July 22, 2015

Mid-SURE

Mid-Michigan Symposium for
Undergraduate Research Experiences

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WELCOME

Thank you for attending the Mid-Michigan Symposium for Undergraduate Research Experiences (Mid-SURE) at Michigan State University. Our goal is to provide a forum for undergraduates in the region to share and discuss their research as well as create networking opportunities with graduate schools and researchers.

Undergraduate students from diverse academic disciplines will present their outstanding research and creative endeavors at Mid-SURE. Approximately 343 students from 117 different institutions are participating in today's event. These students are mentored by 351 faculty members, post-doctoral researchers, and graduate students.

As one of the nation's leading research institutions, MSU offers a breadth of experiences and opportunities that actively engage students in their education. Through undergraduate research and creative activities, students work closely with leading scholars to gain in-depth knowledge about their fields of study and have opportunities to apply classroom learning to real-life situations.

We encourage the student participants, faculty members, research mentors, and guests to walk around the forum and learn about the impressive work of our next generation of scholars and researchers. Thank you for joining us.

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METAL-CATALYZED AHP PRETREATMENT OF WOODY BIOMASS
Aline Silva (Universidade Estadual de Campinas UNICAMP)
Category & Time: Agriculture and Animal Science, Section 3, 3:00 PM - 4:00 PM
Poster: 15
Mentor(s): Hegg Eric (Biochemistry & Molecular Biology)

Second generation biofuels derived from the lignocellulose (i.e. non-food plant biomass) have the potential to replace petroleum-derived nonrenewable fuels. Lignocellulosic material is mainly composed of cellulose, hemicellulose, and lignin, where cellulose and hemicellulose are the source of sugars. Lignin, which mainly contributes to high recalcitrance, impedes the enzymes' access to the polymeric carbohydrate components for their conversion into momomeric sugars. Therefore, pretreatment of this recalcitrant lignocellulose before enzymatic hydrolysis is an unavoidable step in the biological production of biofuels. An effective pretreatment makes lignocellulose susceptible to the action of cellulases and xylanases to efficiently release fermentable sugars. Woody biomass is a promising bioenergy feedstock due to its high density and biomass yields. This study will investigate the potential of metal-catalyzed AHP pretreatment of woody biomass to improve digestibility and maximize the recovery of fermentable sugars.

REPURPOSING AN AMINOMUTASE FROM TAXUS PLANTS: STEREO- AND REGIOSELECTIVE AMINATION OF CINNAMATE EPOXIDES PRODUCES RING-OPENED *ERYTHRO-PHENYLSERINES*
Lawrence Allen (Talladega College)
Category & Time: Biochemistry and Molecular Biology, Section 1, 1:00 PM - 2:00 PM
Poster: 23
Mentor(s): Prakash Shee (Chemistry), Kevin Walker (Biochemistry and Molecular Biology)

β-Hydroxy-α-amino acids occur as proteogenic hydroxy amino acids threonine, serine, and 3-hydroxyproline, a component of collagen. These hydroxy amino acids are key building blocks of medically relevant products such as, vancomycin, chloramphenicol, and lysobactin, all of which contain a β-Hydroxy-α-amino acid moiety. There is great interest in producing β-Hydroxy-α-amino acids by stereoselective synthetic and biocatalytic approaches, where enzyme catalysts plays a significant role because of their high stereoselectivity. The enzyme catalyst used for this research was an MIO-dependent aminomutase isolated from *Taxus canadensis* (TcPAM). In addition to being an aminomutase isomerase, TcPAM also has inherent transaminase activity. Therefore, this enzyme was repurposed to transfer NH2 from styrylalanine to cinnamic epoxides. This study specifically focused on evaluating whether substituents on the aromatic ring of the substrates affected the regiochemistry of amination catalyzed by TcPAM. Hypothetically, electron-donating substituents (EDS) should favor Cβ-amination, making isoserines, whereas electron-withdrawing substituents (EWS) should favor Cα-amination, producing serines. This is the first instance of using an aminomutase to biocatalyze industrially and pharmaceutically relevant hydroxy amino acids. 1 H NMR (Proton Nuclear Magnetic Resonance Spectroscopy) was used to characterize and assess purity of synthesized epoxides. LC-ESI-MS/MS (liquid chromatography-electrospray ionization-tandem mass

spectrometry) was used to quantify the biosynthetic serine and isoserine products for enzyme kinetic measurements of TcPAM for each substituted epoxide substrate.

