FACULTY SPONSOR
EASTERN ILLINOIS UNIVERSITY
UNDERGRADUATE RESEARCH AND CREATIVE ACTIVITY PROPOSAL

Name of Student Sponsored: Nicolas A. Ferry

Name of Faculty Sponsor: Michael A. Menze, PhD

Department: Biological Sciences

Briefly provide your comments about the student's proposed research or creative activity, including an assessment of the validity of the project design.

The project proposed by Nicolas Ferry is part of a larger interdisciplinary collaboration between Dr. Mary Konkle from the Chemistry department and my molecular biology laboratory. The experiments designed by Nicolas integrate perfectly into the larger framework of our goal to develop better and more efficient treatments for type-2 diabetes. Nicolas approached me last semester about the possibility to work on a project in my laboratory that involves a human disease model. I introduced him to my research on type-2 diabetes and he started to design experiments that investigate the interactions among the small protein mitoNEET and enzymes that aid the body in detoxification of harmful substances (e.g. the enzyme catalase). Nicolas independently designed an elegant sequence of three interconnected but not interdependent experiments that will tremendously increase our understanding of the underlying causes of type-2 diabetes. He identified three different methodologies and instruments that are readily available in my laboratory to investigate interactions between the iron-binding protein mitoNEET and the enzyme catalase. He already mastered many techniques during his last semester of research in my laboratory. I highly recommend Nicolas research project with absolutely no reservations. Nicolas experimental design is outstanding and his findings will be highly valued by the scientific community at large.

Explain why you believe the student will be successful in carrying out this proposed project, bearing in mind that the student is primarily responsible for the actual execution of the activity and preparation of the Summary Reflection. Please include comments on the student's communication skills.

Nicolas is one of the few truly outstanding students at EIU. He has a most impressive record of accomplishments including a pre-college GPA of 4.47/4.0 and he maintains a GPA of 4.0/4.0 here at Eastern. Furthermore, he holds an EIU Presidential Scholarship and obtained numerous awards including the CRC General Chemistry Award, the Charles Austin Jr. Scholarship, and the OSA Scholarship Grant Merit Award. Nicolas is highly dependable, intelligent, independent, and has already mastered the techniques he would use to successfully complete his proposed summer research projector. Nicolas easily masters complex molecular biological concepts and techniques and will already present some of the research he performed during the fall semester of 2012 at the 2013 National Conference for Undergraduate Research (NCUR) in La Crosse, WI. I am glad to say that Nicolas is one of the most outstanding students I have been working with during the last 10 years of my career. His exceptional intellectual capabilities combined with his truly outstanding motivation will make him succeed in elucidating the underlying causes of type-2 diabetes. I place Nicolas into the top 1% of all students I worked with in my career and consider him a top candidate for an URSCA summer award. In sum, Nicolas has the research experience, strategy, desire, and materials available to succeed in his research project.

Please specify the account name and number into which faculty funds are to be deposited. *Cannot be a gift account*

Organization #: [redacted] Organization Title: Biological Sciences

I understand that the funds awarded by the Undergraduate Research Council are for the exclusive use of the above named faculty member.

Department Chair Signature — Date: 1/30/13

By signing this application, I agree to supervise the student, the proposed project, and monitor the ethics used in the proposed research. I also understand that the Undergraduate Research Council requires the student to submit a summary report of his/her research to the Honors College Office.

Faculty Sponsor Signature — Date: 01/30/2013

Return this application to Dr. John Paul Stimac, Dean of The Honors College, Booth House, Eastern Illinois University, Charleston, IL 61920. It is the responsibility of the student to inform the faculty sponsor of the proposal due date.

*******Note: It is the student's and the sponsor's responsibility to submit the summary reflection by the deadline. Failure to do so will result in the refusal of future undergraduate research grants for the applicant.*******

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Type-2 Diabetes: Does MitoNEET Impact Mitochondrial and Catalase Functions by Multiple Mechanisms?

Nicolas Ferry, Biological Sciences

R1: Briefly describe your proposed research project (e.g., purpose, hypothesis, research goals).

The American Diabetes Association reports millions of Americans have been diagnosed with Type-2 diabetes, making it the most common form of diabetes[1]. Type-2 diabetes is often brought on by a sedentary life style, common in America, which causes an imbalance in the chemical reactions of the body that sustain life (metabolic disease)[2]. The exact pathology on how type-2 diabetes develops is extremely complex. The pivotal role of mitochondria in cellular energy production suggests that this organelle plays a crucial part in the pathology of type-2 diabetes[2]. The mitochondrion of the cell is the equivalent of the power station of a city. The mitochondrion is a subcellular organelle which generates chemical energy through combustion of electrons from the food we consume with oxygen. The protein MitoNEET has recently come into interest as possible drug target in diabetes treatment. MitoNEET was discovered in 2004 in a test to find physiological targets of Actos (Pioglitazone Hydrochloride), a drug that is commonly used to treat type-2 diabetes[3]. However, MitoNEET was found to be part of the mitochondrion. Due to its location and interaction with anti-diabetic drugs, a role in diabetes treatment and/or prevention is highly likely. Previous protein pull-down experiments in our collaborating lab (Dr. Mary Konkle from the Chemistry department) suggested a direct interaction between mitoNEET and the enzyme catalase. Catalase is a detoxification enzyme found in the mitochondria. Catalase removes these toxic side products (e.g. hydrogen peroxide) from the cell by converting them into water and oxygen[4]. The negative effects caused by the toxins removed by catalase have been hypothesized to be one of the key factors in aging and studies have shown that increased catalase levels increase the life span of mice[5].

