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:
Elucidating the structure-function relationship of human protein kinase MEK1

Proposal Author: **Rebecca Lee** **Cal Poly Email: rlee83@calpoly.edu**
Leandre Ravatt **Cal Poly Email: lravatt@calpoly.edu**

Student ID: **011786329** **Dept.: Biological Sciences**
013127435 **Dept.: Chemistry and Biochemistry**

Signature (Optional):

Signature provides permission to check financial aid eligibility.

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

Is this request to support a Senior Project or thesis? (circle one): Yes **No**

Team Member(s)	Signature	Cal Poly Email	Department
Rebecca Lee		rlee83@calpoly.edu	Biological Sciences
Leandre Ravatt		lravatt@calpoly.edu	Chemistry and Biochemistry

Faculty Advisor: Javin P. Oza **Department:** Chemistry and Biochemistry

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

Anticipated Start Date: Jan 8, 2017 **Anticipated End Date:** Dec 15, 2017

Total Funds Requested ($): 3856.56

Signature of Faculty Advisor: **Date:** 11/6/17

PROPOSAL NARRATIVE

I. Project Title:

Elucidating the structure-function relationships of the human protein kinase MEK1

II. Abstract

MEK1 is an ideal drug target for the therapeutic treatment of cancerous mutations that occur in the MAPK phosphorylation cascade, one of the most common cell proliferation signaling pathways. The study of the specific phosphorylation event between MEK1 and its only known substrate ERK2 has previously been limited due to the difficulty in obtaining the active phosphorylated form phospho-MEK1. Our study aims to overcome this barrier by utilizing the novel technology of the expanded genetic code to produce site-specifically phosphorylated proteins. In order to elucidate the structure-function relationships of MEK1, we are pursuing a hypothesis-driven, structure-guided approach based on conserved kinase motifs for the creation of variants using site-specific mutagenesis. We will optimize immunoassays in order to quantify the enzymatic activity of both the wild-type and mutant forms of MEK1. We will subsequently characterize the enzyme kinetics of MEK1 mutants to deduce the significance of specific residue interactions in the phosphorylation event. Our findings will be the first to establish kinetic parameters for the wild-type phospho-MEK1, further the understanding of MEK1's structure-function relationships in the cell signaling event, and enable the development of more effective MEK1 inhibitors for the treatment of cancerous mutations.

III. Introduction

90% of cancerous melanomas are the results of mutations in the MAPK phosphorylation cascade. The interactions between MEK1 and its substrate ERK2 results in a bottleneck in the cascade, through which all signals must pass, making MEK1 an ideal drug target. However, study of MEK1 has not been trivial, considering that MEK1 is activated upon phosphorylation in the cell, a process which is difficult to replicate using traditional protein expression methods. This limits access to homogenous, active MEK1 samples for biochemical characterization and creates an obstacle in our ability to study and establish kinetic parameters for the active form of MEK1. Our project addresses this barrier by implementing the novel technology of the expanded genetic code to install phosphoserine directly during translation, combining molecular biology with traditional protein biochemistry.

IV. Objective(s)

Our objectives to overcome the field's limitation are:

1. To combine traditional protein expression techniques with an expanded genetic code to produce the active phosphorylated MEK1
2. Develop and conduct kinase activity assays in order to establish the kinetic parameters of MEK1
3. Pursue hypothesis-driven site-specific mutagenesis of the wild-type phosphorylated MEK1 for the elucidation of the kinase's structure-function relationships

V. Methodology

Currently, the technical difficulty in incorporating phosphates into MEK1 in a site-specific manner represents a barrier in our ability to study the structure-function relationships of the kinase. Our project overcomes this limitation by utilizing an expanded genetic code, which leverages an orthogonal translation system to install phosphoserine in response to the Amber codon (UAG). We have combined this biological machinery with traditional *in vivo* protein synthesis for the production of active, phosphorylated MEK1.

Concurrently, we are implementing a hypothesis-driven approach to elucidating the structure-function relationships of the kinase. Based on conserved motifs and current understanding of the protein, we are mutating residues of interest to alanine using site-specific mutagenesis to alanine.

Upon expression of the wild type and mutant variants, we will use an optimized 96-well dot blot assay for the evaluation of MEK1's kinetic parameters. We will subsequently characterize the kinetics of the MEK1 mutants to identify residues vital to ERK2 binding and phosphorylation.

