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Collaborative Research Projects

Collaborative Projects are research projects that engage University and Wyoming Community College researchers and students in projects with the potential to produce publishable results and develop into fundable programs at NIH and other federal agencies. The projects engage undergraduate students at the collaborating institutions and graduate students and postdocs at UW in the collaboration and facilitate the development of undergraduates into independent researchers. Projects also help undergraduates identify and pursue pathways to the baccalaureate degree and graduate training in the biomedical sciences.

Current INBRE Phase 4 Collaborative Projects

Nic Blouin, University of Wyoming-Laramie and David Tanner, Western Wyoming Community College. Microbiome Analysis of Bumble Bee Castes. The current proposal is for an analysis of the microbiomes of queen and worker castes in bumble bees. Bumble bee colonies will be maintained at Western Wyoming Community College (WWCC), and WWCC undergraduate students will be responsible for extracting prokaryotic and eukaryotic DNA. INBRE resources will be leveraged to conduct bioinformatics analysis of microbial community and train WWCC students in computational biology. The results from this study will be published in peer-reviewed journals and used in to further investigate caste determination in eusocial bees. This proposal will enhance WWCC's ability to incorporate research into undergraduate curriculum through the purchase of equipment and association with INBRE Bioinformatics Core faculty.

Brian Cherrington, University of Wyoming- Laramie and Florence Teulé-Finley, UW Casper. The Effects of Citrullination on Tubulin Function. Multiple posttranslational modifications (PTMs) occur on the cytoskeletal protein tubulin, giving rise to the idea of the "tubulin code". One important, yet understudied, PTM of tubulin is the conversion of positively charged arginine residues to neutral citrulline by a family of enzymes termed peptidylarginine deiminases (PADs). Despite our preliminary data showing that tubulin is highly citrullinated, the functional significance of this PTM on tubulin function is unknown. This gap in knowledge underlies a significant biomedical problem because alterations in tubulin arginine residues are known to give rise to abnormal brain development and contribute to neurodegenerative diseases later in life. Our long term goal is to understand how citrullination impacts tubulin structure and function. The objective of this proposal, which is the first step in pursuit of that goal, is to determine the impact of citrullination of specific arginine residues on tubulin polymerization kinetics and binding of the motor protein kinesin. Our central hypothesis is that citrullination alters tubulin kinetics and kinesin binding, with specific arginines having important effects on each. Our hypothesis is formulated based on our preliminary data showing that tubulin is highly citrullinated in multiple cell types. We will test our central hypothesis with the following specific aims: (1) Determine if citrullination alters the polymerization kinetics of and kinesin binding affinity for purified human tubulin; (2) Determine whether specific arginine residues are required for changes in tubulin polymerization kinetics and kinesin binding affinity. In Aim 1, recombinant assembly competent human tubulin will be purified using insect cell culture, in vitro citrullinated, and then examined for changes in polymerization rates and kinesin binding compared to non-citrullinated tubulin. Proposed studies in Aim 2 will mutate specific arginine residues in tubulin to lysine then examine the same parameters as described in Aim 1. We expect that our results will show that citrullination is a critical PTM that has significant

affects on tubulin function. Our work is innovative because we use a novel insect cell model to isolate non-modified tubulin which circumvents the use of commercially available tubulin that displays very high levels of citrullination. The work is significant because it is an important step to characterize a completely novel PTM mechanism underlying the tubulin code.

Maysam Mousaviraad and Danielle Bruns, University of Wyoming-Laramie and Bud Chew, Western Wyoming Community College. Animal and Computational Models for Progression of Cardiac Dysfunction between the Right and Left Ventricles. Heart failure (HF) is an enormous public health problem with staggering morbidity, mortality, and healthcare costs. A heterogeneous clinical condition, HF can develop in the left or right ventricle (LV or RV), ultimately manifesting as a heart which cannot deliver oxygenated blood to the periphery. While most studies have focused on LV, the two ventricles do not adapt to stress in the same manner. The biomechanics of the two ventricles and their interactions in response to pressure overload are not yet clear, which has limited therapeutic success. Progress towards achieving a comprehensive understanding of the essential nature of many of the adaptive phenomena is the goal of this project, which necessitates highly interactive collaborations between experimental and modeling experts. We propose a basic science comparison of the physiology and mechanics which underlie the development of pressure overload in the LV and the RV. We hypothesize that the two ventricles will respond to pressure overload in distinct ways, which will be evident both in overall ventricular function and remodeling, as well as in biomechanical interactions. Computational modeling will identify and quantify the fluid dynamics differences between the two ventricles and their unique responses to pressure overload. We hypothesize that the computational simulations will be sensitive to even slight changes in cardiac functioning, and will be an early indication of cardiac deterioration. This collaborative grant proposal will accelerate an ongoing collaborative endeavor between Drs. Chew (WWCC), Mousaviraad (UW Engineering) and Bruns (UW K&H), advancing each investigator's research productivity and the collaborative capacity for publication and external funding applications. Further, it will involve the training of WWCC students, undergraduate and graduate students at UW in both K&H and Engineering. These students will learn small animal surgical techniques, ultrasound/echocardiography, Doppler Flow Velocity Analysis, histology/biochemistry and computational modeling and post-processing.

