MENTORS DIRECTORY

2025 SUMMER RESEARCH INTERNSHIP AND FELLOWSHIP PROGRAM

Offered by the

West Virginia IDeA Network of Biomedical Research Excellence
(WV-INBRE)

to be held at

The Joan C. Edwards School of Medicine
at Marshall University

And

The Robert C. Byrd Health Sciences Center
Of West Virginia University

Introduction

WV-INBRE is pleased to offer summer research internships to students from colleges and universities participating in the WV-INBRE program. In 2025, the internship period will be from May 27 through July 29, with the Summer Research Symposium to be held on July 29 in Huntington, WV. Listed in this directory are faculty members at Marshall University and West Virginia University who have agreed to participate as mentors in the summer internship program. Mentors have submitted a description of the projects that are available to interns in their laboratories. Please review these carefully so that you are aware of what is available for summer projects.

A listing of mentors with a short description of their research and the general area of their research is presented on pages 3-8. Mentors and project descriptions begin on page 9. Listed for each mentor is an e-mail address, and where available a phone number and website address.

Application forms are available on the WV-INBRE web site: https://www.wv-inbre.net/summer-program-students

For general questions about the summer internship and fellowship program please contact one of the following individuals who are serving as summer research program coordinators.

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Directory of Mentors – Mentors are listed by their location; the first list contains mentors at Marshall University and the second list contains mentors at West Virginia University

Mentors at Marshall University

Marshall University Mentor Listing According to Area of Research

Addiction: Dickson; Henderson; C. Risher; L. Risher
Bioinformatics: Shakirov
Cancer Research: Amin; Dasgupta**;** Koc; Salisbury; Santanam; Valentovic; Varney
Cardiovascular Research: Bihl; Li; Pierre; Santanam; Tian; Wang
Diabetes: Bihl; Kim
Drug Action, Metabolism, and Resistance: Amin; Santanam; Valentovic
GI Research: Arthur; Lu
Genetic Research: Kim; Shakirov
Infectious Diseases: Bogomolnaya; Long; Varney; Yu
Neuroscience: Dickson; Henderson; C. Risher; L. Risher
Obesity Research: Arthur; Kim; Salisbury; Santanam; Varney
Renal Research: Rankin; Valentovic
Toxicology Research: Rankin; Valentovic

Mentors at the West Virginia University Health Sciences Center

WVU Mentor Listing According to Area of Research

Addiction: Setola
Biochemistry: Deng; Holland; Kolandaivelu; Liu; Webb; Ramamurthy; Robart; Robichaux
Bioinformatics: Hu
Biomedical Magnetic Resonance: Driesschaert
Cancer: Bobbala; Hazlehurst; Ivanov; Lockman; Pugacheva
Cardiovascular: Brown; Chantler; Hollander; Levick; Nurkiewicz; Olfert; Sundararajan; Thapa
Diabetes: Leonardi; Widiapradja
Drug Development: Benedito; Geldenhuys; Robart; Setola
GI: Rajendran
Infectious Diseases: Elliot; Holland
Inflammation: Brown
Nanotechnology: Bobbala; Geldenhuys
Neuroscience: Agmon; Bridi; Geldenhuys; Lewis; Morrison; Nelson; Brefczynski-Lewis; Wan
Obesity: Leonardi
Ophthalmology and Visual Sciences: Deng; Du; Kolandaivelu; Ramamurthy; Robichaux
Pharmacology: Geldenhuys
Pulmonary: Hussain; Nurkiewicz
Reproductive Biology: Bowdridge
Tissue Engineering: Pei

II. Mentors at Marshall University

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Project 1
Chemoprevention of head and neck cancer using curcumin analog FLLL12: Curcumin is a dietary compound isolated from the rhizomes of Curcuma longa, commonly known as "haldi" and has been studied extensively for chemoprevention and treatment. Unfortunately, the clinical success was hindered by its poor absorption and rapid metabolic degradation leading to poor bioavailability. To circumvent the bioavailability issue, researchers are undertaking multiple approaches, including the synthesis of more potent analogs with better pharmacokinetic profiles. My laboratory is developing FLLL12 for the chemoprevention of head and neck cancer. We have already shown that FLLL12 is more potent, has favorable pharmacokinetic profiles, and is mechanistically distinct from curcumin (PMID: 25917567, 26511491, 25910231, 34146588). Grants: R15 (funded), R01: Pending

Project 2
Investigating the mechanism of drug resistance
Cancer is the second leading cause of death in the United States accounting for over 600K deaths per year. Intrinsic as well as acquired resistance to anti-cancer drugs are continuously posing challenges to the success of cancer treatments. Understanding the molecular mechanism of drug resistance is the key to overcoming drug resistance and developing new treatment regimen using a combinatorial approach. Keeping this objective upfront, my laboratory is utilizing *in vitro* cell culture and *in vivo* animal models of lung and head and neck cancers to understand the molecular mechanism of resistance to targeted therapy. My laboratory has identified that some head and neck cancer cell lines overexpress p-Met downstream of Src family kinases (SFK) and these cell lines are resistant to apoptosis induced by the combination of erlotinib (EGFR inhibitor) and BKM120 (PI3K inhibitor). The addition of SFK inhibitor or Met inhibitor sensitizes these cells to apoptosis. The laboratory is currently exploring the downstream targets of SFK-Met signaling.