AN INITIAL MUTANT SCREEN FOR LIPID VARIATION IN NANNOCHLOROPSIS OCEANICA CCMP1779. Jonathan Alvaro (Hope College)
Category & Time: Biochemistry and Molecular Biology, Section 1, 1:00 PM - 2:00 PM
Poster: 24
Mentor(s): Christoph Benning (Biochemistry and Molecular Biology), Zhi-Yan Du (Biochemistry and Molecular Biology)

Fossil fuels are quickly disappearing. Under the ever-increasing demand of modern societies for fuel, research into sustainable sources of energy is becoming urgent. Unicellular marine algae have promise for providing sustainable biofuel feedstocks. Unlike traditional biofuel organisms they can be grown in seawater, and do not compete for valuable land area that food production could utilize. Under stressful conditions, microalgae can produce large amounts of storage lipids such as triacylglycerols (TAGs) that can serve as feedstock for the production of biofuels. From a genus of oleaginous marine algae, Nannochloropsis oceanica CCMP1779 offers great promise to biofuel supply because of its attractive characteristics: fast growth and large biomass, high lipid content ability, large scale cultivation using ocean water, and no direct competition with food supply or land requirement. In addition, N. oceanica has a compact genome with approximately 12,000 genes, making it an ideal candidate for genetic studies. The methods of nuclear transformation in N. oceanica have been established recently, facilitating the introduction of foreign DNA and stable integration into the genome of N. oceanica, and easing the insertion of genes and genetic engineering of the oleaginous alga. Currently, hundreds of mutant strains have been generated by insertional mutagenesis. In this project, we performed an initial mutant screen for increased/decreased lipid phenotypes by nitrogen deprivation treatment that can induce lipid accumulation in N. oceanica. Taking advantage of the mutant pool and gas chromatography we performed fatty acid profiling on these mutants to search for strains showing lipid productivity variation

ENHANCING STORAGE LIPID SYNTHESIS IN VEGETATIVE TISSUES OF BRACHYPODIUM DISTACHYON
Kira Bartlett (Clemson University)
Category & Time: Biochemistry and Molecular Biology, Section 1, 1:00 PM - 2:00 PM
Poster: 25
Mentor(s): Christoph Benning (Biochemistry and Molecular Biology), Agnieszka Zienkiewicz (Biochemistry and Molecular Biology)

Biodiesel, one prominent form of alternative fuel, is created in part by lipids from biomass. Plants are one available source of biomass, but generally contain the majority of their lipid content in seeds as triacylglycerols (TAGs). Seeds make up a small percentage of the plant's overall biomass and are often used as food sources or other agricultural products. Therefore, modifying the plant to have increased lipid production in the leaves and stems would enhance its viability as a lipid source for biodiesel. Using *Brachypodium distachyon*, a recently emerged model species for temperate grass research, certain genes were

overexpressed in an attempt to increase the lipid content of the plant. The transgenic *Brachypodium* plants studied expressed the Brachypodium *Wrinkled1* gene under control of a *BdTIFY 3A-like* promoter as well as DGAT1 (diacylglycerol acetyltransferase 1) and LDSP genes (lipid droplet surface protein) from *Nannochloropsis oceanica*. These plants were examined for improved accumulation of TAGs, especially in the vegetative tissues, as well as for overall growth.