Peter Stahl, University of Wyoming-Laramie and Chris Wenzel, Eastern Wyoing College. Passive Motility by Extracellular Matrix Production by Algae in Biotic Crusts. This proposal describes a collaborative project proposing the identification of algae in arid and semiarid biotic soil crusts and elucidation of the role of extracellular matrix production during their life cycle. Building upon preliminary results with Scenedesmus deserticola, a crescent-shaped green algae isolated from biotic crusts on San Nicolas Island (Califormia), demonstrate free living cells are capable of attaching to and moving through confining microarchitectures. S. deserticola accomplishes this by growing celluose filaments from each of its poles. The cellulose filaments can attach to silica-like surfaces, slowly and arbitrarily displacing the cell through its environment as they are extended. The result of this behavior is a passive, almost incidental motility by an otherwise non-motile organism. This project is focused upon the specific question of how this motility may lend S. deserticola an advantage within its natural ecological niche, the complex ecosystems of desert biotic crusts. The research plan is focused around two hypotheses: first, elaboration of copious adhesive extracellular matrix serves to bind soil particles, adding cohesiveness to the crusts and, secondly, the passive motility of S. deserticola serves to distribute individual cells throughout the crust, both horizontally and vertically, allowing the exploration and establishment of symbiotic niches which may be beneficial to the cycling of carbon and nitrogen. The research plan of this proposal focuses upon these fundamental questions, which are directed toward improving soil remediation by reintroducing native microbes and developing biofuel and pharmaceutical uses with non-traditional algae species. The task plan emphasizes undergraduate student involvement combined with field sampling of Wyoming soils to search for similar organisms in local biotic crusts, elucidating the influence of adhesion and motility on algae growth, and exploring motility as an influencer of niche selection.

Past INBRE 3 Collaborative Projects

Brian Cherrington, University of Wyoming- Laramie and Florence Teulé-Finley, UW Casper. The Effects of Citrullination on Tubulin Function. Tubulins are major components of the eukaryotic cytoskeleton and assemble into microtubules that are required for essential cellular process such as intracellular vesicle trafficking. Molecular defects in tubulins are known to cause abnormal brain development and neurodegeneration later in life. Post-translational modifications (PTMs) dynamically alter tubulin stability, polymerization kinetics, and function. Although the effects of PTMs of tubulin such as phosphorylation and acetylation are well documented, the effects of citrullination on tubulin have never been investigated. Citrullination is the post-translational conversion of arginine to citrulline by peptidylarginine deiminase (PAD) enzymes, which alters charge resulting in changes in protein structure and function. Our preliminary work shows that not only is tubulin citrullinated, but that this PTM may be involved in intracellular vesicle transport.

Intracellular vesicle trafficking requires stable microtubule tracks and the binding of the motor protein kinesin to these tracks. Model systems exist to study the kinetics of tubulin polymerization into microtubules and kinesin binding, but commercially available tubulins are highly citrullinated or very divergent from human tubulin at an amino acid level. To circumvent these problems, we propose to express and purify non- citrullinated, assembly competent human tubulin proteins in insect cell culture. Our novel approach is also well suited for the mutation of specific tubulin arginine residues allowing for the investigation of the effects on polymerization kinetics and kinesin binding. Currently, the functional implications of citrullination on tubulin polymerization kinetics and kinesin binding are not known. Our proposed work is highly innovative because it will be the first to investigate PAD-catalyzed citrullination of tubulin and is extremely likely to lead to a mechanistic understanding linking citrullination to important tubulin functions.

Our long term goal is to understand how citrullination impacts tubulin structure and function. The objective of this proposal, which is the first step in pursuit of that goal, is to determine the impact of citrullination of specific arginine residues on tubulin polymerization kinetics and kinesin binding *in vitro*. Our central hypothesis is that citrullination alters tubulin polymerization kinetics and kinesin

binding, with specific arginines having important effects on each. Our hypothesis is formulated based on our preliminary data showing that citrullinated tubulin is present in multiple cell types and our mass spectrometry data identifying specific arginine residues that are citrullinated. Still at issue are the functional consequences of tubulin citrullination. Results from our proposed studies will significantly contribute to our understanding of how the "tubulin code" regulates essential cellular function. The rationale for our studies is that once we know the effects of citrullination of specific arginine residues on tubulin polymerization kinetics and kinesin binding *in vitro*, we can begin to understand the effects of citrullination of tubulin *in vivo* during physiologic and disease states. In order to test our hypothesis and achieve this objective, we propose the following specific aims:

Aim 1: Determine if citrullination alters the polymerization kinetics of and kinesin binding affinity for purified human tubulin. Our *working hypothesis* is that *in vitro* citrullinated purified human tubulin will have altered tubulin polymerization kinetics and kinesin binding compared to non-citrullinated tubulin.