Project 3
Chemoprevention using natural compounds
Chemoprevention means pharmacological intervention before the development of invasive cancer (full-blown cancer) at a precancerous stage with the hope of slowing down or reversing the carcinogenesis process. However, drug-associated toxicity is one of the major concerns in using drugs in prevention settings since the recipients of the chemopreventive drugs are normal subjects with a high risk of developing cancer. Therefore, those agents with non-toxic or minimal side effects would be ideal candidates as chemopreventive agents. Because of their proven high safety margin through centuries of human consumption as food or as traditional medicines, natural compounds present in fruits, vegetables and spices have drawn special attention for chemoprevention. With this objective in mind, my laboratory is investigating the potential of diet-derived natural compounds such as green tea EGCG, luteolin, resveratrol, curcumin, etc. for chemoprevention of head and neck cancer and lung cancer. The project includes testing in vitro and in vivo efficacy and exploring their molecular mechanism of action.

Project 4.
Developing Novel Therapeutics for Cancers

Most of the currently available chemotherapy drugs are DNA-damaging agents that are effective in eradicating cancer cells. Unfortunately, most of them are very toxic. Moreover, their widespread application is compromised by intrinsic and acquired resistance. In collaboration with Dr. Long, we have synthesized a curcumin analog that is highly potent, mechanistically distinct from curcumin and FLLL12 and induces DNA damage. My laboratory will further explore the mechanism of action and develop this novel DNA-damaging compound for the treatment of cancers.

One student can be recruited for each project. These projects will provide opportunities to learn:

- Mammalian cell culture and maintenance
- Cell proliferation and cell death assays
- RNA and plasmid DNA isolation
- Gene expression analysis by PCR
- Protein expression analysis by western blotting
- Flow cytometry analysis
- Measuring protein and nucleic acid concentrations

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Studies on the regulation of intestinal bile acid absorption in obesity.

Obesity and its associated dyslipidemia are the primary drivers of several chronic conditions such as cardiovascular disease and diabetes, which are the most prevalent health disparities requiring urgent health care priorities among the Appalachian population. Energy imbalance and excessive body fat deposition occurring primarily due to excess food consumption and unhealthy diet are the hallmarks of obesity. Recent studies have shown that altered intestinal secretion of bile acids (BA) that facilitates digestion and absorption of dietary fat could be responsible for excess dietary fat absorption in obesity. Moreover, these BA when in systemic circulation also exert endocrine actions regulating a broad spectrum of physiological effects. BA secreted in the intestinal lumen enter systemic circulation through an intestinal absorptive mechanism mediated by Na-bile acid transporter (ASBT) present in the apical membrane of villus cells. Preliminary studies from my lab showed that in diet-induced rat model of obesity, high fat diet increased ASBT expression prior to the onset and progression of dyslipidemia, indicating that the primary event that initiates the development of dyslipidemia in obesity is the increased intestinal absorption of BA by ASBT. Moreover, ASBT is stimulated not only at the cellular level, but also along the crypt-villus and caudal-oral axes in small intestine. This increase of ASBT at three levels in obesity undoubtedly increases net BA absorption and subsequently contributes to enhanced fat absorption in obesity, indicating that altered ASBT regulation may be central to the pathogenesis of obesity. The ongoing research involves understanding the physiological and molecular regulation of intestinal bile acid absorption mediated by ASBT in the intestine using in vivo rat models of obesity. The outcome of the proposed studies will identify novel intestine-specific target/s to reverse ASBT-mediated increased BA absorption in obesity and thereby help mitigate obesity-associated dyslipidemia and other metabolic disorders.

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The Bihl lab studies the role of extracellular vesicles (EVs) in mediating cell-cell and organ-organ communications including adipose tissue with the brain and gut with the brain. The role of stem cell-released EVs in angiogenesis in stroke and diabetic vascular complications is also studied. Our goal is to develop new therapeutical avenues/compounds addressing cerebrovascular diseases. The research approaches include transgenic mouse models in combination with animal surgeries, such as minipump/microinjection for chronic/acute drug administration, telemetric probe implantation for recording blood pressure and heart rate, and animal modeling for MCAO-induced ischemic stroke and brain injection for hemorrhagic stroke.

