational and career goals.**

Participating in UROP has helped me experience the life of a researcher firsthand. In particular, applying for UROP funding has been valuable practice in scientific writing and presenting results. I've learned that I thoroughly enjoy writing about my research, and I now look forward to a career in biomedical research with greater confidence.

Participating in UROP has cemented my desire to pursue a career in biomedical research. Specifically, I aim to complete an MD-PhD program. An additional semester of research would allow me to start pursuing my passions now rather than later. Additionally, I feel ownership over this project, and I want to finish the work that I started. The experiments outlined in this proposal will allow the previous semester's work to be useful in a wider range of applications.

UROP Proposal

Title of Proposal

Investigation of Mechanisms Behind the QUAS/QF System in Prokaryotes

Problem/Topic of Research or Creative Work

The field of synthetic biology uses engineering principles to design and construct genetic circuits for programming cells with novel functions. A main feature of synthetic biology is the transfer of genetic parts from one organism to another with the goal of engineering cells and organisms that can be used for applications in medicine and industry. Many useful gene control systems native to bacteria, such as the LacO or TetR systems, have been successfully integrated into eukaryotic cells (Auslander and Fussenegger, 2016; Auslander et al., 2012; Deans, 2014; Deans et al., 2007; Deans et al., 2016; Fitzgerald et al., 2017; Folcher and Fussenegger, 2012; Kojima et al., 2015; MacDonald et al., Submitted; Ruder et al., 2011; Weisenberger and Deans, 2018; Ye et al., 2013). Moreover, being a non-native component of the genome, they are often orthogonal to the host's natural functions, which minimizes crosstalk between the engineered circuits and the host's cellular machinery. Finally, it is advantageous to investigate the transferability of such systems that enables the reprogramming of cells to behave in predictable ways.

My project will investigate the mechanisms by which the QUAS/QF system functions in bacteria. This system is found naturally in the fungus Neurospora crassa (N. crassa) and has recently been shown to function in Escherichia coli (E. coli) (MacDonal et al., Submitted). The mechanisms by which gene expression is activated, however, have yet to be elucidated and are an important step to realizing further applications with this system.

This will be investigated by studying the dynamics of gene expression via flow cytometry with varying parameters. Green fluorescent protein (GFP) will be placed under direct control of the QUAS/QF system and fluorescent intensity will be measured at regular time points via flow cytometry. These experiments will be important steps to narrow the range of possible mechanisms. Immunoprecipitation experiments and mass spectroscopy will then be used to determine the activity of specific proteins in the bacterial QUAS/QF system. The results of each experiment will inform future experiments.

This project plays an important role in the lab, as understanding the exact mechanism of this system in bacteria is necessary before it can be reasonably used in other applications. Furthermore, the mechanism may shed light on aspects of bacterial gene expression that are as of yet undescribed in science. Novel applications for this technology may also become evident as the QUAS/QF system is probed.

Relevant Background/Literature Review

Researchers recently moved the Q system, a cluster of gene regulatory components which is found naturally in the mold N. crassa, to mammalian cells (Fitzgerald et al., 2017). The QF system is a useful method to control gene expression and is also functional in fruit fly models (Potter et al., 2010). In this system, an activating protein (QF) binds to specific 16mer sites in the DNA sequence (QUAS) and turns on gene expression. In the absence of QF, genes downstream of QUAS are off. Thus, the QF/QUAS system can function as an on/off switch. Our lab has shown the orthogonality and ability of QF to activate genes in genetic circuits next to QUAS sites when introduced to CHO-K1, HEK293, and mouse embryonic stem cells (Fitzgerald et al., 2017). Additionally, this genetic circuit was used to preferentially control the differentiation of

mouse embryonic stem cells (Fitzgerald et al., 2017). Slight variations were found in the function of this system in mammalian cells compared to that in Neurospora crassa (Fitzgerald et al., 2017). This research supports the plausibility of moving eukaryotic genetic parts to different organisms, and also highlights the possibility that the QUAS/QF system may function differently in bacteria than it does in yeast.

Several well-characterized genetic tools will be used in the experimental design to allow for precise investigation of the QUAS/QF system's functionality in bacteria. The T7 promoter has been well-characterized in the field and will be used in this project. The T7 promoter permits transcription to occur exclusively via the T7 RNA polymerase (RNAP), which has been shown to encourage protein production at extremely high levels compared to natural levels (Studier et al., 1990). Moreover, T7 RNAP does not interact with non-T7 promoters; consequently, native gene expression will remain undisturbed, making it a useful orthogonal tool in genetic circuits (Studier et al., 1990). By placing genes of interest before or after a T7 promoter, they will be transcribed only when T7 RNAP is present. The T7 promoter controlled via a lac operator (lacO), is commonly used in prokaryotic synthetic biology (Studier et al., 1990). A T7LacO plasmid will therefore be used as a control to which expression of bacteria containing T7 and the Gal4/GUAS system can be compared. The BL21 (DE3) strain of E. coli will be utilized in gene expression experiments, as T7 RNAP is integrated into its genome and transcribed upon induction with Isopropyl-β-D-thiogalactoside (IPTG). This will allow precise temporal control of gene expression across multiple bacteria cultures.