Aim 2: Determine whether specific arginine residues are required for changes in tubulin polymerization kinetics and kinesin binding affinity. Our *working hypothesis* is that specific arginine residues are required for changes in tubulin polymerization kinetics and kinesin binding, and that mutation of these residues will alter tubulin function.

We expect that the outcomes from our proposed studies will for the first time show that citrullination of arginine residues alter tubulin polymerization kinetics and kinesin binding. These novel results will further our understanding of the role of citrullination of tubulin in vesicle transport in normal cellular function and in disease systems. Importantly, our collaborative project will provide hypothesis driven research training for undergraduate students at UW-Casper and UW. UW-Casper faculty and students will actively participate in collaboration with peers and mentors at UW to establish an interstate research project that will result in high impact journal publications. Our ultimate goal is to use the tools and data generated from this collaborative research project to support a major R series NIH grant application in the future.

Bud Chew, Western Wyoming Community College and Maysam Mousaviraad, University of Wyoming. Collaborative Modeling and Experimental Studies for Biomechanics of Cardiovascular Stiffness Alterations. Altered stiffness is important in many cardiovascular diseases. In hypertrophy, it causes impaired systolic function. Heart failure with preserved ejection function (HFpEF), which has no therapeutic options available, is characterized by diastolic dysfunction due to altered myocardial passive stiffness. The biomechanics of these dysfunctions and the amount of stiffness restoration needed to treat the conditions are not yet clear. Experimental and theoretical efforts have provided insight, but complementary computational studies are required before the essential nature of many of the phenomena are understood. Progress towards achieving that comprehensive understanding is the goal of this project, which necessitates highly interactive collaborations between experimental and modeling experts. Chew and Mousaviraad labs have already established collaborations, which will be intensified and structured towards a collective goal through this project. Pressure-volume loops will be measured in Chew lab, and then translated into time-dependent boundary condition inputs needed for simulations by combining the expertise from both labs in analyzing the experimental data. The computational models will be developed in Mousaviraad lab, and then interpreted and validated through combined efforts in analyzing the simulation results and the relationships to the experimental data. Next, Chew lab will design an in-vivo experimental model called transverse aortic constriction (TAC). Measurements of flow and compliance upstream and downstream of the constriction will be collaboratively evaluated to determine the appropriate fluid boundary condition and material response alterations in the computational model of the dysfunctional arteries. The simulation results will then be validated against the experiments and used to gain insight into the physics behind the altered mechanistic behaviors. The last step will attempt to extend the studies to cardiac dynamics and cardiac dysfunctions. The project will train undergraduate and graduate students and will provide support for a former Chew lab undergraduate student to join Mousaviraad lab for graduate studies.

Kerry Sondgeroth, University of Wyoming and Marie Yearling, Laramie County Community College. The influence of gut microbiome on Toxoplasmosis infection and a CURE for students' experimental design abilities. *Toxoplasma gondii* is an obligate intracellular parasite that is present in 30% of humans worldwide and is a significant health threat in immune compromised individuals. *T. gondii* infection is also the 3rd leading cause of food borne illness in the U.S. that requires hospitalization. The parasite is transmitted orally, usually from undercooked meat, and infection remains for the life of the host. Severity of infection and resultant pathology can be variable in the animal host. This variability is thought to be partially dependent upon the parasite strain, however, since infection occurs orally other factors including the microbial composition of the intestine could alter infection outcomes. The purpose of this study is to evaluate the intestinal microbiome during *T.gondii* infection, and determine if differences in the microbiome play a role in the severity of Toxoplasmosis. Specific Aim 1 tests our hypothesis that the intestinal microbiome composition varies during Toxoplasmosis infection, and this variation is associated with disease severity. Groups of C57/BL6 mice will be obtained from three different suppliers. Some mice will be treated with antibiotics prior to infection to eliminate the bacterial component of the intestinal microbiome. Fecal transplantation will be utilized to determine if microbiome composition restores disease phenotype. During the course of infection, mice will be monitored, and tissue samples collected to determine parasite burden. The small intestine will be evaluated for pathology changes, and sequencing of individual mouse fecal DNA will be used to monitor the microbiome composition at different timepoints throughout the study.

Ultimately, this project will provide information in regards to the role of the intestinal microbiome during Toxoplasmosis.

This collaboration immerses undergraduate students in biomedical research and exposes them to a cross-cutting technology used in many biomedical fields. On the Laramie County Community College (LCCC) campus, students will apply the technical aspects of real-time PCR for quantification of mouse tissue samples. However, the project also allows students to gain experience with more sweeping aspects of experimental design. This will serve as a platform to address students' misconceptions in these experimental design areas as identified through the assessment measures described in Specific Aim 2.