Project 1. Role of adipocyte-derived exosomes in cerebrovascular complications of diabetes

Type 2 Diabetes Mellitus (T2DM) is primarily characterized by hyperglycemia accompanied by adipose tissue (AT) dysfunction. More importantly, T2DM is a risk factor for stroke and other cerebrovascular diseases, which might be related to AT dysfunction. Even though AT is comprised of various cells, previous studies have shown that predominantly adipocytes (Adp) dysfunction contributes to diabetes and related complications. Others and our previous research demonstrated enlarged ischemic stroke size and impaired functional recovery in diabetic mice. However, molecular mechanisms linking dysfunctional AT and stroke in diabetes remain unidentified. One of the underlying mechanisms could be the extracellular vesicle-mediated inter-organ communication, particularly through exosomes (EXs). Previous studies have found that Adp-released EXs play a crucial role in the functionality of AT- AT-released secretomes. Recent studies show that Adp-EXs regulate vascular functioning in diabetes via oxidative stress. Also, Adp-EXs have been found to downregulate the expression of various mitochondrial functioning proteins. Therefore, Adp-EXs could be pivotal in mediating crosstalk between dysfunctional AT and the brain to cause worse outcomes of strokes in diabetes. With our long-term goal to unravel novel therapeutic targets for strokes in diabetes, the current proposal is designed to determine how Adp-EXs facilitate communication between AT and the brain by influencing brain endothelial cells (ECs) which are critical targets in the treatment of stroke. We will critically analyze the content and functional changes of Adp-EXs following diabetes and elucidate the mechanism that drives outcome alterations of strokes (cerebral injury and neurological function). Altogether, we hypothesized that diabetic Adp-EXs aggravate cerebral injury and delay functional recovery in strokes by inducing oxidative stress and mitochondria dysfunction.

The approaches for this project include *in vitro* cell culture and *in vivo* animal models as well. For the *in vitro* model, EXs will be isolated from the adipocytes treated with high glucose or normal glucose media. For the *in vivo* model, EXs will be isolated from the adipose tissue from dib/db diabetic mice. The size and concentration will be measured by using NTA, and the contents will be determined by PCR or western blot. The function of different EXs will be tested on ECs w/wo hypoxia/reoxygenation injury.

Project 2. The role of retinoic acid-inducible gene I in strokes

Subarachnoid hemorrhage (SAH), a medical emergency, is bleeding in the space between the brain and the surrounding membrane and is usually from a bulging blood vessel that bursts in the brain. The key goals for all SAH patients are the prevention of rebleeding, delayed cerebral ischemia, and neuroprotection. Recent studies showed that early brain injuries (EBI) occurring within 72 hours after SAH are important factors correlated with poor clinic outcomes, including microcirculatory dysfunction, blood-brain barrier (BBB) disruption, brain edema, neuroinflammation, oxidative cascade, and death of brain cells. Therefore, protection of the brain from SAH-induced EBI is considered a key therapeutic strategy for SAH. Retinoic acid-inducible gene I (RIG-1) is found to participate in inflammatory responses by activating nuclear factor kappa B (NF-κB)/caspase-3 signaling. RIG-1 is found to be expressed in the brain and is involved in neuroinflammation after cerebral ischemia injury by promoting NF-κB signaling pathway activation. Moreover, RIG-I has been shown controlling disorders associated with altered immunity and inflammation in endothelial cells (ECs). Activation of RIG-I significantly impairs EC function and induces the activation of downstream pro-inflammatory signals, which further participate in vascular pathology and BBB function damage. Collectively, inhibition of RIG-1 could be a potential treatment target for SAH by providing brain protection from EBI.

The objectives are to determine the role of RIG-1 in EBI after SAH by focusing on the EC function on BBB through the RIG-1 neuroinflammatory pathway. The approaches for this project include *in vitro* cell culture and *in vivo* animal models as well. For the *in vitro* model, ECs will be treated with oxyhemoglobin (OxyHb) and siRIG-1 to determine the role of inhibition of RIG-1 in protecting ECs from OxyHb-induced injury. For the *in vivo* model, the mice will be subjected to surgery to induce SAH and treated with siRIG-1 to determine the role of inhibition of RIG-1 in protecting the brain from SAH-induced injury.

Project 3. The role of extracellular vesicles in mediating gut-brain communication

Extracellular vesicles (EVs) serve as cell-to-cell and inter-organ communicators by conveying proteins and nucleic acids with regulatory functions. Emerging evidence shows that gut microbial-released EVs play a pivotal role in the gut-brain axis, bidirectional communication, and crosstalk between the gut and the brain. Increasing pre-clinical and clinical evidence suggests that gut bacteria-released EVs are capable of eliciting distinct signaling to the brain with the ability to cross the blood-brain barrier, exerting regulatory function on brain cells such as neurons, astrocytes, and microglia, via their abundant and diversified protein and nucleic acid cargo. Conversely, EVs derived from certain species of bacteria, particularly from gut commensals with probiotic properties, have recently been shown to confer distinct therapeutic effects on various neurological disorders. Thus, gut bacterial EVs may be both a cause of and therapy for neuropathological complications. We have recently established a method to isolate EVs from gut microbiota. Further studies will investigate the contents and function of gut microbiota-released EVs. The function of gut microbiota-released EVs on brain cells, neurons, and astrocytes will be tested *in vitro*.