The overall purpose of my project is to explore the properties and characteristics of mitoNEET. Since the protein was only recently discovered, very little research has been put forth and not much is known about any purpose it may serve in the body. Studies show that catalase deficiency has a high correlation with diabetes[6]. The link between mitoNEET and anti-diabetes treatment drugs and the connection between catalase and mitoNEET demonstrates that the protein is a key factor in the pathophysiology of type-2 diabetes. My research will specifically focus on the mechanism by which mitoNEET interacts with the enzyme catalase and improves the efficiency by which catalase neutralizes toxic compounds in the cell. I will be investigating this hypothesis by measuring the change in activity of the enzyme catalase in the presence of different amounts of mitoNEET. I hypothesize that mitoNEET administered in the presence of catalase will raise the effectiveness of the enzyme, and thus consequently increase mitochondrial performance and cellular energy production. The knowledge I will gain through these experiments will allow a deeper understanding

on the mechanisms by which type-2 diabetes develops and likely pave the way for novel treatment avenues of this common disease.

R2: Describe the method to be used in the proposed research project including an evaluation of the data. Include a project timeline.

Three sets of interconnected experiments will be performed. Each set will monitor the rate of hydrogen peroxide breakdown by catalase and investigate whether the protein mitoNEET affects this rate. I will use three different approaches to investigate the effect of mitoNEET on the activity of catalase using different signals to judge catalase efficiency. These different signals will be measured with three different instruments. The first instrument that will be used is the UV-Vis spectrophotometer which will measure the change in absorbance of light. The second instrument will be the thermal activity monitor TAM2277 which will measure any change in heat. The third instrument will be the Oroboros Oxygraph-2k which will measure the change in oxygen levels.

Experimental Set 1: Certain properties of compounds allow some to absorb energy from a certain frequency, or type, of light. Hydrogen peroxide is one such compound that absorbs light. An UV-Vis spectrophotometer will be used to analyze the rate of change in light-absorption levels of a hydrogen peroxide solution, and the effects of mitoNEET. As hydrogen peroxide is broken down, the amount of hydrogen peroxide in the solution will decrease. As the amount decreases, the amount of light absorbed will also decrease. This decrease can be measured with the spectrophotometer. An increased reaction rate will demonstrate a positive effect of mitoNEET on catalase. Catalase is an enzyme that aids the body in detoxification of toxins and a higher reaction rate indicates a positive impact on cellular and whole body health. Each test will run for approximately 10-20 minutes. The preparation of the experimental solutions and glassware will take an additional 20 minutes. My tests will be performed in sets of two (control and mitoNEET), with a minimum of three repeats per day. Data collection will require approximately four weeks.

Experimental Set 2: Every chemical reaction consumes or releases heat. Fortunately Novartis Consumer Sciences recently donated an instrument that is capable of measuring minute changes in heat dissipation to Dr. Menze's laboratory. The instrument, a thermal activity monitor TAM2277, will be used to analyze the rate of energy given off in the form of heat from the degradation of hydrogen peroxide by catalase, and the effects of mitoNEET. Using the TAM2277 will allow me to gain even further insights into the thermodynamic parameters such as ΔH (enthalpy). Enthalpy is

the total amount of energy in a system (compound or environment) and the change in enthalpy measures the release, normally in the form of heat, or absorption of energy. Increased levels of heat release rates in chambers of the instrument will demonstrate a positive effect of mitoNEET on catalase. Each test will take about two hours. Tests will be run in sets of two (control and mitoNEET), with a minimum of five repeats. Data collection will require approximately four weeks.

Experimental Set 3: Finally, to thoroughly characterize the interactions between mitoNEET and catalase, an Oroboros Oxygraph-2k will be used. This instrument can directly measure the concentration of oxygen in a sample. Since hydrogen peroxide is detoxified to oxygen I will be able to measure not just an unspecific reduction in substrates (levels of hydrogen peroxide over time) but a specific accumulation of product (oxygen). Increased oxygen production rates in the chambers of the instrument will demonstrate a positive effect of the protein mitoNEET on the rate of hydrogen peroxide degradation by catalase. Each test will take about six hours. Test will be done in sets of two (control and mitoNEET), with a minimum of five repeats. Data collection will require approximately another four weeks. This project will be completed between August 2nd and August 23rd.

The experimental data will be analyzed using statistics including a one way ANOVA. By combining the three above described approaches I will be able to thoroughly characterize the complex interactions between mitoNEET and catalase.

R3: How will this research contribute to the existing knowledge in the field of study? Provide relevant citations from the published literature and place your proposed work into this context.