CONSTRUCTING A DESIGN TO MAKE THE NODAL GENE
Andie Williams (Michigan State University)
Category & Time: Biochemistry and Molecular Biology, Section 1, 1:00 PM - 2:00 PM
Poster: 27
Mentor(s): Erik Martinez-Hackert (Biochemistry & Molecular Biology)

Background: Nodal is a ligand that is a part of the Transforming Growth Factor- Beta (TGF-β) family. The TGF-β family controls cell differentiation and proliferation; and its members play key roles in many diseases including cancer, diabetes, and asthma. Nodal plays a regulatory during embryogenesis. In addition, we now know that nodal plays a role in cancer metastasis. In order to stop nodal from contributing to cancer metastasis, we have to identify its function. Once we understand nodal better we could create a molecule that could inhibit its function. Methods: The purpose of our study is to synthesize nodal in order to be able to clone it into mammalian cells. We designed an expression cassette that has the Luciferase gene (Luci). This helps Nodal secretion and also can be used for tracing nodal during purification. The original Luci expression cassette has an Fc domain. However, the Fc destroys nodal expression. We therefore want to remove the Fc gene from the expression cassette. To do this, we perform a deletion mutagenesis polymerase chain reaction (PCR). We transform the PCR product directly into e. coli cells for cloning. To confirm that the Fc has been removed successfully, we a colony PCR reaction. One colony that is positive for Luci will be amplified and sequenced. Once we have the Fc free cassette, we will insert nodal by restriction cloning. Once we have created this clone we will proceed to isolate nodal. Once we have created this clone we will proceed to isolate nodal. Results: We predict that once nodal is made we will be able to clone it into Chinese hamster ovaries. This will allow us to get a better understanding of how nodal works and find a molecule to inhibit it. Conclusion: Once the Nodal is expressed and purified, we will be able to work more closely with it and determine its role cancer. This work will also allow us to have a better understanding of how the proteins in the TGF-β family work.

GENETIC ENGINEERING OF RHODOPSINS INTO ANCILLARY BICARBONATE TRANSPORTERS IN CYANOBACTERIA
Ana Christine Belza (Michigan State University)
Category & Time: Biochemistry and Molecular Biology, Section 2, 1:00 PM - 2:00 PM
Poster: 29
Mentor(s): Aparajita Banerjee (MSU-DOE Plant Research Laboratory), Sandeep B Gaudana (MSU-DOE Plant Research Laboratory), Cheryl Kerfeld (MSU-DOE Plant Research Laboratory)

The cyanobacterial carbon concentration mechanism (CCM), including its key step of inorganic carbon transport, is being avidly explored as a means for enhancing

photosynthesis in prokaryotic and eukaryotic photoautotrophs. It has been recently reported that overexpression of a bicarbonate (HCO_3^-) transporter in a model cyanobacterial strain, *Synechocystis* sp. PCC 6803, leads to enhanced growth rates and biomass production. HCO_3^-, unlike CO_2, cannot cross the plasma membrane and is hence actively transported into the cytoplasm. Therefore, positioning of a potential HCO_3^- transporter in the plasma membrane of cyanobacteria is a critical prerequisite for successful HCO_3^- transport. We envision that the benefit of enhanced HCO_3^- uptake can be augmented by designing and constructing pumps that are driven by light energy. To this end, we are repurposing bacteriorhodopsin and halorhodopsin, light-driven proton and chloride pumps, respectively, into bicarbonate transporters. Several variants of the rhodopsins, including their fusion to proteins known to localize in plasma membrane as well as signal peptides preceding the rhodopsin candidates, were cloned in cyanobacterial expression systems. Heterologous expression of the engineered constructs was followed by sub-cellular immunolocalization and transmission electron microscopy. Rhodopsins were screened for bicarbonate pumping by assaying if they can enable a high CO_2 requiring strain of *E. coli* deficient in carbonic anhydrase, to grow in ambient air. The results have provided key insights for potential engineering of rhodopsins into light driven bicarbonate transporters for use in cyanobacteria and, prospectively, algae and C3 plants

THE ROLE OF THE ARYL HYDROCARBON RECEPTOR IN MODULATING MITOCHONDRIAL FUNCTION Alexander Best (Tuskegee University)
Category & Time: Biochemistry and Molecular Biology, Section 2, 1:00 PM - 2:00 PM
Poster: 30
Mentor(s): John LaPres (Biochemistry)

The Aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that is responsible for mediating most, if not all, of the toxic effects of a class of planar aromatic hydrocarbon, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The last several years, research has shown that TCDD, via the activation of the AHR, can negatively impact the mitochondria. This negative impact includes alterations in electron transport chain (ETC) function, reactive oxygen species generation and perturbation of the nuclear-to-mitochondrial stress signaling axis. In the absence of ligand, the AHR is primarily thought to be located within the cytosol; however, we have recently shown that a portion of the AHR is found within the intermembrane space of the mitochondria. The link between TCDD and metabolic syndrome and mitochondria dysfunction, and our preliminary results have led us to hypothesize that the AhR can influence mitochondrial function via direct protein interactions and transcriptional regulation of key electron transport chain (ETC) genes. To test this hypothesis, we will use a human B lymphoblastoid cell line that was transformed with EBV, called SKW6.4 cells. We will use these cells to determine if TCDD exposure impacts mitochondrial function in an AHR-dependent manner. To measure mitochondrial function, we will use a Seahorse XF24 Extracellular Flux Analyzer. This machine measures the oxygen consumption rate of cells in culture. We hope to determine the impact of long and short-term TCDD exposure has on these two measures of cellular metabolic activity and determine if the AHR plays a role in modulating the observed impact of TCDD.

INVESTIGATION OF GROWTH AND PHYSIOLOGY IN ARABIDOPSIS THALIANA MUTANT LINES WITH REDUCED UBIQUITIN CONJUGATING ENZYME (UBC22) GENE EXPRESSION

Madalyn Bryant (Fort Valley State University)

Category & Time: Biochemistry and Molecular Biology, Section 2, 1:00 PM - 2:00 PM
Poster: 31
Mentor(s): Thomas Sharkey (Biochemistry and Molecular Biology), Sarathi Wijetilleke (Biochemistry and Molecular Biology)

Studies have shown that an ubiquitin conjugating enzyme (UBC22) is present in peroxisomes of plants and may act as a negative regulator during the conversion of indole butyric acid to indole acetic acid (IAA). IAA is an auxin and is known to inhibit primary root elongation, induce lateral root formation, promote stem elongation, inhibit growth of lateral buds, and regulate plant responses to light and gravity. Two *UBC22* gene knockout mutants (ubc22-1 and ubc22-2) of *Arabidopsis thaliana* were used to investigate the impact of removing the negative regulation of UBC22 on leaf gas exchange, carbon partitioning, and plant growth. A comprehensive growth analysis was carried out to measure leaf area, root and stem length, growth rates, and dry weights throughout the growth cycle. Photosynthesis and respiration rates were measured to determine net carbon gain. Leaf anatomy and thickness measurements were taken to examine leaf cell density and mesophyll structure. Chlorophyll content and total protein extraction and quantification were also carried out. All collected data will be fitted to the *Arabidopsis* Leaf Area Growth Model to analyze the differences in carbon partitioning between the mutant lines and the wild type (Col-0). The role of UBC22 in regulating auxin biosynthesis and subsequently carbon assimilation, partitioning, and growth will be discussed along with its potential for crop improvement.

EFFICIENT THICK LAYER SPECTRO-ELECTROCHEMISTRY

Dawei Chen (Michigan State University)

Category & Time: Biochemistry and Molecular Biology, Section 2, 1:00 PM - 2:00 PM
Poster: 34
Mentor(s): Denis Proshlyakov (Chemistry)

Redox reactions are crucial life-sustaining processes. The study of these reactions, especially those that are catalyzed by enzymes, is of interest by revealing key mechanisms of enzymatic activity. Investigating these reactions within enzymes consists of two requirements. The first is a flexible and efficient method of communicating electrons to and from these enzymes. The second is a method of observing the changes within these enzymes during the redox reaction. This study attempts to perform a redox reaction while simultaneously observing optical changes within the analyte using UV-Visible spectroscopy. These reactions are usually limited to molecules with strong optical absorption such as chromophores. The main barrier of efficiently conducting a redox reaction is the rate of diffusion of charge through the solvent. To solve this, the working electrode uses a carbon fiber brush to increase surface area. However, the solution needs to be circulated between the brush hairs, which was accomplished using a micro stir-bar

inside the cuvette. Dimensional constraints of a traditional optical cuvette limit the options of properly circulating the analyte through the working electrode. To overcome this barrier, a 3D-printed cuvette was tailored to reduce the 13 volume of solution and distance between the working electrode and the stir bar without blocking the optical window. An additional benefit is the conical shape of the analyte chamber which further improves diffusion by facilitating vortex flow through the working electrode. We will demonstrate the change in reaction rate of this 3D-printed cell in comparison to a traditional, working cell cuvette.