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Our research focuses on characterization of molecular mechanisms utilized by Gram-negative bacteria *Salmonella enterica* ser. Typhimurium and *Serratia marcescens* to survive host response and to develop better treatment options. In particular, we are interested in the following important questions:

Project #1: Identification of natural functions of drug efflux pumps during infection
Non-typhoidal *Salmonella enterica* serotypes including Typhimurium are the leading cause of bacterial food-borne enteritis in the United States. Until recently, *Salmonella* isolates were highly susceptible to most of the commonly used antibiotics but in the last decade the emergence of multidrug resistant *Salmonella* has been reported worldwide. *Serratia marcescens* is an opportunistic pathogen with increasing clinical importance. *S. marcescens* can cause meningitis, endocarditis, infections of airway and urinary tract, especially in immune-compromised patients. Efficiency of antibiotic therapy for these patients in some cases is extremely low due to the high intrinsic antibiotic resistance of *S. marcescens.* One mechanism for resistance of bacteria to antibiotics is through antibiotic efflux via multidrug efflux pumps. However, little else is known about the natural functions of these pumps during infection. We found that at least one pump called MacAB present in both bacterial species protects them against reactive oxygen species (ROS). We are interested in identification of natural substrates of this pump; how these substrates protect bacteria from ROS and how substrate production is regulated. Our studies will advance our understanding of the natural functions of bacterial efflux pumps beyond excretion of antibiotics and will aid to develop alternative strategies to control bacterial infections and augment conventional antimicrobial therapy.

Project #2: Defining the role of secreted DUF1471-containing proteins in adaptation of bacteria to different environments
Bacteria are able to successfully exist in ever-changing environment. For a quick adaptation to a new niche, bacteria rely on secondary metabolites, peptides and secreted proteins. These molecules can participate in a number of important biological processes: signal transduction within population, production of new compounds (for example, antibiotics), formation of biofilms, and also play an important role in virulence. Gram-negative bacteria from *Enterobacteriaceae* family secrete in the environment a number of proteins containing DUF1471 domain with unknown function and a similar structure. The physiological role of these proteins in maintaining of bacterial viability remains unexplored. We hypothesize that bacteria utilize DUF1471-containing proteins as a network of signals to accelerate adaptation to a new environment. Our studies will be focused on identification of DUF1471-containing proteins needed for survival during infection and during antibiotic exposure.
WV-INBRE participants will receive training in standard microbiological techniques, molecular cloning, generation of mutants, DNA and protein analysis, and animal handling.

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The following projects are available in my laboratory:

1. **Anti-cancer activity of nicotinic antagonists in lung cancer:** Smoking bears a strong correlation to the development of a type of lung cancer called lung adenocarcinoma. In our laboratory we study the signaling pathways of how nicotine and NNK (components of cigarettes) promote the growth of lung cancer. Specifically, students working on this project will examine whether compounds which block the effect of nicotine can be useful for lung cancer therapy. Other techniques the students will learn are (i) to measure the effects of nicotine on the growth of human lung cancer cells (ii) the measure the anti-cancer activity of compounds (that inhibit the effects of nicotine) in human lung adenocarcinoma.

2. **Capsaicin and small cell lung cancer:** Capsaicin is the major active ingredient of chilli peppers. Preliminary data in our laboratory shows that capsaicin can inhibit the growth of human small cell lung cancer cells. We are interested in investigating molecular pathways contribute to this process. If you are interested in this project, you will learn (i) to perform specific assays to determine whether capsaicin can cause cell death in human small cell lung cancer cells (ii) to examine the biochemical mechanisms underlying this growth-inhibitory activity of capsaicin.

TECHNIQUES:

The techniques that are routinely performed in our laboratory:

1. Cell culture techniques
2. Preparation of lysates, nuclear, membrane and cytosolic fractions
3. Assays to study cell growth and cell cycle progression
4. Detection of proteins using Western Blotting
5. Measurement of tumor angiogenesis.
6. Animal studies: anti-cancer studies on nude mice models

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Systems genetics and recombinant inbred mouse panels for discovery of the mechanisms driving drug addiction
Drug addiction is a critical public health issue with genetic and environmental causes for which the underlying biological mechanisms remain largely unknown. To uncover these mechanisms, the Dickson Lab uses construct-valid behavioral techniques within the context of a systems genetics approach. Systems genetics using experimental mouse populations enables discovery of novel genetic and genomic mechanisms influencing disease by associating genetic and phenotypic variation. The intravenous drug self-administration paradigm is the gold-standard of volitional drug use assessment in rodents due to its ability to index drug taking and seeking at many stages of drug use including initiation, maintenance, and relapse. Through integration of a systems genetics approach and construct-valid behavioral techniques such as intravenous drug self-administration, novel and unexpected genetic mechanisms underlying the complex psychological phenotype of drug addiction and behaviors that predict drug use and addiction can be discovered.

Students in the Dickson lab can expect to learn about:

- Systems genetics as an approach to biological discovery
- The importance of genetic diversity in the laboratory mouse in the context of systems genetics
- The use of recombinant inbred mouse panels in the context of systems genetics
- Intravenous drug self-administration as an approach to identify biological and psychological mechanisms driving addiction

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Examining the effect of flavors on vaping-related behaviors

