Warren J. Baker Endowment
for Excellence in Project-Based Learning
Robert D. Koob Endowment *for Student Success*

CAL POLY

Proposal Cover Page

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

Proposal Author: Betina Concepcion **Cal Poly Email:** bconcepc@calpoly.edu

Student ID: 009884559 **Signature (Optional):**
Signature provides permission to check financial aid eligibility.

Previous Baker/Koob Endowment funding? (circle one): **Yes** **No**

Team Member(s)	Signature	Cal Poly Email	Department
Betina Concepcion		bconcepc@calpoly.edu	Chemistry and Biochemistry
Mona Kamranikia		mkamrani@calpoly.edu	Chemistry and Biochemistry

Faculty Advisor: Javin P. Oza **Department:** Chem & Biochem

Faculty Advisor email: joza@calpoly.edu **Telephone:** 805-259-5440

Anticipated Start Date: Jan 9, 2017

Anticipated End Date: Dec 15, 2017

Total Funds Requested ($): 4,720.79

Signature of Faculty Advisor: **Date:** 11/14/16

PROPOSAL NARRATIVE

I. Project Title:

Leveraging biotechnologies to understand the mechanisms by which California market squid achieve camouflage

II. Abstract

The California market squid has specialized skin that is able to manipulate light through reversible condensation and assembly of reflectin proteins, allowing the squid to tune its skin iridescence for precise camouflage and underwater communication. Differential phosphorylation of reflectin proteins is observed to be contaminant with the reversible condensation and hierarchical assembly of reflectin proteins. Our mechanistic understanding of the role of protein phosphorylation in reflectin-mediated iridescence remains incomplete due to the inability to obtain large quantities (>1mg) of a homogenous population of phosphorylated reflectins. To overcome this limitation, we propose to leverage state-of-the-art biotechnologies. These include 1) an engineered *E. coli* strain with an expanded genetic code and 2) an orthogonal translation system capable of site-specific incorporation of phosphoserine into proteins. We will aim to mimic the squid by incorporating phosphoserine into reflectin in a genetically encoded manner. To accomplish this, *the learning-objectives-by-doing in this interdisciplinary project will be: cloning the reflectin A1 gene, constructing mutants, protein expression in a bacterial system, and purification of site-specifically phosphorylated variants of reflectins.* Our work will provide the foundation for elucidating the role of phosphorylation in reflectin-mediated camouflage in marine organisms.

III. Introduction

The iridescence in the California market squid (*Loligo opalescens*) is achieved by unique protein biochemistry capable of manipulating light. The proteins responsible for this phenomenon have been discovered *by scientists at UC Santa Barbara [1], with whom we will collaborate for this project*. These proteins, reflectins, are particularly interesting because they are able to sustain iridescence in a reversible manner, allowing the squid to repeatedly change its appearance. It is found that the reversible nature of this phenomenon is explained by the reflectin proteins' capacity to transition from a soluble state to an aggregated state in a reversible manner. It is hypothesized that modification of reflectins, called phosphorylation, may be the driver of reflectin aggregation. Phosphorylation has been observed to modulate the activity of thousands of proteins in nature, so this is a plausible mechanism by which

squid tune their appearance.
Testing this hypothesis has not been trivial since our capacity to obtain phosphorylated reflectin proteins is technically limited. We aim to use state-of-the-art biotechnologies to overcome this limitation – through the use of an expanded genetic code which allows us to install phosphates into proteins in a site-specific manner. Accomplishing this would allow us to mimic the reflectins as they exist within the squid, and therefore would allow us to evaluate the mechanisms by which the squid tunes reflectin aggregation to achieve camouflage.

IV. Objective(s)

Our learn-by-doing objectives are:
- To clone the wildtype Reflectin A1 into the pCRT7 expression vector
- To create mutants of Reflectin A1 that contain the UAG-Amber codon for phosphoserine incorporation
- To optimize expression of Reflectin A1 in the genomically engineered *E. coli* strain capable of phosphoserine incorporation
- To express and purify Reflectin A1 and its phosphorylated variants.