Chris Wenzel, Eastern Wyoming College and Pete Stahl, University of Wyoming. Passive Motility by Extracellular Matrix Production by Algae in Biotic Crusts. This project involves the identification of algae in desert biotic soil crusts and elucidation of the role of extracellular matrix production during their life cycle. Building upon preliminary results with *Scenedesmus deserticola*, a

crescent-shaped green algae isolated from biotic crusts on San Nicolas Island (California), demonstrate that free living cells are capable of attaching to and moving through confining microarchitectures. *S. deserticola* accomplishes this by growing celluose filaments from each of its poles. The cellulose filaments can attach to silica-like surfaces, slowly and arbitrarily displacing the cell through its environment as they are extended. The result of this behavior is a passive, almost incidental motility by an otherwise non-motile organism. This project is focused upon the specific question of how this motility may lend *S. deserticola* an advantage within its natural ecological niche, the complex ecosystems of desert biotic crusts. The research plan is focused around two hypotheses: first, that the elaboration of copious adhesive extracellular matrix serves to bind sandy soil particles, adding cohesiveness to the crusts and, secondly, that the passive motility of *S. deserticola* serves to distribute individual cells throughout the crust, both horizontally and vertically, allowing the exploration and establishment of sybiotic niches. The research plan of this proposal focuses upon these fundamental questions, which are directed toward addressing more applied themes of informing soil remediation by reintroducing native microfauna and developing and accessing new industrial markets with non-traditional algae species. The task plan combines field sampling of Wyoming soils to search for similar organisms in local biotic crusts, elucidating the influence of adhesion and motility on algae growth, and exploring motility as a influencer of niche selection.

Bud Chew, Western Wyoming Community College and Wei Guo, University of Wyoming. Restoring Cardiac Function in Failing RBM20-/- Rats. Loss-of-function mutations in RNA binding motif 20 (RBM20) are associated with end-stage dilated cardiomyopathy (DCM) and heart failure (HF) progression [1-7]. Whether restoring RBM20 function in human patients with RBM20 mutations can prevent HF progression remains unknown. Reducing RBM20 levels in a mouse model to knock out the titin (TTN) isoform N2B (KO) results in diastolic dysfunction and improves diastolic stiffness [8], and inhibition of RBM20 in cardiac muscle reduces ventricular wall stiffness in a transverse aortic constriction mouse model, thus improving diastolic function [9]. Thus, restoring RBM20 function should be a feasible strategy for improving TTN-based ventricular wall stiffness. However, no studies have examined the role of RBM20 in HF treatment. Here, we will use the Rbm20 KO rat model to assess whether targeting RBM20 can rescue HF and/or prevent HF progression. Rbm20 KO rats, which are available in the Guo lab, begin to manifest the DCM phenotype at ~6 months of age, with more severe symptoms at ~9 and 12 months. We will restore RBM20 function in these rats using recombinant adenovirus-associated virus (AAV)- mediated gene therapy and will assess changes in heart pathology, cardiac function, and hemodynamics during development. AAV-mediated gene delivery and therapy are efficient and safe, and have been successful in some recent clinical and research applications [10-17]. We will also assess whether cardiac function in RBM20 KO rats can be improved acutely, using sympathetic stimulation via infusion of the neurotransmitter norepinephrine, and the beta-1 agonist isoproterenol. Finally, we will assess whether 30 days of infusion of angiotensin II, which acutely increases blood pressure through vasoconstriction, and chronically results in cardiac hypertrophy and increased cardiac stiffness, interacts with RBM20 mediated HF. Therefore, in this project, we will determine (1) whether restoring RBM20 can improve HF symptoms and/or prevent HF progression, and (2) whether HF caused by RBM20 KO can be improved acutely by sympathetic stimulation and/or angiotensin II. Specific Aim 1: Develop an AAV capable of delivering RBM20 to cardiac muscle in rats. Specific Aim 2: Assess the therapeutic role of restoring RBM20 in HF progression and improvement, using pressure-volume loop analysis, echocardiography, isolated myocardial contractility analysis, and molecular techniques. Specific Aim 3: Assess the acute therapeutic role of sympathetic stimulation and beta-agonists in HF induced by RBM20 KO using pressure-volume loop analysis. Specific Aim 4: Assess cardiac function of RBM20 KO and WT rats after 30 days of infusion of angiotensin II via subdermal osmotic pump, using echocardiography, isolated myocardial contractility analysis, aortic flow probe, and pressure-volume loop analysis.

Sadanand Dhekney, UW Sheridan Agriculture Experiment Station and Vikram Chhatre, WY INBRE Bioinformatics Core, University of Wyoming. Bioinformatics Collaborative Grant: Understanding genetic architecture of stress tolerance in Wyoming grapevine varieties. Grape and its products are a valuable source of antioxidants including anthocyanins and other phenolic compounds that are well known for their health beneficial properties. Global shifts in climate change have resulted in an increase in drought and soil/water salinity worldwide. Such factors can adversely affect grapevine growth, yield and wine quality thereby limiting production in areas otherwise suitable for grape cultivation. The goal of the proposed project is to increase our understanding of grapevine response to salt stress, at the transcriptome level by identifying differences in responses between salt susceptible and tolerant species. We will study changes in gene expression patterns of salt stressed grapevines using whole transcriptome analysis to identify genes and their genetic elements involved in stress regulation and tolerance pathways. The specific objectives of the project are : a) impose salinity stress treatments on salt-susceptible 'Frontenac Gris' and salt tolerant 'Richter 110' grapevine cultivars to isolate total RNA and 2) analyze differences in gene expression of susceptible and tolerant cultivars using RNA sequencing technology. We hypothesize that any potential differences in the salinity response of susceptible and tolerant species can then be used to improve existing grapevine cultivars using precision breeding. Community college students involved in this research project will gain research experience in diverse areas of molecular biology, physiology and bioinformatics. Results from the project will expand our understanding of the response of Vitis species to salinity stress and assist in the genetic improvement of elite cultivars using precision breeding technology.