While nicotine is the primary addictive component of all tobacco and vaping products, flavor additives have now been found to alter the neurons that are critical for addictive behaviors. As the use of electronic cigarettes continue to grow, it is critical that we understand how all constituents of vaping e-liquids effect the neurons that mediate addiction. The Henderson lab directly studies how nicotine and flavors trigger addiction. We use mice that are trained to use vaping devices to model human smoking and vaping behaviors. In these experiments we directly study how combining flavors with nicotine can increase drug reinforcement and vaping initiation. We then conduct follow-up experiments to examine changes in neurobiology and neurophysiology. These include the use high-powered fluorescence microscopy to examine structural changes in the dopamine neurons that play a major role in addiction neurocircuitry and electrochemistry to examine functional changes in the release of dopamine in the brain. Together, these experiments allow us to determine how entire brain circuits are modified by vaping constituents and trigger changes that reinforce vaping-related behaviors. For more information, visit the Henderson lab website: www.hendersonlab.org

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Genetics of Obesity, Type 2 Diabetes, and Hyperlipidemia

My research interest is in understanding the etiology and pathogenic mechanisms underlying type 2 diabetes, obesity, and hyperlipidemia, which have strong implications for cardiovascular diseases (CVD). Type 2 diabetes is the most common form of human diabetes, accounting for over 90% of cases and obesity at such epidemic proportions creates serious public health problems. The prevalence of atherogenic dyslipidemia including hypercholesterolemia has increased considerably. Atherogenic dyslipidemia is causally linked to the development and progression of atherosclerotic CVD. There is substantial evidence demonstrating that genetic factors are strongly involved in the development of type 2 diabetes, obesity, and hyperlipidemia, and I have focused my attention on the link between gene dysfunction and these diseases and its interaction with diets. As an internship project in our laboratory for the Summer Research Program, I propose to study candidate genes and pathways for diabetes, obesity, and hyperlipidemia loci identified in a genetic mouse model and their interactions with diets. This study will ultimately provide ready targets for the disease therapies in humans. Experimental methods involved in this internship research will include enzyme-linked immunosorbent assay, colorimetric assay, polymerase chain reaction (PCR), western blot analysis, and real-time quantitative PCR. DNA, RNA and protein will need to be isolated from mouse tissues. Instruments involved in this project include gel electrophoresis, western blotting apparatus, microplate readers, spectrophotometer, imaging system, thermal cyclers, EchoMRI, and comprehensive lab animal monitoring system.

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Ovarian cancer, worldwide, is the most common cause of gynecologic cancer death. Primary treatment consists of a combination of surgical and platinum-based therapy. Despite success in attaining remission in many cases of ovarian cancer, over half of women with ovarian cancer experience of recurrence with chemoresistance and metastasis, specifically in high grade serous ovarian cancer (HGSOC). Interestingly, mitochondrial dysfunction is emerging as one of the major contributors of aggressiveness and chemoresistance in HGSOC due to its central role in energy metabolism.

Energy requirements for tumor growth in epithelial high-grade serous ovarian cancer (HGSOC) are fulfilled by a combination of aerobic glycolysis and oxidative phosphorylation (OXPHOS). Although reduced OXPHOS activity has emerged as one of the major contributors to tumor aggressiveness and chemoresistance, up-regulation of mitochondrial antioxidant capacity has been shown to be required for matrix detachment and colonization into the peritoneal cavity to form malignant ascites in HGSOC patients.

To evaluate modulation of OXPHOS in HGSOC tumor samples and ovarian cancer cell lines, we will perform:

1) Proteomic analyses of proteins involved in mitochondrial energy metabolism and biogenesis and formation of reactive oxygen species (ROS) by immunoblotting and flow cytometry, respectively.

2) Cell culture studies using drugs directed against mitochondrial targets such as those involved in transcription and translation machineries.

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Project 1. Explore thymidine phosphorylase (TYMP)'s role in obesity, metabolic dysfunction-associated fatty liver disease (MAFLD), and atherogenesis.

Obesity is a major independent risk factor for metabolic dysfunction-associated steatotic liver disease (MASLD), type 2 diabetes (T2DM), cardiovascular disease (CVD), and some cancers. Dysregulated lipid metabolism and chronic inflammation as well as epigenetic changes have been recognized as key contributors to the development of obesity and atherogenesis. TYMP is an enzyme in the pyrimidine salvage pathway. Our recent study revealed that TYMP possesses signaling functions and is essential for platelet activation and thrombosis, suggesting that TYMP has unknown functions. This project is to explore TYMP's role in regulating glycolysis, lipid metabolism, obesity, fatty liver disease, and atherogenesis. Interns participating in this project will have opportunities to learn TYMP's role in obesity-associated complications.

Project 2. Explore the role of sodium/potassium ATPase alpha 1 subunit in thrombosis.

Our ongoing studies demonstrated that **sodium/potassium ATPase alpha 1 subunit haplodeficiency** significantly prolonged the time to form an occlusive thrombus in mice. This phenotype was only found in males but not in females, suggesting sex hormones may play a role. This project will test the hypothesis that cross-talk between sex hormones and NKA α1 plays a critical role in regulating platelet activity and thrombosis. Interns participating in this project will have opportunities to learn platelet and plasma isolation, platelet function assay, and in vivo thrombosis models.