The protein mitoNEET was only recently discovered and very little is currently known about this novel protein[7]. Several studies have demonstrated a role of mitoNEET in type-2 diabetes pathology and or treatment. One such study found that the compound pioglitazone, an anti-diabetes drug, interacts directly with mitoNEET and inhibits iron transfer[(7-10)]. Two functions have been suggested for mitoNEET to date: a) transfer of high energy electrons taken from food molecules during energy production in the mitochondrion and/or b) donating a bound iron molecule from its 2Fe-2S cluster to another protein that uses iron as a functional group[9]. These finding are important due to the role of iron overload in diabetes and also suggest that mitoNEET improves the efficiency of mitochondrial respiration, or energy production. My study will work to further characterize the relationship between mitoNEET and type-2 diabetes. The main function of catalase is to detoxify hydrogen peroxide[11]. Hydrogen peroxide is a free

radical in the body and is linked to decreased mitochondrial function and insulin resistance[12]. Several studies demonstrated the positive effects of increased levels of catalase on cellular life span and mitochondrial functions[5,12]. Since type-2 diabetes involves decreased levels in mitochondrial function, catalase contains the ability to counter this decrease. My studies will investigate if mitoNEET expression will allow for increased efficiency of catalase without the need to overexpress the protein in cells. With so little information currently available about mitoNEET, any discoveries will contribute greatly to the understanding of mitoNEET and type-2 diabetes.

R4: What background and skills qualify you to successfully complete this project? How does this project develop/strengthen your professional skills?

I have had research experience at EIU for a semester in Dr. Menze's laboratory. Before starting my project described here I began preliminary readings during my sophomore year and it was my plan to conduct research under the guidance of Dr. Daniel who is a microbiologist in the Biology department. However, I had to postpone my research with Dr. Daniel in order to participate in the EIU study abroad program in the spring of my sophomore year (Spring 2012). Dr. Daniel than went onto sabbatical leave and I was unable to continue my planned research project under his guidance. Upon my return, I began my current research project under the guidance of Dr. Menze. I started to measure the activity rates of degradation of compounds by enzymes, primarily the enzyme catalase. This research project is in collaboration with Dr. Mary Konkle from the Chemistry department. I will already be presenting the results that I gathered in just one semester at the National Conference on Undergraduate Research (NCUR 2013). With my work in the laboratory of Dr. Menze during fall of 2012 (and continuing now), I have gained the knowledge necessary to perform all tests and techniques outlined in this proposal. My preliminary data using UV-VIS spectrophotometry showed an increase in activity of catalase in the presence of mitoNEET in some experiments. However, these results were variable and make a more thorough investigation using several different experimental approaches necessary. I know in detail how to use the various tools necessary for my experiments and how to analyze the data that will be collected using the various tools and approaches. This project will deepen my strengths in using the UV-Vis spectrophotometer and will also allow me to gain deeper insights to develop the skills necessary to operate the thermal activity monitor TAM2277 and the Oroboros Oxygraph-2k. To be trained in a variety of physiological and biochemical techniques will give me a highly competitive edge when applying for medical school program in the fall of 2014. Achieving scientific success through hard work and being able to contribute to the understanding of type-2 diabetes will continue to serve me

throughout my life. I plan to continue a career in biomedical research. Being able to perform research in Dr. Menze's laboratory during the summer would certainly be of enormous value and help me to achieve my professional and career goals. My project will also give me many opportunities to increase my communication skills since I will need to communicate my findings in shorter weekly lab reports to Dr. Menze, a detailed written report to the Honors College, and in form of a talk at the National Meeting for Undergraduate Research (NCUR) 2014 in Lexington, KY. My project will also allow me to increase my organizational skills by being able to plan all of the experiments in consultation with my mentor Dr. Menze.

R5: Indicate how award funds will be used. Specify a plan for the dissemination of the results from this project.

The funds will be used to make this project possible by allowing me to remain in Charleston over the summer. The award funds will allow me to pay for housing, food, and tuition during the summer break. Without the support of these funds, I would be forced to return home for the summer or to spend a large amount of my time working one or several summer jobs. Both of these alternatives would prevent me from being able to continue my research project.

Results produced by my project will be submitted for presentation at national and regional conferences, including the 2014 National Conference on Undergraduate Research (NCUR) in Lexington, KY, the 2013 National Collegiate Honors Council (NCHC) conference in New Orleans, LA, and the 2014 Society for Integrative and Comparative Biology meeting in Austin, TX.

The intended audiences will include medical personnel and research faculty at EIU and other universities with an interest in mitochondrial function, type-2 diabetes, or those with an interest in proteins.

My results will also form the basis of my Honors Thesis. I hope to be able to generate enough data during the summer to be able to submit my findings to a professional journal such as the *American Journal of Physiology* and *Diabetes*. A large amount of hard work is needed to gather enough data for a journal publication and the summer will allow me to gather the amount of data for a possible publication. Without the opportunity to perform research this summer my chance to gather enough findings for a manuscript are much lower.

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