

Undergraduate Research Grants Application Form

Due May 7, 2012

1. Student Information

First Name Ngoc-Han Last Name Tran

Major Biological Sciences Student ID# ~~992299~~

Email Address ~~ngoc.han@sjcsu.edu~~ Phone ~~(408) 924-5283~~

Class Standing: Freshman Sophomore Junior Senior

GPA ~~3.8~~ Have you been the recipient of this award before? Yes No

2. Faculty Information

First Name Lionel Last Name Cheruzel

Email Address lionel.cheruzel@sjcsu.edu Phone (408) 924-5283

Department Chemistry College/School College of Science

3. Project Information

Title of Project Characterization of Light-Activated Hybrid P450 BM3 Enzymes as Biocatalysts

Project Timeframe: Start Date 08/01/12 End Date 05/30/13

Will this project use (check all that apply)

- Animal Subjects
- Biohazards/Human Blood
- Human Subjects
- Recombinant DNA
- Radiation/Isotopes/Lasers
- Controlled Substances

TO BE COMPLETED JOINTLY BY THE STUDENT AND THE FACULTY MENTOR:

In a few sentences, describe the goals of the research or creative project you will be working on together.

In response to a growing interest in technology that utilizes renewable energy in order to reduce the dependence on fossil fuels, Dr. Cheruzel's research laboratory has focused on developing biocatalysts from hybrid P450 enzymes capable of selective oxidation of organic substrates using sunlight and dioxygen. The oxidation of an unactivated C-H bond, while being essential in many synthesis pathways, is a very challenging reaction to perform in the laboratory. Cytochromes P450 are heme-thiolate enzymes capable of selectively catalyzing such oxidation reaction utilizing two reducing equivalents and molecular dioxygen, which makes their use as biocatalysts highly desirable.

We have previously demonstrated the successful engineering of various hybrid P450 BM3 enzymes constituting of a photosensitizer covalently attached to P450 BM3 heme domains. These enzymes are capable of selectively hydroxylating lauric acid under visible light activation with high total turnover numbers. Interestingly, the mutants in our series of hybrid enzymes display large differences in activity toward the hydroxylation of lauric acid despite very small structural changes.

The goal of this project is to rationalize these observed differences by monitoring and studying various properties of the hybrid enzymes such as: 1) enzyme stability under heat degradation, 2) electronic property of the P450 BM3 heme active site, 3) energy transfer and kinetic of electron transfer between the photosensitizer compound and the heme active site, and 4) stability of reaction intermediates under anaerobic environment.

Findings from these characterization techniques would provide new insights into the hybrid enzymes mechanism and aid in the design of future hybrid P450 BM3 mutants in order to optimize their catalytic activity under light activation.

These hybrid enzymes could be a valuable alternative approach for biotechnological applications using light to initiate hydroxylation reactions.

Enumerate the general activities the **student** will perform as part of their participation in this project. Provide a rough timeline.

As part of this project, the student will conduct the following experiments to characterize the hybrid P450 BM3 enzymes in order to gain insights into their mechanism. Within the allocated timeline indicated, the proposed experiments consist of:

1. Preparation of samples and solutions at known concentration for quantitative measurements
2. August 2012-October 2012: UV-Vis spectroscopic monitoring of enzyme stability, formation of reaction intermediates and spectroscopic profiles of the hybrid enzymes using a newly acquired UV-Vis spectrophotometer equipped with a temperature controller unit available in Dr. Cheruzel's laboratory.
3. November 2012-February 2013: Using luminescence spectroscopic techniques on a fluorimeter instrument available in the chemistry department's core facility to determine characteristic properties of the photosensitizer and the heme active site.
4. March 2013 - May 2013: Monitoring the formation and decay of reaction intermediates under oxygen-free environment using an anaerobic chamber.
5. September 2012 - February 2013: Kinetic measurements to measure rate of formation of reaction intermediates and more importantly rate of electron transfer in the hybrid enzyme system.

While being familiar with many techniques in the laboratory as well as with handling the hybrid enzymes, the student will take on new challenges in learning many spectroscopic techniques to widen her portfolio. The student will have a full-time commitment to the project while school is not in session and a part-time commitment during the academic year.

Enumerate the general activities the **faculty mentor** will perform as they supervise or guide the student throughout this project.