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My lab in the Marshall University School of Pharmacy is focused on discovering new treatment strategies for multi-drug resistant infections. We are currently investigating the repurposing potential of disulfiram (Antabuse) to treat vancomycin-resistant *Staphylococcus aureus* and fluconazole-resistant *Candida* infections. In *S. aureus,* it was discovered that disulfiram is able to lower the minimum inhibitory concentration (MIC) of vancomycin to increase it susceptibility to this first-line antibiotic for MRSA infections. Mechanistic studies have revealed that disulfiram functions as an antimetabolite and this action may counteract the vancomycin-resistance mechanism in *S. aureus*. In Candida, disulfiram was found to be a fungicidal agent and have synergism with copper, but through a fungistatic mechanism. The contrasting mechanisms are also being investigated. Researchers who work in the lab will learn techniques to evaluate antimicrobial synergy via the checkerboard assay and time-kill studies. Researchers will further use plate readers, HPLC, PCR and flow cytometer conduct mechanistic experiments.

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Peroxisomes and metabolic liver disease

Chronic alcohol and high fat diet consumption may cause metabolic liver disease designated alcohol-related liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD), respectively. Both ALD and NAFLD range from simple steatosis (fatty liver) to steatohepatitis (liver inflammation), fibrosis and cirrhosis, and even liver cancer. Fatty liver is benign, but it is sensitive to developing to advanced liver disease like fibrosis, cirrhosis and liver cancer. Impaired fatty acid oxidation is one of major reasons for the development of fatty liver. Fatty acids are mainly oxidized in mitochondria, but they can also be oxidized in peroxisomes. Usually, very long chain or side chain fatty acids are metabolized in peroxisomes, and the resultant short chain fatty acids will be further oxidized in mitochondria. Peroxisomal fatty acid oxidation is regulated by a transcriptional factor peroxisome proliferator activated receptor α (PPARα), and PPARα agonist WY-14,643 can induce peroxisome proliferation, which enhance peroxisomal fatty acid oxidation, and ameliorate alcoholic fatty liver. We are examining how peroxisome proliferation influences the development of metabolic fatty liver disease in mouse models.

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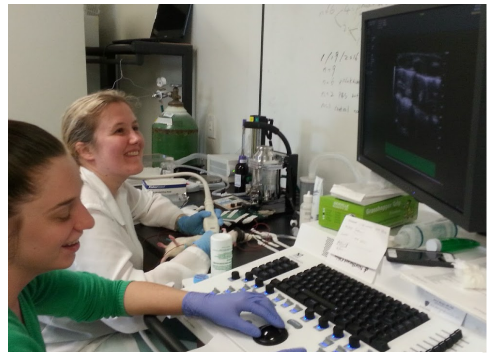
The Pierre lab studies specific intracellular pathways involved in the integrated response of the myocardium to hemodynamic and metabolic disturbances. Our goal is to develop new paradigms to therapeutically address cardiovascular diseases based on the Na/K-ATPase signaling complex. We examine these issues by combining techniques of molecular and cell biology with *ex-vivo* (biochemistry and cell physiology, isolated heart perfusion, primary cardiac cell cultures, histology) and *in-vivo* assessments of cardiac function in genetically altered mice (echocardiography, measurement of blood pressure by tail-cuff and telemetry, cardiac and vascular catheterization). In the interdisciplinary environment provided by MIIR, interns are exposed to the pre-clinical models and key techniques that are currently available to cardiac and vascular physiologists and pharmacologists.



Echocardiographic assessment of rodent cardiac function by Dr. P. Marck and undergraduate fellow A. Bryant.

Project 1. Cardioprotection by Na/K-ATPase ligands in acute myocardial infarction
Rationale: In addition to pumping ions, Na/K-ATPase interacts with neighboring membrane proteins and takes part in signaling complexes to send messages to various intracellular organelles. We believe that understanding these pathways and targeting the Na/K-ATPase receptor function will lead to novel interventions for the treatment and prevention of ischemia and reperfusion injury.
Method: the INBRE fellow will learn the isolated Landendorff-perfused mouse heart preparation and expose it to novel compounds targeting the Na/K-ATPase cardioprotective signaling pathway. This includes analysis of contractile function in real time and assessments of activation of the Na/K-ATPase cardioprotective pathway biochemically. The effectiveness of promising compounds will be further tested *in vivo* following experimentally-induced acute myocardial infarction (AMI). Mice will be subjected to an acute occlusion of the left descending anterior artery (LAD) for 30 min, and cardiac function and remodeling will be monitored after 1 and 2 weeks of reperfusion. In addition to functional echocardiographic assessments, the fellow will conduct morphometric and histological studies as well as biochemical (western blot) and qPCR evaluation of fibrosis, inflammation, and hypertrophy markers.

Project 2. Role of α1 Na/K-ATPase in adverse cardiac remodeling and heart failure
Rationale: Heart failure (HF), a chronic incurable illness, is the common end-stage of heart diseases caused by an array of highly prevalent conditions such as hypertension and coronary heart diseases. A greater and broader protection must be achieved to face the unmanageably high HF morbidity and mortality rates amidst the exploding incidence and prevalence of the condition worldwide. Targeting the Na^+/K^+-ATPase receptor function may lead to novel interventions
Method: Using our newly developed model of cardiac-specific KO of Na^+/K^+-ATPase α1, we will assess the role of Na^+/K^+-ATPase α1 in the development of hypertrophy, fibrosis and heart failure in mice subjected to Angiotensin II infusion by osmotic minipumps. In addition to functional echocardiographic assessments, the students will conduct morphometric and histological studies as well as biochemical (western blot) and qPCR evaluation of fibrosis, inflammation, and hypertrophy markers.