Ami Erickson, Sheridan College and Nic Blouin, University of Wyoming. Biodiversity discovery & analysis of soil microbial communities: stress response to increased salinity. High salinity soils represent a significant abiotic stress in Wyoming and worldwide with respect to agricultural production, with an estimated 20% of Wyoming agricultural land affected worldwide. Saline water and soil can impact plant productivity by amplifying water stress, causing cellular toxicity or competing with mineral nutrient uptake (Parida and Das, 2005). Up to 50% reductions in productivity are associated with high salinity soils. Vegetable production is a growing industry in Wyoming, but a primary limitation to the ability to expand crop production is the availability and quality of irrigation water. Many regions in Wyoming have groundwater that is high in soluble salts. Increasing ground water removal for development, agriculture operations and energy extraction, as well water loss due to drought and climate change can further decrease groundwater quality by concentrating salts and other minerals. Irrigation with saline water can have longterm negative impacts on soil quality. As water evaporates or transpires from the soil, the salt is left within the soil matrix increasing the salinity of the soil (Legras and Lifran, 2006). Thus, understanding salt-tolerance mechanisms and developing salt-tolerant crops are essential for maintaining the world's food security (Negrão et al., 2017 and references within). Soil microbial communities in the soil specifically associated with plant roots have significant impacts on crop yields and fitness. Where studied, microbial communities associated with plant health have known stress tolerances and these studies suggest that some microbes necessary for plant vigor, normal fruit development, and

quality cannot survive in high saline stress. There has been much research into plant physiology with respect to salinity stress; however, soil community responses, root, and rhizosphere community responses to salinity stress are largely unknown (Yang et al., 1016; Shabsala & Mumus, 2017). We propose to characterize the bulk soil, rhizosphere, and surface sterilized root microbial communities associated with soil irrigated by mountain run-off (Sheridan municipal water, low salinity, LS) and ground water (well water, high salinity, HS) in the Big Horn Basin (Table 1). Microbiome (amplicon) sequencing of bacteria and eukaryotes (e.g., fungi) will co-occur with water salinity measurement, soil chemistry and plant growth metrics to shed light on the response mechanisms induced by salt stress. Analysis will correlate differences in microbial community members with elevated salinity, providing targets for future work in terms of characterizing the functional biology of soils where elevated salinity exists to determine which pathways may be damaged or compensated for in stress conditions.

Marie Yearling, Laramie County Community College and Kerry Sondgeroth, University of Wyoming. How the intestinal microbiome affects the severity of Toxoplasmosis infection. *Toxoplasma gondii* is an obligate intracellular parasite that is present in 30% of humans worldwide and is a significant health threat in immune compromised individuals. *T. gondii* infection is also the 3rd leading cause of food borne illness in the U.S. that requires hospitalization. The parasite is transmitted orally, usually from undercooked meat, and infection remains for the life of the host. Severity of infection and resultant pathology can be variable in the animal host. This variability is thought to be partially dependent upon the parasite strain, however, since infection occurs orally other factors including the microbial composition of the intestine (aka microbiome) could alter infection outcomes. The purpose of this study is to evaluate the intestinal microbiome during infection, and determine if differences in the microbiome play a role in the severity of Toxoplasmosis. Our hypothesis is the intestinal microbiome composition varies during Toxoplasmosis infection, and these variations are associated with disease severity. It has been acknowledged that mice with the same genetic background from different suppliers, react differently during infection, thus we are incorporating the comparison of mice from three different supplies into our experimental design to determine if these differences may be significant. Our hypothesis will be tested with three specific aims. Specific Aim 1: Determine if there are differences in the intestinal microbiomes during Toxoplasmosis infection. The purpose of this aim is to evaluate the intestinal microbiome of mice prior, and during infection with *T.gondii*. The mice will be infected, and monitored for disease severity using multiple parameters including weight, histopathology, brain cyst count, and parasite burden in tissue. The expected results for this aim will be that mice with more severe toxoplasmosis infections will be associated with differences in their intestinal microbiomes in comparison to mice with less severe infections. Specific Aim 2: Determine if the intestinal microbiome is sufficient to alter Toxoplasmosis severity. The purpose of this aim is to eliminate the intestinal microbiome of mice prior to infection, then monitor the changes in microbiome during infection with *T. gondii*. Mice will be treated with a cocktail of antibiotics to eliminate most of their intestinal microbiome, then infected with *T.gondii* and monitored for disease severity as in specific aim 1. The expected results for this aim will be that antibiotic treatment "resets" the microbiome and alters disease severity. Specific Aim 3: Determine if replacement of the intestinal microbiome is sufficient to alter Toxoplasmosis severity. The purpose of this aim is to utilize fecal transplantation to influence disease severity. Three pools of mice feces will be collected, that correspond to each of the suppliers. Mice will be treated with antibiotics to eliminate their original intestinal microbiome, then transplanted with homogenized feces. The mice will acclimate for 2-4 weeks, and then be infected with *T.gondii*. The infection severity will be monitored as in specific aim 1. The expected results for this aim will be that fecal transplantation alters disease severity to more closely mimic the original donor. If we determine that the intestinal microbiome alters *T. gondii* severity, then we can more closely evaluate microbiome interactions with the immune system cells to determine how this alteration occurs. Determining a mechanism for *T.gondii* disease severity could provide innovative therapeutic approaches for both animals and humans diagnosed with this disease.