Since Dr. Cheruzel's hire in the department of Chemistry at San Jose State University in Fall 2009, his research laboratory has been devoted to the development of hybrid P450 BM3 enzymes for the selective hydroxylation of organic substrates. Our early success in the development of the hybrid enzymes has led to two publications in peer-reviewed journals with SJSU undergraduates as co-authors. These publications established the feasibility of the proposed project as well as Dr. Cheruzel's ability to train and mentor undergraduate students.

From his training, the faculty mentor has all the expertise in the spectroscopic techniques. The faculty mentor will train the student on specific techniques and guide the student through the experimental design, data collection and data analysis while ensuring the student's safety. The faculty mentor will also ensure that the student is provided with any equipment and materials needed for the completion of the project. Participation of the faculty mentor in this project also includes active discussion with the student about experiments to be conducted, results obtained from experiments already completed, interpretation of the data and relevance of finding in context with literature and theoretical principles.

The faculty has full confidence in Ngoc-Han's ability to perform the proposed experiments and believe that involvement in this project would greatly broaden her knowledge as she is gearing up to pursue her educational and career goals.

How will the student's participation in this project contribute to their educational and/or career goals?

The student's educational and career goal is to obtain a PhD in molecular biology and conduct research in health-related topics. The student's participation in this project will undoubtedly provide valuable experience for the student, which will be a tremendous asset for the student in further career trainings especially with spectroscopic techniques. The interdisciplinary nature of the research project will allow the student to apply materials learned in classes and acquire new knowledge. Aside from many essential project-specific skills directly applicable to the student's future area of research, the student will learn other crucial skills in the general research approach with designing and conducting experiments as well as in analyzing and presenting data. The student will be able to attend scientific meetings and is expected to present the results of this project both in writing and orally. The student will also learn to interact with the faculty mentor and other members of the large research group.

Overall, participation in this project will strengthen the student's resume as a candidate for graduate programs. This project will also prepare the student for graduate school environment as well as increase the student's chance of success in graduate school and well beyond.

4. Budget and Justification (Budget itself to be submitted on Budget Form)

Explain how the funds requested for this project will be expended. Grant funds can be used to cover project related software, equipment, materials, supplies, travel, data collection and analysis, and other project related expenses. Up to one half the grant can be used as student assistant funds. Funds may not be used for faculty compensation or travel or to purchase food.

One third of the budget (\$350) will be dedicated to provide travel funds and registration fee for the student to attend a scientific meeting in Spring/Summer 2013 in order to present her findings from this study. The targeted meetings are either the American Chemical Society National Meeting held in New Orleans in April 2013, or the 18th International Conference on Cytochrome P450 held in Seattle in June 2013. Attending either of these meetings will be a great opportunity for the student to showcase her work and will also be an invaluable educational experience for the student.

The remainder of the funds will be used to purchase supplies necessary for conducting the proposed research. The supplies include UV-Vis cuvettes (\$200) and optic filters (\$200) for the fluorimeter and the UV-Vis spectrophotometer. Pipet and pipet tips (\$250) will also be purchased to handle the protein and to prepare solutions of known concentration.

5. Signatures

Student Signature

[Redacted Signature]

Date

05-02-2012

Faculty Signature

[Redacted Signature]

Date

05-02-2012

Department Chair
Signature

[Redacted Signature]

Date

5-2-12

Save & Print Form

Submit completed application with supporting documents to the Center for Faculty Development (IRC 213) or email to cfcd@sjsu.edu no later than 5:00 p.m. **Monday, May 7, 2012.**

Undergraduate Research Grant Budget Form

Project Title

Characterization of Light-Activated Hybrid P450 BM3 Enzymes as Biocatalysts

Budget item	Quantity	Unit Cost	Total Cost
Supplies			
UV-Vis cuvettes	2	\$100.00	\$200.00
Optic fibers	2	\$100.00	\$200.00
Pipet	1	\$150.00	\$150.00
Pipet tips	2	\$50.00	\$100.00
			\$0.00
			\$0.00
Travel			
Air plane ticket to conference	1	\$250.00	\$250.00
Conference registration fee	1	\$100.00	\$100.00
			\$0.00
			\$0.00
Other			
			\$0.00
			\$0.00
Student Assistant Funds Specify number of hours and hourly rate			\$0.00
Total Costs (may not exceed \$1000)			\$1,000.00

Include separate lines for software, equipment, materials, supplies, travel, data collection and analysis. You may also use the "other " line to insert additional lines as needed.