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The following projects are available in my laboratory:

Project #1: Chloroanilines are commonly used chemical intermediates in the manufacture of dyes, drugs, agricultural herbicides and fungicides and thousands of other products. Exposure to a chloroaniline can result in a number of toxicities including toxicity to the blood, liver and kidney. This project seeks to determine the chemical species (parent compound or metabolite) responsible for liver and kidney damage and the mechanism by which toxicity occurs.

Project #2: Halogenated benzenes and phenols are common intermediates in the synthesis of a wide range of commercial products and appear as environmental pollutants in many parts of the world. Many of these compounds and/or their metabolites target the kidney and can induce kidney injury. This project will examine the nephrotoxicity induced by these compounds, examine structure-toxicity relationships as well as mechanisms by which these important chemicals harm the kidney.

Assays and Instrumentation: Projects that will investigate nephrotoxicity will use in vitro assays that involve isolation of rat kidney cells, measurement of enzyme release from treated and control cells, and potentially, the measurement of cellular ATP levels and other mitochondrial functional parameters. Additional techniques may involve Western blotting, quantifying urinary contents (protein, glucose), and measuring blood urea nitrogen and glucose levels Instrumentation will primarily involve the use of balances, centrifuges, and UV-visible spectrophotometers. High pressure liquid chromatography and thermocycler use is also possible.

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Recently, much progress has been made towards understanding how neurons, the cells responsible for the processing and transfer of information in the central nervous system (CNS), interact with non-neuronal brain cells. However, we have still only begun to scratch the surface about how non-neuronal cells contribute to the structural and functional maturation of the neuronal junctions known as synapses. Our work focuses on identifying and elaborating the genes, molecules, and signaling pathways that are crucial for linking non-neuronal cells with the synaptic structures that have been shown to be severely disrupted in nearly all known neurodevelopmental and psychiatric disorders. The long-term goal of our research is to contribute to novel therapeutic strategies to prevent or repair the impaired synaptic connectivity that occurs during abnormal brain development and following CNS injury or insult.

Two primary projects are currently ongoing in the Risher lab:

1) Astrocytes, the primary glial cell type in the brain, secrete a variety of factors that promote synaptogenesis during development and after injury. One family of synaptogenic proteins, the thrombospondins (TSPs), acts through a neuronal receptor, calcium channel subunit α2δ-1, which is known to be altered in some patients with epilepsy, intellectual disability, and autism. Our recent work using rodent models revealed that the interaction between TSP and α2δ-1 differentially promotes synaptic connectivity between males and females. We are currently investigating the genetic, cellular, and molecular mechanisms that underlie these differences, as well as determining the functional relevance of this disparity between the sexes.

2) Neonatal abstinence syndrome (NAS) is a devastating consequence of the national opioid epidemic that is showing striking incidence rates in West Virginia and Central Appalachia. NAS infants are essentially born with an addiction to opiates, and they enter an intense state of withdrawal after cessation of placental exchange-mediated drug exposure. The babies require constant supervision and, approximately 50% of the time, pharmacological intervention before being able to be discharged from the NICU. The long-term effects of NAS on cognition and behavior are predicted to be numerous, but there is currently not much known about how prenatal opioid exposure affects brain development. We have strong preliminary evidence that astrocyte-mediated synaptogenic signaling is among the developmental processes that are significantly disrupted with prenatal opioid exposure (POE). We are now conducting experiments to try to understand the extent to which prenatal opioids influence the formation and maturation of synaptic circuitry in cell culture and rodent models of NAS.

In the Risher lab, students will be exposed to a variety of cellular, molecular, genetic, and imaging techniques. Commonly used methods include animal handling (mouse/rat), primary cell culture, organotypic brain slice culture, Western blotting, immunohistochemistry, plasmid DNA transformation and transfection, confocal microscopy, electron microscopy, 3D reconstruction-based image analysis, genotyping, viral vector work, and single-cell RNA sequencing. Students will have the opportunity to meet regularly with Dr. Risher as well as in a group setting such as our monthly lab meetings.

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Our laboratory is interested in understanding how adolescent binge drinking influences brain function and contributes to the development of alcohol use disorder. Using a rodent model of adolescent binge drinking, our laboratory and others have demonstrated that there are acute and long-term changes to neuronal structure, function, and behavior across multiple cognitive domains.

Over the last few decades, it has become apparent that non-neuronal cells called astrocytes which outnumber neurons and ensheathe many neuronal connections, play an important role in synapse formation, synapse maintenance across the life-span, and synaptic recovery following injury. However, how astrocytes contribute to neuronal and synaptic remodeling following ethanol exposure is not fully understood. Understanding how astrocytes contribute to the long-term effects of adolescent binge drinking in a rodent model is crucial for understanding the impact that underage alcohol exposure can have on the adult brain and how early onset drinking may contribute to the development of alcohol dependence later in life.