Ami Wangeline, Laramie County Community College and Nic Blouin, WY INBRE Bioinformatics Core, University of Wyoming. Bioinformatics Collaborative Grant: RNAseq and gene model improvement of *Alternaria astragali.* The current proposal is for ongoing genomic level work on the selenophilic fungus, Alternaria astragali, by sequencing the transcriptome and conducting downstream gene expression analysis. Most fungi are sensitive to selenium. However, A. astragali is not only tolerant, but grows well in the presence of high levels of this element. Overall, this award will enhance our research program by providing a timely and relevant scaffold on which to conduct novel research. Specifically, this project will provide opportunities for undergraduate science majors to build on our previous organismal work by using the genome to answer authentic physiological questions on selenium metabolism in fungi. In addition, pre-health students will have the opportunity to study specific gene expression profiles during in-class work with this project. Genomic DNA has been sequenced for A. astragali, providing a basis for completing the proposed research and allowing progression to examination of the transcriptome, specifically targeting selenium metabolism though comparing expression under Se specific parameters. Further, this genome will be compared to several other Alternaria genomes present in the Alternaria Genomes Database (http://alternaria.vbi.vt.edu) to look for alterations in the sulfur assimilation pathway, unique metabolites, and the presence of enzymes known to harbor selenium in other organisms. Lastly, A. astragali has been found to produce two compounds antagonistic to cancer cells in vitro; having this transcriptome should provide new avenues to elucidate these molecules.

Chris Wenzel, Eastern Wyoming College and John Oakey, University of Wyoming. Passive Motility by Extracellular Matrix Production by Algae in Biotic Crusts. This proposal describes a collaborative project that proposes the identification of algae in desert biotic soil crusts and elucidation of the role of extracellular matrix production during their life cycle. Preliminary results with Scenedesmus deserticola, a crescent-shaped green algae isolated from biotic crusts on San Nicolas Island (California), demonstrate that free living cells are capable of attaching to and moving through confining microarchitectures. S. deserticola accomplishes this by growing celluose filaments from each of its poles. The cellulose filaments can attach to silica-like surfaces, slowly and arbitrarily displacing the cell through its environment as they are extended. The result of this behavior is a passive, almost incidental motility by an otherwise non-motile organism. This project is focused upon the specific question of how this motility may lend S. deserticola an advantage within its natural ecological niche, the complex ecosystems of desert biotic crusts. The research plan is focused around two hypotheses: first, that the elaboration of copious adhesive extracellular matrix serves to bind sandy soil particles, adding cohesiveness to the crusts and, secondly, that the passive motility of S. deserticola serves to distribute individual cells throughout the crust, both horizontally and vertically, allowing the exploration and establishment of sybiotic niches. The research plan of this proposal focuses upon these fundamental questions, which are directed toward addressing more applied themes of informing soil remediation by

reintroducing native microfauna and developing and accessing new industrial markets with non-traditional algae species. The task plan combines field sampling of Wyoming soils to search for similar organisms in local biotic crusts, elucidating the influence of adhesion and motility on algae growth, and exploring motility as a influencer of niche selection.

Eric Atkinson, Dept. of Biology, Northwest College and Matt Carling, Department of Zoology and Physiology, University of Wyoming. Which factors influence the distribution and impacts of diseases in wild birds? Understanding the ecology of infectious diseases has become increasingly critical to the health of both humans and the animals upon which we rely. Similarly, as our climate and the environment continues to change, understanding how those processes may impact the distribution of infectious agents is also becoming more important. Using wild bird populations as a model, we propose a multi-faceted research program with the following aims: 1. Investigate the impact of disease state on fitness-related parameters in Dark-eyed Juncos. 2. Investigate how habitat, land use and elevational gradients influence disease state in wild birds and establish a long-term disease monitoring program across such gradients. 3. Train undergraduates in the scientific process and expose them to techniques and tools useful in myriad biomedical, ecological or evolutionary biology fields. 4. Use the data generated in this proposal to help establish a long-term collaboration between Atkinson and Carling and to submit a proposal to an external funding source. Using a multitude of molecular biological techniques, and incorporating aspects of field-based eco-physiology, our work will investigate how carrying chronic pathenogenic infections influences metabolic parameters and how environmental variation influences the distribution of disease causing agents. The work we propose here will be the bedrock for a budding collaboration between us (Atkinson and Carling), which we anticipate will result in a long-term 'cross-pollination' experiment between students at Northwest College and the University of Wyoming. Not only will this research provide new insights into the ecology of diseases infect wild animal populations, but it will serve as a training program for undergraduates interested in pursuing biology based careers.