PHLOEM-MEDIATED LIPID SIGNALING INVOLVED IN DROUGHT TOLERANCE
Henry Csikszentmihalyi (Oberlin College)
Category & Time: Biochemistry and Molecular Biology, Section 3, 2:00 PM - 3:00 PM
Poster: 37
Mentor(s): Allison Barbaglia (Biochemistry and Molecular Biology), Susanne Hoffmann-Benning (Biochemistry and Molecular Biology)

Developing new ways of adapting crops to drought conditions could be critical to feeding a climate change-stricken world. Plants have developed long-distance signaling pathways, most often running through the phloem, to better adapt to changing environments and stressors (an important trait given their sessile nature). Using Arabidopsis and its pre-mapped genome, this lab identified several predicted lipid-binding proteins and lipids that we believe to be critical in plant response to drought conditions. Since hydrophobic lipids were unexpected to exist in the aqueous environment of the phloem, we are investigating the affinity of these phloem proteins for specific lipids as well as their role in phloem-mediated long distance signaling. We have found the proteins to be quite lipid-specific (the precise lipid matters during the binding event). We have also utilized overexpression of the genes that code the proteins of the pathway, finding that hardier, larger, and more drought resistant plants grow from the overexpressing seeds. We are in the process of determining the exact cause of the increase in drought resistance (are the roots bigger which allows the plant to be hardier? Or is the abiotic signaling pathway more sensitive to drought due to an increase in the proteins critical for the signaling?). We have also knocked out the genes, and not surprisingly, the resulting plants experienced a failure to thrive at the same level as the wild type. We will continue our research and characterize the specificity of the protein-lipid interaction to increase our understanding of function and physiological consequences.

CLONING OF BRG1 SHRNA BY SWITCHING ANTIBIOTIC MARKERS IN MACROPHAGE CELLS
Aja Green-Walker (Michigan State University)
Category & Time: Biochemistry and Molecular Biology, Section 3, 2:00 PM - 3:00 PM
Poster: 41
Mentor(s): Mohita Tagore (Biochemistry and Molecular Biology), Monique Floer (Biochemistry and Molecular Biology)

Macrophages are important cells of the immune system that are formed in response to an infection or accumulation of damaged or dead cells. In macrophages DNA is tightly wound around nucleosomes into a complex called chromatin. Through a process called chromatin remodeling, this complex can be "opened" so that specific genes are expressed. But it is not known how chromatin remodelers and transcription factors interact with each other in macrophages. Transcription factors are proteins involved in the process of converting, or transcribing, DNA into RNA. The relevance is to understand how macrophages function when responding to bacterial challenge. This is because little information is known regarding how Brg1, a protein that works to activate or repress transcription, and transcription factors contribute to inducible gene expression in macrophages. To acquire knowledge regarding this we will inhibit Brg1 in macrophages. To achieve stable Brg1 knockdown in macrophage cells, we will be cloning a Brg1 shRNA to the pLKO.1 vector having a blasticidin resistance marker for selection. As a result of this it is anticipated that the transcription factor, Pu.1, will not be able to bind to its target site. With this, we hypothesize that Brg1 mRNA knockdown using Brg1 shRNA will prevent inducible gene expression in macrophages.

VARIATION OF THE ACYLSUCROSE BIOSYNTHESIS PATHWAY CATALYZED BY ACYLSUCROSE-ACYLTRANSFERASE-2 AND 3 IN WILD TOMATO SPECIES
Abigail Miller (Michigan State University)
Category & Time: Biochemistry and Molecular Biology, Section 3, 2:00 PM - 3:00 PM
Poster: 42
Mentor(s): Pengxiang Fan (Biochemistry and Molecular Biology)

