I. Project Title
   a. Investigating the mechanisms of tunable camouflage through emerging biotechnologies to produce previously intractable squid proteins

II. Project Completion Date
   a. January 15th, 2020

III. Student(s), Department(s), and Major(s)
   (1) Logan Williams, Biological Sciences, Biological Sciences
   (2) Jason Lorenz, Microbiology, Biological Sciences
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IV. Faculty Advisor and Department
   a. Dr. Javin Oza, Chemistry and Biochemistry

V. Cooperating Industry, Agency, Non-Profit, or University Organization(s)
   a. N/A

VI. Executive Summary
   The below report details the results of the research supported through the generous funding provided 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 our lab has been able to make significant advances towards our goal of creating a high quality expression and purification platform for squid protein Reflectin. Furthermore, we have continued our developments in piloting that platform to synthesize non-standard amino acid incorporating Reflectin to better understand the mechanisms of tunable squid camouflage. In preforming this research, multiple Cal Poly undergraduates have developed skills in experimental planning, troubleshooting, and science communication that could only be developed through undertaking scientific research firsthand.

VII. Major Accomplishments
   (1) The Creation of pUC-RefA1HTag as a More Robust Reflectin Expression System
      a. Addition of a 6XPoly-Histidine Tag to Reflectin
         i. After moving away from the issues associated with the pCRT7 vector, pUC vector became the workhorse for Reflectin expression. While pUC’s improved control over expression and non-toxic inducer reagent increased consistency
of protein expression experiments, protein purification was further improved by adding a 6X poly-histidine ‘tag’ to the C-terminus of the Reflectin protein. This allowed for the purification of Reflectin by immobilized metal affinity chromatography, making it easier to produce large quantities of purified Reflectin for future experiments.

b. Creation of Autoinduction Media
   i. The manual induction of cell culture required to express Reflectin protein was one of the larger procedural obstacles impeding Reflectin research progress. In order to eliminate this procedural hurdle, a pilot autoinduction media recipe was developed that would eliminate the need for lab personnel to directly monitor and induce cell culture to express Reflectin protein.

(2) Development of New Methods in Reflectin Purification
   a. Synthesis of pH-Intervention Technique to Resolubilize Reflectin Protein
      i. Reflectin forms highly insoluble structures known as “inclusion bodies” when expressed in vivo. Resolubilizing these structures is a time-consuming process in most Reflectin research. In order to save time in purifying Reflectin protein from inclusion bodies, we synthesized a novel experimental procedure for resolubilizing inclusion bodies in more acidic conditions before neutralizing the resolubilized protein before downstream purification steps. While still in the development phase, this technique shows promise for decreasing the time required for future Reflectin purification experiments.

(3) Troubleshooting Platforms for Non-Standard Amino Acid Incorporation in Reflectin
   a. Investigating the Viability of SepOTS-λ and B40 Systems for Non-Standard Amino Acid Incorporation
      i. Through extensive expression tests with control protein GFP, we were able to determine that older cell systems using SepOTS-λ and B40 based non-standard amino acid incorporation systems in BL21 E.coli cell lines were incapable of synthesizing non-standard amino acid incorporating protein. While the origin of this inability to produce non-standard amino acid containing protein remains elusive, this discovery has allowed us to switch focus to new non-standard amino acid incorporation systems (including the pSLO system developed in-house by other lab members) that have a higher likelihood of producing recombinant protein.

   b. Preliminary Development of New Reflectin Mutants for Non-Standard Amino Acid Incorporation
      i. In conjunction with the move towards pSLO as a new expression system for non-standard amino acid incorporating protein, new mutant varieties of the Reflectin gene have begun development for use in future experiments. Namely, variants of Reflectin that contain sites for non-standard amino acid incorporation at the protein’s N terminus have been generated, with C terminus variants to follow. These mutant Reflectin varieties will allow for new approaches to understanding mechanisms of Reflectin activity, and enable new investigations in harnessing the capabilities of Reflectin for biotechnologies.

VIII. Expenditure of Funds
Baker Koob Endowment funds were used to purchase:

(1) Purification reagents and dialysis cassettes in order to purify expressed proteins.
Consumables for SDS-PAGE analysis for protein expression

PCR materials to create our mutant library.

DNA sequencing services to validate mutants

Additional miscellaneous reagents for transformation and protein expression, including non-standard amino acids.

Funds initially provided for travel to UC Santa Barbara were no longer needed thanks to teleconferencing. These funds were used instead for the purchase of a laptop used for data collection, analysis, maintenance of a lab electronic notebook, and presentation preparation to communicate research progress.

IX. Impact on Student Learning

The research opportunities enabled by the Baker and Koob Endowments have provided multiple undergraduate students the opportunity to develop their skills as scientists in a manner simply not possible through conventional coursework. More specifically, this research experience has taught students how to perform a range of biochemical techniques, how to thoroughly troubleshoot a complicated system, and how to plan and achieve research goals in a timely manner.

In pursuing the rather complex end goal of this project (the creation of a library of Reflectin mutants to be expressed from a robust system to better understand the mechanisms of Reflectin activity), students have been exposed to a number of varied biochemical techniques. While a conventional lab course may seek to develop student familiarity with a couple aspects of a single extended procedure, this research experience allowed students to cultivate expertise in numerous critical lab protocols in their entirety. Students learned how to culture cells, how to purify protein through immobilized metal affinity chromatography, how to design primers to mutate native DNA sequences, how to validate protein expression results through an SDS-PAGE, how to purify and quantify plasmid DNA, and how to perform a plethora of other lab techniques that will certainly be necessary in their future careers. Furthermore, these students learned how such techniques fit together into a larger experimental workflow, understanding why each step they were performing was necessary to achieve their end goals. This whole-system understanding is only made possible when one is exposed to the long-term aims of an undergraduate research progress, and thus could only be taught through the experience funded by this grant.

Understanding the larger experimental workflow and purpose also allowed students to engage in developing a critical scientific skill often neglected in conventional lab courses: troubleshooting your experiment. Given the constraints of time and course material that most lab courses have, it is typically impossible to investigate abnormal experiment results to understand their origins. However, troubleshooting skill is instrumental in the success of any scientist, and our students were allowed to develop troubleshooting aptitude through their research experience. When experiments failed or produced abnormal results, the less-structured nature of a research project allowed students the opportunity to develop their own troubleshooting experiments to explore why their previous efforts might have failed. Whether the subject matter was a failed protein expression, PCR, or transformation, students were frequently challenged to think deeper about why their experiments might have failed, and in so doing came to understand the intricacies of their experiments on a deeper level.

Additionally, it is worth noting how valuable this research experience has been in allowing undergraduate students to develop their skills in experimental planning and team communication. While still under the watchful guidance of a Principal Investigator, students were given the freedom to plan their own schedules and supervise their own experiments. This required that students learn to plan their protocols well in advance, to ensure that they would have the necessary time and materials to complete their protocols in the timeframe desired. This also taught students the importance of coordinating with one’s colleagues, as it
was often the case that a single experiment would be started by one researcher before being handed off to another researcher for completion. Being sure to communicate effectively with one’s peers was paramount to research success. By placing students in an environment where planning and communication were key, students were able to cultivate workplace skills that will benefit them immensely in the years to come.

All of this learning and development was made possible through the donations of the Warren J. Baker Endowment and the Robert D. Koob Endowment. The members of the Oza Lab would like to offer our personal thanks to the Baker and Koob Endowments for enabling this Learn-by-Doing experience that immensely improved our skills as undergraduate scientists, and has helped each of us discover our own passions as future scientists.