LONG TERM GRANT APPLICATION
Cover Sheet

Amount Requested: $1018.95

Project Information

Belnap, Deborah
Geilmann, Shelby

Student Participant (Last, First)

Investigating the Inhibition of APEH in Disease

Project Title (10 words or less)

Covey, Tracy	2503
Faculty Mentor Name (last, first)	Mail Code
College of Science	Chemistry and Biochemistry
College (Weber State is the University, NOT college)	Department

This project ___ DOES/__X_ DOES NOT require review by the WSU Institutional Review Board for Human Subjects or the WSU Animal Care and Use Committee.

Student Signature — 11/2/18 Date

Project Mentor Signature — 11-2-18 Date Received by Mentor. Must be 10 business days before final deadline.

2503 Campus Mail — x8665 Phone Ext.

Undergraduate Research Committee Representative — 11-2-18 Date Received by URC Rep. Must be 5 business days before final deadline.

Faculty Mentor Department Chair — 8 Nov 2018 Date

Please check if attended Research Proposal Workshop:

☑ DATE WORKSHOP ATTENDED **OCTOBER 23, 2018**

LONG TERM GRANT APPLICATION
Budget Worksheet

BUDGET ITEM	Department or College Funds	Outside Agency Funds	Personal Funds	Undergrad. Research Funds	GRAND TOTAL
Materials	Amino Acids and Proteins $700 BCA Assay Kit $180.00 Western Blotting material $107 Solvents and Buffers $50			Lipid Peroxidation Products $565.00 Protein Isolation DEAE columns $270.00 Cell Culturing Reagents $133.95 Shipping $50	$2055.95
Equipment					
Research Scholarship (max request $2,500.00)					
Mileage to gather Data (.38 per mile)					
GRAND TOTAL	$1037			$1018.95	$2055.95

Project Description

(Approximately 2 pages)

All living cells contain proteins that function as catalysts to help speed up biochemical reactions. These proteins are called enzymes. There are several types of enzymes, each with their own function. An enzyme of interest to us is called APEH (acyl-peptide enzyme hydrolase). APEH works to degrade damaged proteins by cleaving peptides so that amino acids, the building blocks to proteins, can be recycled and reused to make other proteins. This plays an important role in clearing the cell of unnecessary and possibly harmful materials. Previous studies have found that the activity level of APEH is decreased in certain diseases such as Type II diabetes (Covey, et al., 2018) and Alzheimer's disease (Palmieri, et al. 2017). Decreased APEH activity may lead to an increase of oxidative stress that promotes disease progression. **This project involves investigating the mechanism of APEH inactivation in disease.**

To start, we used online prediction programs and computer modeling to develop our hypothesis: APEH is inactivated by lipid peroxidation products that are produced at high levels in disease. Cysteine is a reactive amino acid that is often covalently modified by these unwanted products in disease. Online prediction programs suggest that APEH has a reactive cysteine and molecular modeling illustrated several cysteine amino acids located in the active site of APEH (Figure 1). We predicted that unwanted lipid peroxidation products can bind to a cysteine in APEH active site and cause its activity to go down.

Figure 1: Cysteine amino acids near the active site of APEH (Figure built in Maestro)

We began by testing APEH activity in the presence of different lipid peroxidation products to see if any of them decreased the activity of APEH. Disease and oxidative stress increase the production of

reactive free radicals in the cell that create these peroxidation products (Sayre, et al., 2002). We tested several unwanted lipid peroxidation products and found that APEH activity is inhibited with Hydroxynonenal (HN), Hydroxyhexanal (HX), and Prostaglandin (PG) (Figure 2). Based on computer modeling and our APEH activity results, we predict that lipid peroxidation products of a certain size and reactivity will inhibit APEH.