V. Methodology

We have selected Reflectin A1 due to its prominence in the squid Bragg lamellae, capacity for reversible condensation, and numerous predicted sites of phosphorylation. The wildtype reflectin A1 gene will be cloned into the pCRT7 phosphoprotein expression vector using the Gibson assembly method for cloning. Site-directed mutagenesis will be used to incorporate Amber codons at previously-identified *in vivo* phosphorylation sites. To accomplish this, we will design primers that ingrate desired changes in the gene in a site-specific manner. Plasmids containing the wildtype and mutant reflectin A1 genes will be transformed into the genomically recoded *E. coli* strain C321.ΔA.ΔSerB along with the SepOTS plasmid for phosphoprotein expression *in vivo*. We will optimize expression of phospho-reflectin A1 through conditions including time and temperature of protein expression. Reflectin A1 is expected to be partitioned into *E. coli*'s inclusion bodies, and proteins will be purified from this fraction of the cell lysate.

VI. Timeline

Winter 2017: Clone Reflectin A1.
Spring 2017: Generate mutants of Reflectin A1.
Summer 2017-Fall 2017: Optimize expression and purification of Reflectin A1.

VII. Final Products and Dissemination

Final products of our work will be:
- Purified Reflectin A1 and its phosphorylated variants

We will disseminate our product through material transfer to our collaborators at UC Santa Barbara who will conduct experiments to evaluate the role of protein phosphorylation on Reflectin A1's tunable iridescence. We will also disseminate our accomplishments to the field through presenting at the national American Chemical Society conference as well as the American Society for Biochemistry and Molecular

Biology upon completion. Our department has committed to funding our travel to present at conferences. We also aim to publish our results in a peer-reviewed journal.

VIII. Budget Justification

We are requesting funds for three primary categories, 1) reagents and consumables to execute the proposed work, 2) travel expenses for three over-night trips to Santa Barbara to work with our collaborators, 3) services, and 4) a computer to be used solely for research purposes.

Reagents and consumables: The reagents and consumables we are requesting will sustain our interdisciplinary work which spans microbiology, molecular biology, and protein biochemistry. We are not requesting equipment as we will use existing infrastructure in the Department of Chemistry & Biochemistry to execute our work. A significant faction of this budget is dedicated toward the detection of phosphorylated reflectin proteins to validate our final product through corroborating assays.

Travel: Our interdisciplinary and collaborative effort will require us to travel to UC Santa Barbara to work in the lab to Prof. Dan Morse given that they have infrastructure and expertise that we do not. We anticipate three trips to Santa Barbara, each comprising 2-days and 1-night in duration for intensive efforts in protein expression and purification. Travel funds will allow us to pay for gas and hotel accommodations, as well as meal expenses.

Services: We will require external services for the synthesis of DNA primers for mutagenesis, and subsequent DNA sequencing for validating that we have generated the desired reflectin mutants.

Computer: We are also requesting funds for a simple computer for our research efforts. This will enable us to keep an electronic notebook, to keep up with journal articles, prepare presentations, and prepare reports for our proposed project.

Budget			
	cost	quantity	subtotal
Reagents/Consumables			
Phos-tag Biotin	328.22	2	656.44
Streptavadin-HRP conjugate	206.3	2	412.6
anti-phosphoserine antibody	319	1	319
Yeast Extract	97.53	1	97.53
Tryptone	118.15	1	118.15
Glucose	24.25	1	24.25
Ampicillin	33.86	1	33.86
kanamycin	16.26	1	16.26
Anhydrotetracycline	134.92	1	134.92
HEPES	42.16	1	42.16
O-phospho-L-Serine	75	1	75
Acrylamide	32.41	1	32.41
protein purification misc	600	1	600
Electroporation Cuvettes	92.55	1	92.55
Competent cells	82.83	2	165.66
Travel to Santa Barbara			
Hotels, gas, food			700
Computer for research lab			800
Services			
DNA/primer synthesis			200
DNA sequencing			200
Total			**4720.79**