VI. Timeline

Winter 2018:

- Create phospho-MEK1 mutants based on literature knowledge and conserved kinase motifs

Spring 2018

- Express and purify wild-type and mutant phospho-MEK1 variants
- Optimize 96-well dot blot parameters for MEK and ERK detection

Summer 2018:

Conduct baseline kinase activity assays with wild type phospho-MEK1

Conduct kinase activity assays of phospho-MEK1 mutants

VII. Final Products and Dissemination

Final products of our work will be:

- Purified, phosphorylated MEK1 and hypothesis-driven mutants
- Evaluation of the structure-function relationships of MEK1 and the nature of its interaction with its substrate ERK

Upon completion of the Baker-Koob funded research, we will disseminate our findings mainly through presentation at national conferences such as the 2019 American Chemical Society (ACS) and American Society for Biochemistry and Molecular Biology (ASBMB) conferences. Additionally, we intend to publish our results in a peer-reviewed journal. The studies conducted in this project will also be incorporated into Rebecca Lee's senior research project.

VIII. Budget Justification

Budget			
	cost	quantity	subtotal
Misc. Reagents/Consumables			
Phos-tag Biotin	328.22	1	328.22
Anhydrotetracycline	134.92	1	134.92
HEPES	42.16	1	42.16
Ammonium Persulfate	18.49	1	18.49
Acrylamide	32.41	2	64.82
TEMED	20.42	1	20.42
Protein Purification			
Ni-NTA resin (100 ml)	570.31	1	570.31
Dialysis Cassetes	129.61	2	259.22
Antibodies			
Anti-phospho-MEK1	399	1	399
Anti-MEK1	405	1	405
Anti-Phospho-ERK2	389	1	389
Anti-ERK2	425	1	425
Services			
DNA/primer synthesis			300
DNA sequencing			500
Total			**3856.56**

We are requesting funds to cover the expenses associated with reagents and consumables required to conduct the proposed work. The proposed work requires specialized reagents such as **antibodies** for wild-type and phosphorylated versions of the proteins MEK1 and ERK2. These antibodies will allow us to detect and quantify enzymatic reactions. Since our proposed work will require us to generate mutations in the enzyme MEK1, we will require **DNA synthesis and sequencing services**. We have negotiated discounted rates to maximize our budget. Since we aim to generate several MEK1 mutants, we will require larger quantities of **protein purification reagents** to accommodate our workflow. The remaining **miscellaneous reagents** will be used for protein expression and SDS-PAGE analysis. This is not the full list of reagents our project will require; we will use existing resources in the Oza Lab to support much of our work. These reagents will include cloning and mutagenesis reagents/enzymes, micro- and molecular biology reagents, protein characterization and dot blot assay reagents. The Oza Lab and the Center for Applications in Biotechnology at Cal Poly will also provide access to several key pieces of equipment including incubators, images, spectrophotometers, and many others.

IX. Bibliography

1. Roskoski, Robert. "Allosteric MEK1/2 Inhibitors Including Cobimetanib and Trametinib in the Treatment of Cutaneous Melanomas." Pharmacological Research, vol. 117, Mar. 2017, pp. 20-31. EBSCOhost, doi:10.1016/j.phrs.2016.12.009.
2. Roskoski, Robert. "MEK1/2 Dual-Specificity Protein Kinases: Structure and Regulation." *Biochemical & Biophysical Research Communications*, vol. 417, no. 1, 06 Jan. 2012, pp. 5-10. EBSCO*host*, doi:10.1016/j.bbrc.2011.11.145.
3. Pirman, Natasha L.; Barber, Karl W.; Aerni, Hans R.; Ma, Natalie J.; Haimovich, Adrian D.; Rogulina, Svetlana; Isaacs, Farren J.; Rinehart, Jesse (2015-09-09). "A flexible codon in genomically recoded Escherichia coli permits programmable protein phosphorylation". *Nature Communications*.
4. Oza, Javin P.; Aerni, Hans R.; Pirman, Natasha L.; Barber, Karl W.; ter Haar, Charlotte M.; Rogulina, Svetlana; Amrofell, Matthew B.; Isaacs, Farren J.; Rinehart, Jesse (2015-09-09). "Robust production of recombinant phosphoproteins using cell-free protein synthesis". *Nature Communications*.

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PROPOSAL BUDGET

Student Applicant(s): Rebecca T. Lee Leandre Ravatt		
Faculty Advisor:	Javin P. Oza	
Project Title: Elucidating the structure-function relationships of the human protein kinase MEK1		**Requested Endowment Funding**
Travel	*subtotal*	**$0**
Travel: In-state		**$0**
Travel: Out-of-state		**$0**
Travel: International		**$0**
Operating Expenses	*subtotal*	**$ 3056.56**
Non-computer Supplies & Materials		**$3056.56**
Computer Supplies & Materials		**$**
Software/Software Licenses		**$**
Printing/Duplication		**$**
Postage/Shipping		**$**
Registration		**$**
Membership Dues & Subscriptions		**$**
Multimedia Services		**$**
Advertising		**$**
Journal Publication Costs		**$**
Contractual Services	*subtotal*	**$800**
Contracted Services		**$800**
Equipment Rental/Lease Agreements		**$**
Service/Maintenance Agreements		**$**
	TOTAL	**$3856.56**