Example STEM-Biology IDEA Grant Project Proposal

Project Proposal (800-word limit)

Present a plan for a project that you will pursue over the summer. Your proposal should describe the problem to be investigated, the hypothesis to be tested, the idea/initiative to be developed/evaluated, or the creative endeavor to be undertaken and include a timeline. Describe how you chose your supervising professor and how you expect to work with him/her (and community partner organization, if applicable). If you are doing a project in partnership with a community organization, the proposal should also outline the community organization's need and how your project will address this need, as well as the organization's willingness to have you execute the project.

A key step in the Biochemical Platform of the Biomass to Fuels (or chemicals) Process being developed by the Department of Energy is the hydrolysis of cellulose to glucose through fungal cellulases. This key step must occur prior to the fermentation of glucose to ethanol or other products by microbial biocatalysts. Major cellulase (enzymes) producers, such as Novozymes, estimate the cost of fungal cellulases to be approximately $0.50 per gallon of cellulosic ethanol produced, about 40 to 100 times higher than the enzyme cost in the starch to ethanol process. Seeing that the price of enzymes is about 25% of the total cost of cellulosic ethanol production, new strategies for reducing enzyme loading are required before cellulosic ethanol can become cost-competitive with gasoline**.**

Previous work by the Biofuels Research Group in the Department of Chemical and Biomedical Engineering has identified a novel solvent, N-methyl morpholine oxide (NMMO),for biomass treatment. Employment of the solvent results in enhanced enzymatic hydrolysis rates and product yield. As a result, this is a very promising development that, when coupled with fermentation, will result in an economic process for biofuels production. The missing link in the work is a lack of fundamental understanding of how enzymes break down NMMO treated biomass, as this will aid in developing reactors aimed to produce biofuels. Thus, the main focus of my summer work is carrying out experiments that will aid in developing this fundamental understanding.

Throughout the enzymatic process of producing sugars through hydrolysis, many steps are believed to occur. The first step involves the process of adsorbing the enzymes onto the cellulose surface. Seeing that this occurs at the molecular level and that only nanogram amounts of enzyme are adsorbed; it can prove to be quite difficult to measure both the kinetics as well as the amount of enzymatic material adsorbed. Additionally, once the required enzyme is adsorbed onto the cellulose surface, the enzyme proceeds to bond with the individual cellulose structures. In doing so, the enzyme is thus able to assist in carrying out the hydrolysis reaction.

As with the adsorption process, it can prove to be very difficult to measure the amount of enzyme that bonded to the individual cellulose molecules, for just because the enzyme bonds to the cellulose does not indicate that it is in a reactive state. Consequently, a device that has the ability to directly measure the nanogram amount of enzyme adsorbed onto a cellulose surface as well the amount of enzyme that proceeded to bond to cellulose molecules can prove invaluable in regard to this field of study. A device that has this potential and which will be used in this work is a quartz crystal microbalance (QCM). The experiments I will be performing using a QCM are outlined below.

1. Preparation of cellulose thin films: I will learn how to prepare cellulose thin films on a quartz crystal surface so that it can be used in a QCM. Seeing that this is a crucial step in starting the research, this will prove to be a heavy focus in the beginning portion of my summer work. I will treat cellulose with NMMO and work on procedures to coat crystals with the pretreated cellulose.

2. Enzymatic hydrolysis of cellulose thin films using QCM:

    a. The effect of enzyme concentration on the rate of hydrolysis: It has been determined that the addition of extra enzyme to the solution increases the yield of sugars; however, it is not yet certain that this correlation is linear. Hence, it is my goal to determine this effect in the course of my summer work.

b. The effect of inhibition or deactivation on the overall hydrolysis process: It is known in literature that glucose formed in hydrolysis inhibits the enzyme from further hydrolysis of cellulose. Experiments will be performed using QCM with varying amounts of glucose added to the enzyme mixture to study the effect of glucose concentration on rates and yields of subsequent reactions.

c. The effect of solvent concentration (NMMO) on the rate of hydrolysis: A hypothesis in the Biofuels group is that certain amounts of NMMO if present in the pretreated biomass will aid in the hydrolysis reactions due its effect on cellulose structure. Moreover, it also reduces the amount of water used in washing process resulting in a more economical process. Hence the focus of this aim would be to run experiments using a QCM in which controlled amounts of the solvent NMMO will be added to the enzymes to see the effect of solvent on the hydrolysis reactions. This has not been studied till date.