The apical cells of glandular trichomes on the leaves and stems of wild tomato plants produce diverse natural pesticides called acylsucroses. These compounds have esterified acyl chains on a sucrose backbone. In the acylsucrose-biosynthesis pathway, there are several steps that contribute to the variety of acylsucroses. The second and third step have functionally different enzymes, Acylsucrose Acyltransferase-2 and 3 (ASAT2 and ASAT3), which introduce the diversity of acyl chains on the sucrose molecule. *S. pennellii* and *S. lycopersicum* have ASAT2 and ASAT3 homologous genes; however, these enzymes have a different function in each species. The evolutionary history behind the functional diversity of ASAT2 and ASAT3 is still unknown. In this research, ASAT2 and ASAT3 were cloned and the activities were tested in multiple wild tomato species to determine the genetic basis for the different function, and to compare this to the geographical location of the accessions. Through site-directed mutagenesis, it was found that there are certain key amino acid residues that can change the substrate specificity of *S. pennellii* ASAT2. These residue changes reflect the regions in South America where the plants originated, outlining the question of where the divergence of this function took place. This indicates that these residues are responsible for the evolutionary story of the acylsucrosebiosynthesis pathway and the diversity of acylsucroses. These findings help elucidate a specialized metabolic pathway, and perform manipulation of key enzymes that help in plant defense.

ASSESSMENT OF DIFFERENT PERMEABILIZATION METHODS OF MINIMIZING DAMAGE TO THE NATURAL KILLER CELLS FOR DETECTION OF EAT-2 AND IRS-2 BY FLOW CYTOMETRY

Minjae Kim (Michigan State University)
Category & Time: Biochemistry and Molecular Biology, Section 4, 2:00 PM - 3:00 PM
Poster: 46
Mentor(s): Sungjin Kim (MMG)

Various fixation and permeabilization techniques have already been developed for detection of intracellular antigens by flow cytometry; however, there are few studies using flow cytometry to detect the frequency of intracellular signaling molecules in natural killer cells, particularly IRS2 and SH2D1B. This research investigates the effect of several permeabilization methods on detection of IRS2 and SH2D1B in peripheral blood. Detecting molecules of interest while maintaining intracellular components intact were the main considerations of the study. The suggested method would be applicable for intracellular detection of IRS2 and SH2D1B by flow cytometry in NK cells.

THE STEREOCHEMICAL AND MECHANISTIC STUDIES OF TYROSINE AMINOMUTASE IN ORYZA SATIVA Zayna King (Medgar Evers College of the City University of New York)
Category & Time: Biochemistry and Molecular Biology, Section 4, 2:00 PM - 3:00 PM
Poster: 47
Mentor(s): Kevin Walker (Department of Chemistry and Department of Biochemistry & Molecular Biology), Tyler Walter (Department of Chemistry)

β-Amino acids serve as building blocks for biologically active compounds and important metabolites. A unique family of aminomutase enzymes contain a methylideneimidazol-4-one (MIO) prosthetic group that helps isomerize α- to β-amino acids. Recently, an MIO-dependent tyrosine aminomutase (*Os*TAM) isolated from Japanese rice *Oryza sativa* was discovered. This is the first aminomutase from a cash crop and the first TAM isolated from a plant. The β-tyrosine product catalyzed by *Os*TAM was derivatized as the *N*-(2-(S)-methylbutyramide) methyl ester to assess the stereochemistry as (3*R*) by gas chromatography/mass spectrometry (GC/EIMS). Deuterium-labeled α-tyrosines were incubated with OsTAM to assess the stereochemistry of the hydrogen abstraction at C_β and rebound at C_α. The β-tyrosines from the deuterium labeling studies were derivatized as ethyl formamide methyl esters and analyzed by GC/EIMS. OsTAM removed the pro-(S) hydrogen and moved it to C_α with retention of configuration (R.O.C.). The R.O.C. pathway was assessed by ^{2}H-NMR analysis of a [^{2}H]-labeled β-tyrosine product. Overall, the *Os*TAM retains the configuration each migration terminus. Further, like other TAMs, *Os*TAM makes a mixture of 3R- and 3S-β-tyrosine with a K_M = 600 μM and a $k_{cat} \simeq 4\ s^{-1}$. However, by contrast, long incubation time (24 h) and changes in pH did not affect the stereoselectivity of *Os*TAM like other bacterial TAMs.