IX. Bibliography

1. Levenson R., Bracken C., Bush N., Morse DE., (2016) J Biol Chem 19;291(8):4058-68

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PROPOSAL BUDGET

Student Applicant(s): **Betina Concepcion** **Mona Kamranikia**	
Faculty Advisor: Javin P. Oza	
Project Title: Leveraging biotechnologies to understand the mechanisms by which California market squid achieve camouflage	**Requested Endowment Funding**
Travel *subtotal*	**$700**
Travel: In-state	**$700**
Travel: Out-of-state	**$0**
Travel: International	**$0**
Operating Expenses *subtotal*	**$ 3,620.79**
Non-computer Supplies & Materials	**$2,820.79**
Computer Supplies & Materials	**$800**
Software/Software Licenses	**$**
Printing/Duplication	**$**
Postage/Shipping	**$**
Registration	**$**
Membership Dues & Subscriptions	**$**
Multimedia Services	**$**
Advertising	**$**
Journal Publication Costs	**$**
Contractual Services *subtotal*	**$400**
Contracted Services	**$400**
Equipment Rental/Lease Agreements	**$**
Service/Maintenance Agreements	**$**
TOTAL	**$4,720.79**

CAL POLY

California Polytechnic State University
San Luis Obispo, CA 93407

Chemistry and Biochemistry Department
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Dear Baker & Koob Endowment committee,

I am excited to write a letter of support for the project led by Betina Concepcion and Mona Kamranikia. They are proposing to pursue a previously intractable problem in protein biochemistry – to generate phosphorylated proteins in a predictable, site-specific manner. Through this effort, they will generate phosphorylated variants of the squid protein Reflectin A1. *This has never been done before.* The phosphorylation of proteins is a ubiquitous phenomenon employed by many organisms to control functions of proteins in a rapid and dynamic manner. Mimicking this biochemical process in the lab has been limited by technology and resulted in many unanswered questions in cellular biochemistry. The proposed project aims to utilize biotechnologies that my lab is established at Cal Poly to overcome this limitation. In brief, we are able to utilize an *E. coli* strain with an expanded genetic code to include phosphorylation events into the protein while it is being synthesized. Betina and Mona will use this capacity to mimic phosphorylation of squid reflectin proteins for the first time.

The proposed project builds on recent discoveries at UC Santa Barbara by the lab of Prof. Dan Morse, which have shed light on the biochemistry by which the California market squid achieves tunable iridescence (e.g. camouflage). Outcomes of the proposed work will enable the field to test the hypothesis that reflectin A1 phosphorylation enables the reversible protein condensation, which results in iridescence. Therefore, Mona and Betina will collaborate with Prof. Dan Morse's lab throughout their project to enrich their success.

To accomplish the proposed work, Betina and Mona will master basic methods in microbiology, molecular biology, and protein biochemistry as well as state-of-the-art methods in molecular cloning and protein expression. This project will also require them to master scientific literacy and science communication. Therefore, the proposed project is a wonderful platform for them to learn-by-doing. I believe that Mona and Betina possess the abilities to successfully execute the proposed work. Through summer research efforts, they have demonstrated the qualities of a successful student – the drive to learn, the drive to excel, commitment and persistence to succeed. Furthermore, they have committed a minimum of 10 hours per week to conducting this research during the academic year, plus full time in the summer. The funds that are requested in the proposal appear sufficient to accomplish the proposed work. The department of Chemistry & Biochemistry is dedicated to undergraduate research and provides research space and equipment. My lab is housed in the Center for Applications in Biotechnology, and we are equipped with high caliber instrumentation necessary to execute most of the proposed work. Protein purification expertise remains at UC Santa Barbara, so students will travel to Santa Barbara to execute key steps in the lab of Prof. Dan Morse in a collaborative capacity.

The proposed project is aligned with the core competencies of my lab and those of the collaborating of Prof. Dan Morse at UC Santa Barbara. Given the funding and the opportunity, the chances of success are high for this team and their proposed work.

Mona and Betina are intelligent women with strong intrinsic motivation to succeed, and I am happy to support them on their scientific journey.

Thank you for your consideration,

Javin P. Oza
Assistant Professor
Department of Chemistry & Biochemistry