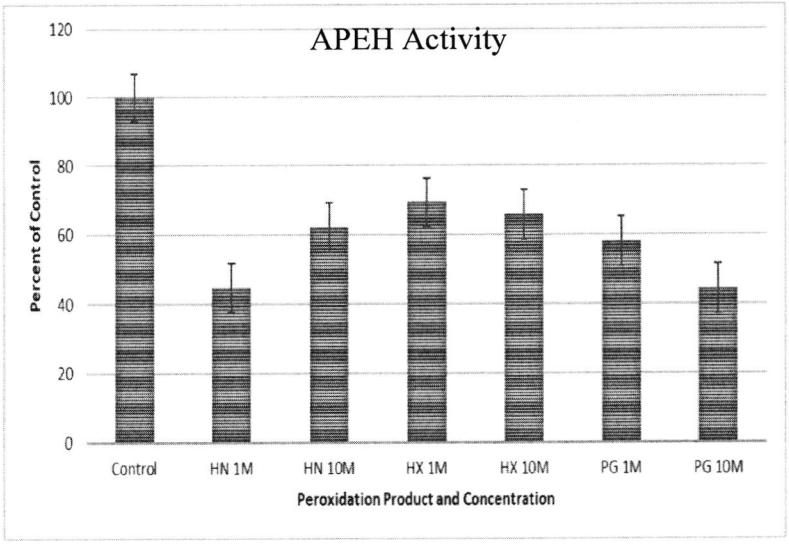

Figure 2. APEH activity in the presence of lipid peroxidation products

The next step in this project will be to test the inhibitory ability of different molecules similar in size to Prostaglandin, but different in their reactivity to cysteine. If these products do not inhibit APEH, that will support our hypothesis about Cysteine being the reactive amino acid in the enzyme (Fowler, Blanford, de Visser, & Warwicker, 2017). We also plan on isolating APEH to make sure that the inhibition is coming from this enzyme and not some other factor, since the HeLa cells we have been testing this on have multiple proteins contained inside them. Information about these chemicals will be helpful in adding to the breadth of our knowledge regarding this enzyme and the relating diseases. It is also possible that APEH activity could be used as a potential biomarker in diseases like Type II Diabetes in the future.

We plan on presenting our research at the Utah Conference of Undergraduate Research as well as the Weber State Undergraduate Research Symposium, both of which are held at Weber State University in the Spring of 2018. Once completed, we will publish our work in a peer reviewed biochemistry journal.

Our mentor Dr. Tracy Covey introduced the necessary background knowledge that we used for this project, as well as trained us in the methods we used. Dr. Covey has done previous experiments and research on APEH, and the research we have done will add to the cumulative data on the subject. Both of our science and chemistry backgrounds have aided in our ability to carry out this project. We began the project in May of 2018, meeting twice a week for three hour blocks each time and continued the work throughout the Summer semester. We have continued the project into Fall semester, testing for synergy and planning for the next steps, such as researching new chemicals to test and ways to isolate our enzyme, as described previously. This project has benefitted our understanding of enzymes and their role in disease, as well as improved our lab techniques and project planning. This experience will be valuable for both of us as we move forward in our education and future careers in science. Thank you for considering our proposal.

Dependent _____X_____ Independent
(student helping faculty do research) (student doing own research)

Project Methods & Timeline

(Approximately 1 page)

Timeline:

- Purchase peroxidation products for testing our refined hypothesis (November 2018)
- Isolate APEH with column chromatography and test inhibitors on purified protein (Dec 2018-Jan 2019)
- Format, graph, and evaluate data (Jan –Feb 2019)
- Prepare for presentations: compare data, draw conclusions (Feb 2019)
- Present at UCUR (February 22nd, 2019)
- Prepare for and present at WSU Undergraduate Research Symposium (February 22nd – March 25th)
- Make revisions and work toward publications (March – April 2019)
- Submit Paper at Peer-reviewed journal (May 2019)

Budget Explanation

(Approximately 1 page)

We are requesting a total of $1018.95 for the purposes of our research project. The bulk of the money will be spent in purchasing the lipid peroxidation products that will be tested ($565.00). We also need money for the materials that will be used to isolate our protein ($270.00). The rest of the budget ($133.95) will be used to purchase materials necessary to grow the cells we need that contain our enzyme and estimated shipping ($50.00). See budget breakdown:

Lipid Peroxidation Product	Vendor	Item	Price
24-hydroxy Cholesterol	Cayman Chemical	10009931-1mg	55.00
7-ketocholesterol	Cayman Chemical	16339-10mg	86.00
Linoleic acid	Cayman Chemical	90150-100mg	22.00
9-oxo-ODE	Cayman Chemical	38420-50ug	162.00
15-oxo-EDE	Cayman Chemical	37730-50ug	124.00
Prostaglandin E1	Cayman Chemical	13020-1mg	49.00
Prostaglandin J2	Cayman Chemical	18550-100ug	32.00
Prostaglandin A1	Cayman Chemical	11001-1mg	35.00
Protein Isolation			
DEAE columns	GE Lifescience	17505501 (5 x 1mL)	270.00
Cell Culturing Reagents			
TrypLE Express (Trypsin Reagent)	ThermoFisher	12604021	74.25
DMEM (Cell culture media)	ThermoFisher	2 x 10566016	59.70
Estimated Shipping			50.00
Total			1018.95

References

Covey, T. M., Marshall, I., Prince, D., Johnson, H., Ruiz, D., & Nichalaou, M. (2018). Analyzing the activity and expression of Acyl Peptide Enzyme Hydrolase in the blood serum of patients with Type II Diabetes. BIOS (in press)

Palmieri, G., Cocca, E., Gogliettino, M., et. Al. (2017). Low Erythrocyte Levels of Proteosome and Acyl-Peptide Hydrolase (APEH) Activities in Alzheimer's Disease. J. Alzheimer's Disease (60) 1097-1106.