CHARACTERIZATION OF PHOTOSYNTHETIC PRODUCTIVITY AND GROWTH IN ARABIDOPSIS MUTANTS
Linh Pham (Humboldt State University)
Category & Time: Biochemistry and Molecular Biology, Section 5, 3:00 PM - 4:00 PM
Poster: 55
Mentor(s): David Kramer (DOE-PRL), Stefanie Tietz (DOE-PRL)

Changes in environmental conditions, such as water content, CO2 concentration, and light intensities, can trigger many different regulatory mechanisms in plants'photosynthesis. High light intensities, for example, can activate a variety of cyclic electron pathways that control the ratio of ATP/NADPH. The type of pathway triggered is dependent upon factors such as the amount of CO2 available for the Calvin Cycle and amount of water that has been oxidized for the light reaction. Faulty activation of these cycles can lead to accumulation of energy in photosystem-II and high concentration of electrons in the thylakoid membrane, allowing formation of reactive oxygen species (ROS) and destruction of photosystem-II. Therefore, a plant's ability to control these mechanisms and express this control in its high photosynthetic productivity under varying environmental conditions is capable of adapting to and repairing damages from environmental stresses very well. In the rapidly changing climate, plants with high photosynthetic productivity in varying conditions can possibly be the key to increasing food and biofuel production. The purpose of this study is to examine ten mutants of Arabidopsis with interesting photosynthetic phenotypes for their photosynthetic productivities and correlation between their productivity and growth. To achieve this purpose, imaging chambers that can capture Arabidopsis' fluorescence under flat and fluctuating light, spectroscopy, PhotosynQ, confocal microscopy, and biomass measurements are used.

SUBSTRATE PREFERENCES IN CYCLIZATION BY POPB
Miranda Smith (Michigan State University)
Category & Time: Biochemistry and Molecular Biology, Section 6, 3:00 PM - 4:00 PM
Poster: 61
Mentor(s): Mike Sgambelluri (Biochemistry and Molecular Biology), Jonathan Walton (Plant Research Laboratory)

Properties such as stability, rigidity, and high membrane permeability make cyclic peptides promising pharmaceutical tools. The Walton Lab has identified prolyl oligopeptidase B (GmPOPB) as the enzyme responsible for macrocyclization of α-amanitin from the linear propeptide GmAMA1. α -Amanitin, a bicyclic octapeptide found in *Galerina marginata*, is a member of a class of compounds known as amatoxins, which are responsible for most fatal human mushroom poisonings. In order to produce novel cyclic peptides, variations of linear peptide GmAMA1 have been produced in E. coli and screened for cyclization by GmPOPB. LC-MS analysis of the reaction products showed that GmPOPB is capable of producing a wide array of cyclic octapeptides, but there was decreased efficiency when certain residues were altered. This analysis will serve as a guide in producing combinatorial libraries of novel cyclic peptides to be screened for useful activities.

BRANCHED-CHAIN AMINO ACID BIOSYNTHESIS IN ARABIDOPSIS THALIANA
Sarah Sprenger (Michigan State University)
Category & Time: Biochemistry and Molecular Biology, Section 6, 3:00 PM - 4:00 PM
Poster: 63
Mentor(s): Anqi Xing (BMB)

Unlike animals, plants are able to produce Branched-Chain Amino Acids (BCAAs) isoleucine, leucine and valine. Because plant-based diets may lack sufficient BCAAs, it is

important to understand the regulation of their biosynthesis. The committing enzymes Isopropylmalate Synthase(IPMS), Acetohydroxy Acid Synthase(AHAS) and Threonine Deaminase(TD) that catalyze BCAA biosynthesis are feedback inhibited by one or more end products. In a previous study, we identified Arabidopsis thaliana feedback insensitive mutants of IPMS1 and AHAS_S1. Mutants showed significant changes in Leu and Val, however Ile levels was only marginally affected. This is consistent with the hypothesis that Ile biosynthesis is regulated separately by TD. In this study, the Ile toxic analog O-methyl-threonine (OMT) was used to select TD feedbackinsensitive mutants. Screening identified four mutations on TD, one on AHAS_SI, and three mutants with unkown mutations. Progenies of mutants were sowed on plates containing different OMT concentrations and all mutants showed OMT resistance. Two TD mutants which showed strong OMT resistance, OMTr6 and OMTr11, were selected for further characterization. Segregation analysis with mutant (Col-0)/Ler F2 populations genetically validated the TD mutations in OMTr6 and OMTr11 as causal mutations. Amino acid profiling was performed on OMTr6 and OMTr11 seedlings, which were found to have an average of 102-fold and 30-fold increase in Ile respectively. OMTr6 also had a 3.68-fold increase in Leu, while OMTr11 had no significant changes in other BCAAs. Eventually, the TD mutants will be crossed with previously identified IPMS1 and AHAS_S1 mutants to further understand the regulation of BCAA biosynthesis.