Recent experiments have shown that when placed upstream of a promoter, QUAS maintains a tight off state in the absence of QF and shows moderate gene expression when QF is present (MacDonald et al., Submitted). When the QUAS is placed downstream of the promoter, however, gene expression is seen in the absence of QF, and there is a two-fold increase in expression that is maintained longer when compared to the control (MacDonald et al., Submitted). The mechanisms by which high gene expression is achieved in this system are unknown.

Specific Activities to be Undertaken and Timeframe for Each Activity

Jan 6 - 10: Prepare for flow cytometry experiments by transforming necessary bacterial cultures and planning experiments in full detail
Jan 13 - 17: Perform experiments to measure the effects of nutrients in media on gene expression using flow cytometry
Jan 20 - 24: Test the effect of the activating and binding domains of QF on gene expression in constructs without the QUAS
Jan 27 - 31: Determine if glucose further prevents leakage of protein product. This will be especially useful to shed light on the toxicity of QF.
Feb 3- 7: Analyze data from the experiments listed above and interpret results. Repeat experiments as necessary Feb 10 - 14: Research literature and protocols regarding immunoprecipitation. Seek advice and demonstrations from labs familiar with these techniques, such as Dr. [redacted] laboratory
Feb 17 - 21: Order necessary supplies and prepare reagents for immunoprecipitation experiments
Feb 24 - March 6: Perform immunoprecipitation experiments on upstream and downstream constructs of the T7 promoter, QUAS, and GFP and perform mass spectroscopy to determine the identity of proteins involved in gene expression

March 9 - 13: Spring Break
March 16 - 27: Analyze results of IP and mass spectroscopy experiments. Allow time for troubleshooting. March 30 - April 3: Review results with Dr. [redacted] and discuss future experiments for the project
April 6 - 10: This time is allotted to performing experiments determined by Dr. [redacted] and I to be relevant to the project. These will be determined based on past results but may include more measurements of expression dynamics or protein chemistry experiments.
April 13 - 17: Complete any remaining experiments and prepare slides for lab presentation April 20 - 21: Present results to the entire lab
April 22 - 29: Study and take final exams

Dr. [redacted] will regularly check on the status of the project and offer advice. Moreover, she will always be available for private meetings to discuss troubleshooting, progress, and offer ideas as needed. Lab meetings in large and small group formats will provide an opportunity to discuss my research with peers and exchange advice as appropriate.

Relationship of the Proposed Work to the Expertise of the Faculty Mentor

An expert in the field of synthetic biology, Dr. [redacted] will be an invaluable resource throughout this project. She completed postdoctoral training at Johns Hopkins University under the mentorship of James Collins, an expert in bacterial synthetic biology. She also developed the first genetic circuit for mammalian cells. Dr. [redacted] primary research focus is to develop genetic circuits in mammalian cells that control stem cell differentiation. As such, Dr. [redacted] is familiar with prokaryotic and eukaryotic systems and will be an effective mentor as I work to uncover the mechanisms behind a eukaryotic system functioning in a prokaryotic host.

Moreover, this project may require the construction of several genetic circuits. Dr. [redacted] is well-versed in molecular biology techniques and will be a powerful resource as I troubleshoot problems. She has been an impactful mentor to me while working on past projects and continues to be readily available for consultation. I am confident that this research project will allow me to learn much more about synthetic biology from Dr. [redacted] than would otherwise be possible.

Relationship of the Proposed Work to Student's Future Goals

Working in Dr. [redacted] lab for applied synthetic biology has solidified my goals to pursue a career in scientific research. Science has captivated me from a young age, and I plan to pursue this passion after obtaining a Bachelor's Degree in biomedical engineering by completing an MD-PhD program with the end goal of pursuing a career in biomedical research. Working on this project will provide me with valuable experience that will prepare me for a successful future. This project will likely lead to a publication, which will be a noticeable achievement as I apply for graduate schools. Moreover, the experience of working through a project, troubleshooting problems, designing experiments, and analyzing data will further develop my scientific mind.

I envision myself applying synthetic biology to addressing medical problems in my professional career. Though my work in the future may not involve bacterial gene expression, it will necessitate a strong background in molecular biology techniques and research. Investigating the QUAS/QF system in bacteria is an important stepping stone toward a fulfilling career in biomedical research.

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