Fowler, N. J., Blanford, C. F., de Visser, S. P., & Warwicker, J. (2017). Features of reactive cysteines discovered through computation from kinase inhibition to enrichment around protein degrons. *Nature Scientific Reports*, 1-12.

Sayre, L. M., Zelasko, D. A., Harris, P. L., Perry, G., Salomon, R. G., & Smith, M. A. (2002). 4□Hydroxynonenal□Derived Advanced Lipid Peroxidation End Products Are Increased in Alzheimer's Disease. *Journal of Neurochemistry*, 2092-2097.

LONG TERM GRANT APPLICATION
Additional Questions

1. What funding have you received from OUR in the past? Where has your previous project been disseminated?

No, we have not received any funding in the past.

2. Is this project part of a required course? If so, please indicate the support (monetary and in-kind) provided for this project by the academic department.

No, the project in not part of a required course.

3. What additional sources of funding have been solicited? Is your department willing/able to fund any equipment they will be retaining?

Any other sources of funding are presented in the table above.

4. Where do you plan to disseminate the results of this project?

In a published peer-reviewed journal and at the Weber State and Utah Undergraduate Research Conferences.

5. If you are requesting a Research Scholarship, please list all significant time commitments (5+ hours per week) that you expect to maintain over the duration of your project including, for example, class and work schedules.

Not applicable

LONG TERM GRANT APPLICATION
Faculty Recommendation Form

Student Name (last, first): Shelby Geilman and Deborah Belnap_____

Project Title: ___ Investigating the Inhibition of APEH in Disease _____

Mentor Directions: After carefully reviewing the proposal and assessing both the viability of this project and the qualifications of the student requesting funding, answer the questions found below. Please expand the sections as necessary (**do not attach separate letter**). If the project involves the use of human subjects or protected animals, be sure the student secures IRB or ACUC approval. If the project receives funding, it is your responsibility to work closely with the student, monitor the ongoing progress of the project and budget, and evaluate the project's results. Failure to do so will jeopardize funding for this project and any future projects.

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1. How long and in what capacity have you known this student?

I have known Shelby and Deborah for about 2 years. They were both students in my classes where they were both excellent. Additionally, I have worked with them as research students since spring 2018.

2. Briefly describe the proposed project. Is this part of a larger research project? Is this part of a course? If so, how is the project apart from the nature and scope of activities normally taken for the course (Please attach a copy of your course syllabus)?

This project is part of an overall interest in APEH in disease, although this aspect of the project stands on its own research question that Shelby and Deb are addressing. The work they are doing is anticipated to lead to a publication since it is both novel and important for better understanding APEH in disease states.

3. Give an assessment of the project's significance to the student's discipline and of the project's educational and/or professional benefit to the student.

This project is a biochemistry-based project that incorporates structural biology, computer modeling, cell culturing, and mechanism of disease. Exposure to these different areas in answering a research question will benefit both students as they pursue their careers. Furthermore, working on an undergraduate research project solidifies knowledge they are learning in courses and allows them to expand their critical thinking and data analysis skills.

4. Comment on the qualifications of the student to successfully complete this project, both in terms of the project's scope and its time frame.

Both Shelby and Deborah are extremely well-equipped to finish this project in the given amount of time. They worked over the summer on the project and have proven to be talented undergraduate researchers. They have a good grasp on setting up and running the associated experiments. The data shown in this proposal was collected and analyzed by Shelby and Deborah and is just an example of what they accomplished. I have been impressed at their work-ethic and commitment to the project. I don't anticipate them having any problems in completing this project since they have already been working on it for awhile.

5. Comment on the justification and appropriateness of the project budget, including the necessity of a Research Scholarship (if requesting one).

The budget is for materials to finish this project so it can be written up for publication. Deborah and Shelby started the project in Spring 2018 and have 2-3 figures completed. They have refined their hypothesis as they collect data and would like to test the refined hypothesis. Following these tests, we anticipate having enough data to submit it for publication.

6. Describe your role in the project.

My main role is as an advisor to the project. I helped train Deborah and Shelby at the start of the project. Once they were trained on techniques, they have been able to work independently on setting up experiments, performing the assays, and analyzing the data.

7. Include anything else that you think will be helpful to the committee in evaluating this application.

This project ____ DOES _X_ DOES NOT require review by the WSU Institutional Review Board for Human Subjects or the WSU Animal Care and Use Committee.

Tracy Covey
Project Mentor Signature

11-5-18
Date

2503
Campus Mail Code

x8665
Phone Extension