CHARACTERIZATION OF MEMBRANE PROTEIN TSPO FROM THE CYANOBACTERIUM FREMYELLA DIPLOSIPHON

Zachary WareJoncas (St Olaf College)

Category & Time: Biochemistry and Molecular Biology, Section 7, 3:00 PM - 4:00 PM
Poster: 66
Mentor(s): Andrea Busch (Biochemistry)

The tryptophan rich sensory protein TSPO is membrane protein found in many species across all kingdoms. TSPO appears to be involved in early stress responses for many species and is implicated in the binding of steroid compounds and tetrapyrroles. In the oxygenic photosynthetic cyanobacterium Fremyella diplosiphon TSPO (i.e. FdTSPO) is regulated by light quality, becoming upregulated in green light conditions. FdTSPO functions in early responses to abiotic stress and binds tetrapyrroles. Here, we aim to determine the molecular function of FdTSPO through assessment of its ligand binding characteristics.Heterologous expression of FdTSPO in E. Coli has been undertaken. Optimization of protein expression and purification conditions/methods and protein analyses are engaged to ensure isolation of native state protein for binding assays. In addition to examining the natural FdTSPO protein variant, the protein is being mutated to isolate variants with conserved motifs in mammalian TSPO which are known to be involved in ligand binding. Once the expression and stability of both the natural protein and mutant variants have been maximized, ligand binding assessment will be performed using tryptophan fluorescence assays.These efforts are anticipated to contribute to a more complete understanding of the function of the FdTSPO protein, specifically in terms of ligand binding capability. Additionally through comparisons of natural vs. mutated Fremyella TSPO variants we seek to determine key structural features required for ligand

binding. Ultimately, we hope to contribute to an understanding of the evolution and divergence of the TSPO protein across kingdoms.

GENE KNOCKDOWN IN MORMYRID ELECTRIC FISH USING SPLICE-BLOCKING MORPHOLINO

Sophia Sdao (Michigan State University), Fernando Fernandez (University of Puerto Rico Cayey)

Category & Time: Integrative Biology, Section 6, 3:00 PM - 4:00 PM
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Mentor(s): Jason Gallant (Integrative Biology)

The electric organ (EO), used for predation as well as navigation and communication, has convergently evolved from skeletal muscle in six separate lineages of fishes. Recent studies have begun to investigate the molecular mechanisms of these organs; however tools to manipulate gene function in vivo are presently lacking. Toward this end, we sought to interfere with a gene of known importance in the EO: the sodium channel gene scn4aa. Scn4aa is normally expressed in the muscles of teleost fishes, but is expressed only in EOs of electric teleost fishes, where it has undergone significant positive selection affecting ion channel kinetics. In this study we developed a splice-blocking vivo-morpholino that specifically targets the scn4aa mRNA and examined its effects on electric organ discharge (EOD) production. We injected Brienomyrus brachyistius daily for four consecutive days with either morpholino (N=2) or saline (N=2) and assessed EOD amplitude. We found a significant decline in EOD amplitude over four days of treatment with morpholino compared to saline. To verify that the morpholino had the intended effect, we performed RT-PCR. We expect that in morpholino-treated individuals that the RT-PCR product will be longer than in saline-treated individuals, because the morpholino forces the inclusion of a normally spliced intron. We will then verify the identity of the amplified RT-PCR products by cloning and sequencing. The results of this study outline and validate a simple and relatively inexpensive approach (i.e. vivomorpholinos) for interrogating the role of specific candidate genes in the evolution, development, and physiological function of EOs.