Bud Chew, Department of Biology, Western Wyoming Community College and Jun Ren, School of Pharmacy, University of Wyoming. Environmental Exposure and Cardiometabolic Syndrome. The obesity epidemic has gripped the developed world for the past decades and is resistant to various approaches for obesity management. Recent evidence depicts a tie between environmental exposure to hazardous substances and the risk of chronic metabolic diseases. Exposure to ambient air pollution increases the risk of cardiometabolic disease, although this is not well understood. It is speculated that exposure to particulates or volatile organic chemicals (VOC) can induce or exacerbate cardiometabolic diseases, which manifests as insulin resistance, dyslipidemia, obesity and cardiovascular complications. A recent survey of 72,000 individuals living near 258 heavily polluted areas (Superfund sites) reported a significant higher prevalence of type 2 diabetes (T2D) due to VOC exposure. Moreover, individuals working in a polyvinyl chloride plant exhibited a high prevalence of fatty liver disease and insulin resistance. Therefore, exposure to VOC may disrupt cardiometabolic processes, induce or exacerbate obesity, diabetes and cardiovascular complications. Uncorrected obesity is an independent risk factor for cardiovascular disease and is becoming a global health threat. Insulin resistance seems to be a pivotal cause of obesity and T2D. Multiple signaling pathways have been implicated; autophagy has emerged as a critical regulator of insulin resistance. Autophagy is a highly conserved cellular process for degradation of aged or damaged organelles. Our recent studies show that exposure to acrolein, an abundant VOC, interrupts autophagy, insulin sensitivity and cardiac function. Our earlier finding reported that like acrolein, 4-hydroxy-nonenal (HNE), an aldehyde generated by oxidized lipids, is also a potent inhibitor for autophagy and disrupts cardiac homeostasis. Thus, aldehydes generated by VOC-induced oxidative stress or metabolism could be one mechanism by which VOC exposure induce interruption of autophagy. Thus, our central hypothesis is that aldehydes generated upon exposure to VOC diminish insulin sensitivity in heart tissues through autophagy interruption, which triggers a series of metabolic changes that accelerate ectopic lipid deposition, obesity risk and cardiac defects. Collectively, these changes contribute to T2D and increased cardiovascular risk.

Ami Erickson, Department of Biology, Sheridan College and Sadanand Dhekney, Department of Plant Sciences, University of Wyoming. Studying Grapevine Cellular and Physiological Response to Abiotic Stress. The goal of the project is to increase our understanding of Vitis response to drought and salinity stress, which can be potentially applied for improving grapevine abiotic stress tolerance via precision breeding technology. The specific objectives include Specific objectives include 1) evaluate cellular changes and physiological responses to salinity and drought induced water stress, 2) insert the SOS2 and AVP1 genes in embryogenic cultures of target grape cultivars and rootstocks and 3) screen genetically modified grapevines, rootstocks and grafted combinations for drought and salinity tolerance in greenhouse trials. Grape is the 10th most valuable agricultural crop in the United States. Global shifts in climate change resulting from rising temperatures and drought can severely affect grape yield and quality attributes, and limit distribution in regions otherwise suitable for grapevine cultivation. We will study differences in tissue development, leaf water potential, gas exchange and transpiration rates of various grapevine cultivars exposed to abiotic stress. Candidate genes for salt tolerance will be inserted in grapevine embryogenic cultures to generate modified plants that will be screened for abiotic stress tolerance in greenhouse trials. The research would ultimately increase our understanding of vine response to abiotic stress at the cellular level. Information obtained on differences in drought physiology of various cultivars will also serve as a starting point to dissect the underlying molecular mechanisms involved in drought stress and can be utilized for improving grapevine abiotic stress tolerance using precision breeding technology.

Hayley Lanier, Department of Zoology and Physiology, University of Wyoming at Casper and Merav Ben-David, Department of Zoology and Physiology, University of Wyoming. This project will develop a research and teaching collaboration among investigators and students from the University of Wyoming (UW) main campus, University of Wyoming at Casper (UWC) and Casper College (CC) to look at the role of relatedness in habitat usage and spatial overlap among individuals. Building on a long-term study of least chipmunks (Tamias minimus) conducted in the Medicine Bow National Forest, this project harnesses molecular biology and bioinformatics techniques to address fundamental questions in spatial ecology and to train undergraduate students in biomedical techniques and wildlife biology. Since 2006, the UW Wildlife Ecology and Management class (ZOO 4300/5300) at UW (Laramie) has been studying population dynamics in least chipmunks through an annual trapping and tagging project. The proposed population genomics work will add to and build on those student questions, allow the research team to examine at the factors influencing spatial

