

Warren J. Baker Endowment

for Excellence in Project-Based Learning

Robert D. Koob Endowment for Student Success



FINAL REPORT

Final reports will be published on the Cal Poly Digital Commons website (<http://digitalcommons.calpoly.edu>).

I. **Project Title:** Leveraging biotechnologies to understand the mechanisms by which California market squid achieve camouflage

II. **Project Completion Date:** 1/15/2018 (project still continues in the Oza Lab, funded by Dr. Oza's startup funds)

III. **Student(s), Department(s), and Major(s)**

- (1) Betina Concepcion, Chemistry and Biochemistry, Chemistry
- (2) Mona Melody Kamranikia, Chemistry and Biochemistry, Biochemistry
- (3) Daniel Sanderson, Chemistry and Biochemistry, Biochemistry
- (4) Logan Williams, Biology, Biology

IV. **Faculty Advisor and Department**

Dr. Javin Oza, Chemistry and Biochemistry

V. **Cooperating Industry, Agency, Non-Profit, or University Organization(s)**

University of California Santa Barbara

VI. **Executive Summary**

The following report details the projects and progress that has been made under the generous funding by the Warren J. Baker Endowment for Excellence in Project Based Learning and the Robert D. Koob Endowment for Student Success. Over the past year, this project has made advancements towards the goal of creating a method that utilizes novel biochemical techniques to isolate an appreciable amount of homogenous Reflectin A1 proteins in their phosphorylated isoforms. Through this project, students at Cal Poly have had the opportunity to apply themselves in novel research and experience the rigor of scientific research.

VII. Major Accomplishments

(1) *Transformation of pGEx-Erk2 into E. coli strain BL21**

The success of this experiment demonstrated that our transformation methods were feasible and ruled out the possibility of inherent procedural errors. At this point, we were unsure whether this bacterial transformation method would work on our genomically recoded *E. coli* (*rE.Coli*) strains as we had not had any success in the past, but it was an important step forward. As an added benefit, this transformed strain was capable of expressing the human protein Erk2, which was used by another team in the Oza lab.

(2) *Cloning of RefA1 gene into pCRT7 expression vector*

a. Development primers for Gibson Assembly

During any downtime while working on cellular transformations, we began work on the cloning portion of our project. We utilized a software called Benchling with an “Assembly” tool that allows us to computationally stitch together genes and desired plasmid backbones along with the primer sequence necessary to execute the Gibson assembly in the lab. After creating the primer sequences, we are able to edit them in order to create the desired experimental conditions. This tool removes a lot of the hard work of primer design and should prove itself to be incredible powerful in the future as we dive further into the Gibson Assembly.

b. Isolation and Validation of Gibson Assembly

A successful Gibson Assembly product of pCRT7-RefA1 plasmid was obtained and validated via sequencing. The validated plasmid was transformed into multiple stocks of *rE.coli* cells for storage as a glycerol stock, and kept in the -80C freezer. This result was the culmination of the summer's work, as the confirmation that we had integrated the RefA1 insert into a pCRT7 backbone enabled the team to begin attempts at the expression of RefA1 from *rE.coli*. Additionally, this confirmation allowed work on the mutagenesis of pCRT7-RefA1 strains with amber codon replacements at phosphorylation sites 1 and 3 on RefA1. It was in these two areas (the expression of RefA1 protein and the mutagenesis of new variants) that the majority of the team's work was focused in the Fall 2017 quarter.

(3) *Educational Experience at UC Santa Barbara*

Our team spent multiple days in the Fall 2017 quarter at University of California, Santa Barbara, shadowing Dr. Morse's research group. Dr. Levenson, staff scientist in Dr. Morse's lab taught us the process of large scale protein expression and isolation of the squid reflectin (RefA1) using HPLC and FPLC methodologies. Additionally, consultation with students in Dr. Levenson's team has enabled our team to refine some of our protocols involving cell culturing and protein expression, all in efforts improve our effectiveness as researchers and increase the quality of our results.

VIII. Expenditure of Funds

Funds were spent primarily on reagents and a laptop for the research team as proposed. Two primary deviations from the budget occurred. First, even though students did travel to UC Santa Barbara as proposed, costs associated with the travel could not be reimbursed through Baker-Koob due to account coming to a close; instead, Dr. Oza reimbursed the travel costs from his startup funds. The \$700 allocated for travel were spent on general Oza Lab purchasing which included the data analysis software package "Sigmaplot". Second deviation to the budget was the purchase of a programmable repeat pipettor to enable students to conduct a higher throughput of experiments with reduced experimental error. This purchase did not displace any required materials listed in the original budget, these required materials were purchased from Dr. Oza's startup funds on an ongoing basis.

IX. Impact on Student Learning

Over the past year, this project has allowed for significant opportunities for multiple students to apply themselves in challenging scientific work. Work in the Oza Lab has allowed for students to apply knowledge gained from course work in a more open-ended setting. This setting has provided students the chance to experience the rigor of scientific investigation that cannot be attained through instructional lab work, while still under the guidance of an experienced mentor. Through this, students have developed a wide range of real-world lab skills (both technical and non-technical), that simply could not be taught in a lecture hall. Following the needs of the project, students learned advanced biotechnology techniques, such as designing specific primers to make mutations to a unique plasmid, and protein purification and clean-up through High Performance Liquid Chromatography and Fast Protein Liquid Chromatography. Students also learned to develop long-term planning skills, as they created schedules to pan out multi-week experiments around the sporadic scheduling of full-time students. Through the collaborative visit to UCSB's Marine Biotechnology Center, students learned how to communicate their research to fellow scientists in their field, as well as how to exchange practical advice with fellow researchers in order to improve the minutiae of their experimental protocols.

The projects conducted in the Oza Lab compound upon the material of Cal Poly's instructional courses, but also provides an education in skills not easily taught through lectures and structured lab work. In this lab, students are pushed to troubleshoot issues through their own critical thinking, whereas this is not possible in teaching labs due to time restrictions. Due to this unique environment, members of the Oza Lab have gained experience in adjusting their own procedures as well as planning out which experiments to conduct on a daily basis in order to best assess and remedy problems. This type of work environment makes students flexible with their planning and comfortable with short term goal adjustments in order to achieve long term success.

Furthermore, work in the Oza Lab has given students the chance to effectively "test out" their choice in career path as a scientific researcher. This opportunity is something that is not openly accessible to most undergraduate students. By spending time in the Oza Lab, students gain real hands-on experience with technical lab skills and non-technical skills, such as planning and organization, that are applicable regardless of their post college endeavours, whether they be industry, graduate education, or other fields entirely.

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Endowment and the Robert D. Koob Endowment. The members of the Oza Lab would like to personally thank the Baker and Koob Endowments for their generous contributions that have led to this exciting and unique opportunity.