Additionally, following my summer work I plan to pursue involvement in the Honors Thesis Program, employing my summer findings throughout the research group to carry out further experimentations in the subsequent Fall and Spring semesters.

Example Project Proposal (800 word limit)

Previous studies have shown that deletion of the genes encoding the Kv1.3 potassium ion channel in mice (thus producing knock-out, or KO, mice) results in heightened olfaction, resistance to obesity, and increased metabolism. While performing memory tests on KO mice, I observed they also mimic the hyperactive behavioral phenotype seen in animal models of attention deficit/ hyperactivity disorder (ADHD). Based on this observation and the fact that a wholly representative animal model of the ADHD diagnosis has yet to be discovered, my research seeks to determine whether KO mice are suitable behavioral models of the ADHD phenotype and, if so, whether their hyperactive behavior can be corrected with methylphenidate treatment.

Because ADHD is often associated with increased anxiety, the anxiety levels of KO mice as compared to wildtype (WT) mice were analyzed as a preliminary survey for the possession of additional ADHD-type behaviors in the fall of 2012 and the spring of 2013. Though the KO mice mimic the hyperactivity seen in ADHD, a relation has also been found between decreased olfactory abilities and increased anxiety. It was thus hypothesized that the KO mice would have equal or decreased anxiety levels as compared to their WT counterparts due to their superior olfactory abilities. Anxiety levels were determined using three manual behavior screens: the marble burying test, the elevated plus maze, and the light/dark box.

In the marble burying test, mice were placed in a cage containing bedding and a grid of marb les; the number of marbles buried over 30 minutes was measured. In the elevated plus maze, mice were placed in the center of a cross-shaped maze with two enclosed arms and two open arms. The mice were free to enter and exit the arms for a five minute period while the amount of ti me spent in each arm was measured. The lightdark box experiment involved placing mice in a box c ontaining a white and a black chamber separated by a black divider.

The divider contained a small opening, thusallowing the mice to enter and exit the chambers dur ing a five minute period while the amount of time spent in each chamber was recorded. The number of marbles buried, the amount of time spent in the enclosed arms, and the amount of time spent in the dark chamber are positively correlated with increased anxiety. After determining the anxiety levels of the KO mice, I will quantify their hyperactive behaviors through the use of metabolic chamber analysis and a modified object-memory test in the spring and fall of 2013. Metabolic chamber analysis involves placing a mouse in a cage equipped with a motion-sensitive infrared laser, thus enabling the differences in locomotor activity between KO and WT mice to be recorded. Because the instrument is sealed, an indirect measure of calorimetry will also be made and used to compute basal metabolic rate and ingestive behaviors. The modified memory test is a technique developed by me and my faculty advisor and involves placing a mouse and several plastic toys in a large test cage. The mouse is free to interact with the toys for a five minute period while the amount of time spent focusing on the toys is recorded and used to indicate attention span.

In the final stage of my research, the mice will be subjected to treatment with methylphenidate in the fall of 2013 and spring of 2014. Because KO mice appear to mimic the behavioral phenotype seen in ADHD animal models, I expect treatment will result in the decreased locomotor activity and increased attention span observed in ADHD mice models treated with this drug. The metabolic chamber and attention-span tests will be repeated post-treatment and the results will be compared to the pre-treatment results as well as to results from published literature to determine whether methylphenidate has a noticeable effect on

behavior. If WT behavior patterns are generated through the treatment, it will be concluded that KO mice are suitable behavioral models of ADHD.

This research is being performed under the guidance of Dr. Debra Ann Fadool in the Biological Science department. Because I developed the idea for this project while performing behavioral testing in Dr. Fadool's lab in the spring of 2012, she is the supervising professor for my research. Dr. Fadool has helped me choose which tests to use to measure the anxiety and hyperactivity levels of the mice and has also helped connect me with other professors in the biology department and medical school who possess equipment I will need to use. She has provided me with WT and KO mice on which to perform my anxiety and behavioral tests and will also advise me while I gather, analyze, and prepare to present my findings for my honors thesis defense as well as for biological conventions and competitions.