overlap among individual chipmunks and the fitness implications of that habitat usage. Through the work of students in ZOO 4300/5300 this fall, chipmunks are currently being trapped, tagged, and radio collared. These chipmunks will be radio-tracked daily until they go into hibernation mid-October, providing evidence on shared hibernacula. DNA will be extracted from chipmunk blood samples and reduced-representation genomes (ddRADseq) will be sequenced for each individual. The resulting data will be cleaned, aligned, and analyzed using bioinformatics approaches to evaluate relatedness among individuals relative to habitat overlap, differential reproductive success, and gene flow among habitats. Undergraduate researchers, as well as students in courses in Laramie and UWCasper, and throughout the state, will benefit through either direct participation (wildlife biology techniques, DNA extraction, processing of next-generation sequencing data) or through indirect involvement (e.g., data analysis, class modules on population genomics). The rich dataset that exists though the previous 10-year trapping history and the diverse array of techniques provide a unique opportunity to train undergraduates in scientific inquiry and the development of scientific questions. Not only does this help build their skills as scientists, this experience will help them identify pathways to baccalaureate degrees, biomedical careers, and graduate school. The resulting data will also be used to develop funding proposals for a long-term project integrating research and education directed at an external funding source.

Steve McAllister, Department of Biology, Central Wyoming College and Baskaran Thyagarajan, School of Pharmacy, University of Wyoming. Analysis of mechanisms by which TRP protein activation protects from vascular. Metabolic syndrome comprising of obesity, impaired glucose metabolism, dyslipidemia and cardiovascular complications leads to stroke, a major cause of death worldwide. This necessitates the development of a strategy that can treat high blood pressure, dyslipidemia, hyperglycemia and vascular dysfunctions. Contemporary research demonstrates a role of transient receptor potential (TRP) proteins in the regulation of metabolic syndrome. Specifically, transient receptor potential vanilloid 1 (TRPV1) channel protein expressed in non-neuronal vascular smooth muscle cells (VSMC) has been identified as a new target to treat atherosclerosis and hypertension. In our efforts to investigate the role of TRPV1 in high fat diet (HFD)- induced obesity in mouse model, we discovered that HFD significantly suppressed the expression and activity of TRPV1 and dietary capsaicin (CAP; TRPV1 activator) decreased HFD-induced weight gain. Moreover, treatment with atorvastatin (ATS, 10 micro M; an HMG CoA reductase inhibitor that is used to treat hyperlipidemia) increased the expression of TRPV1 and potentiated CAP stimulated currents and Ca2+ influx in vitro. Consistently, the use of statins to treat hyperlipidemia decreases hypertension and the incidence of stroke in patients with coronary heart diseases. Therefore, we hypothesize that "TRPV1 expression and activation are important for vascular functions. HFD-induced hypercholesterolemia leads to lipid accumulation and oxidative stress to cause hypertension. TRPV1 activation prevents hypertension by upregulating lipolysis and decreasing oxidative stressis". This research proposal is developed to systematically 1). Analyze the effects of CAP and ATS feeding on HFD-mediated hypertension and vascular dysfunctions.; 2). Investigate the effects of TRPV1 activation on PPARα expression and its interaction with sirtuin-1 and 3). Evaluate the mechanism by which ATS potentiates TRPV1 expression and activity in VSMC. The outcome of this work will provide new insight into the role of TRPV1 in the regulation of vascular functions and advance strategies to treat obesity and vascular dysfunctions.

Florence Teulé-Finley, Department of Biology, University of Wyoming at College and Patrick Johnson, Department of Chemical Engineering, University of Wyoming. Generation of Electrospun Spider Silk Nanofiber Mats for Medical Applications. Alternative improved wound dressings that are more suited to a specific application must be investigated to address a real public health need when standard commercial bandages may fall short. Wound dressings promote healing by providing a protective barrier against microorganismal infections. According to the American Burn Association, hospitals in the US treat close to 450,000 patients for burn injuries each year. In 2014, the Center for Disease Control (CDC) reported that 29.1 million or 9.3% of the US population suffered from diabetes and most of these patients may eventually suffer chronic non healing dermatologic/skin ulcers. Additionally, every year, millions of surgical procedures are performed and all of these will generate wounds that need to be treated. The focus of this research is to generate of spider silk-like nanofiber mats from recombinant spider silk-like proteins (SSLP) to provide improved alternative wound dressings for both acute and chronic wounds. These materials may be more suited to a specific application due to their unique structure function relationships allowing a better control over physical factors such as pore size, gas permeation and high surface volume ratio. The first aim of the project will be to optimize the production of selected engineered SSLP variants through recombinant technology in bacteria (Escherichia coli) and to purify these through affinity chromatography. After lyophilization (freeze drying process that preserves the integrity of proteins), these spider silk-like proteins will be electrospun into nanofiber mats under various conditions and the mechanical and chemical properties of these materials formed, as well as their interactions with mammalian cell cultures will be analyzed.