We have three ongoing projects: 1. Investigating the acute and long-term effects of binge drinking on astrocyte function. 2. Investigating the role of astrocytes in the development of addiction. 3. Investigating how changes in astrocyte function following adolescent binge drinking influence recovery from secondary injury later in life, e.g., following traumatic brain injury. Techniques used to answer these questions include: intracranial survival surgery for injection of adenoassociated viruses and insertion of optic fibers for optosensors to evaluate calcium and neuro/gliotransmitter release, immunohistochemistry, Western blot, qPCR, neuronal-astrocyte primary co-culture, confocal microscopy, 3D morphometric analysis of astrocytes, and a battery of behavioral paradigms including conditioned place preference, fear conditioning, open field, social interaction, and plus maze.

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Obesity increases the risk for 10 different cancers including breast cancer. We have shown that adipose tissue from the breast tumor microenvironment releases factors that induce signaling in breast cancer cells that stimulates cancer cell migration and invasion. We are investigating the signaling mechanisms by which obesity associated secreted factors stimulate breast cancer cell migration and invasiveness. We hypothesize that the primary pathway involved is the mTOR pathway. Students in my lab would have the opportunity to study these questions in several lines of human breast cancer cells. Our methods are largely molecular biology based; therefore, students would have the opportunity to use real time PCR machines, electrophoresis equipment, and laminar flow tissue culture hoods. Students will also have a choice as to what technique they would like to learn during their internship. Techniques in lab will include, but are not limited to, real-time PCR, western blot, chromatin immunoprecipitation analysis, interfering RNA approaches to gene knockdown and proliferation assays.

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The following projects are ongoing in my laboratory:

Project 1: Vaping and exercise: Vaping is highly rampant among young individuals. This study will test the effects of vaping on cardiometabolic markers. This study will also test if exercise can help these individuals from some of the harmful effects of vaping.

Project 2: Heart fat and health: Obesity is very high in West Virginia. There are several fat tissues in the body including the one that is in or around the heart. We are studying the heart fat from patients to understand its role in cardiovascular disease.

Project 3: New pain medications: There are millions of individuals who suffer from chronic pain. The current treatments that they are provided are not effective. Our lab is researching alternatives to the current medications for pain.

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Our research interests focus on telomeres, the evolutionarily conserved protein-DNA complexes that cap linear eukaryotic chromosomes, promote genome maintenance and regulate cellular lifespan. Telomere length shortens with each somatic cell division and is often viewed as the most accurate cellular marker of biological age. Proper maintenance of telomere length has important implications for aging, stem cell-related diseases and cancer. Although considerable variation in mean telomere length exists in yeast, plants and humans, mechanisms underlying telomere length homeostasis are largely unknown

Project 1. Genetic and epigenetic architecture of natural telomere length variation.
The main objective of current research in the lab is to elucidate the genetic and epigenetic causes of telomere length variation using the genetically facile plant Arabidopsis thaliana as a model. To achieve this goal, we use a plethora of cutting edge natural variation resources available for this organism and a collection of powerful molecular, genomic and epigenetic tools. We recently identified a major effect QTL that explained 48% of telomere length variation in recombinant inbred Arabidopsis populations, with the underlying natural polymorphism mapping to the NOP2A gene. Mutations in mammalian NOP2 orthologs lead to uncontrolled proliferation of cancer cells, and their expression serves as a prognostic marker of tumor development. INBRE program participants will work with laboratory personnel on understanding the role played by NOP2A and other genes in telomere length control. Our studies will have an impact on understanding genetic differences in telomere length between individuals and populations, and may provide novel insight into the molecular basis for different rates of aging and predisposition to telomere-associated stem cell, cancer and age-related diseases.

Project 2. Analysis of the interplay between telomere biology and ribosome biogenesis.
We have recently identified several components of rRNA maturation machinery, including RPL5, that impact species-specific telomere length set point in plants. Interestingly, human *RPL5* inhibits tumorigenesis, and its inactivation is the most common (11-34%) somatic ribosomal protein defect in multiple tumor types. Indeed, important similarities exist between human diseases known as telomeropathies and ribosomopathies, and our findings argue that components of rRNA maturation machinery may impact species-specific telomere length set point across eukaryotic evolution. IMBRE participants will work with mutants of ribosome biogenesis genes in plants to uncover specific mechanisms linking telomeres with ribosome biogenesis.

All participants will receive training in molecular cloning, RNA, DNA and protein analysis, aspects of genetic manipulations and bioinformatics.

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Our laboratory is focused on exploring new interventions that will reduce the adverse effects of drugs. We have recently focused on examining ways to reduce the toxicities of cancer chemotherapy agents. Projects available in my lab:

Project #1. Reducing serious cancer chemotherapy side effects. This is an ongoing project that has been funded by a federal grant from NIH. Our laboratory is evaluating new compounds that may reduce the adverse effects experienced by individuals treated with cancer chemotherapy drugs. All cancer chemotherapy agents induce side effects and reducing these side effects will allow a better quality of life for the individual and potentially improve the success of the cancer chemotherapy agent. A long-term goal is to develop methods to improve the effectiveness of the cancer chemotherapeutic agents while lessening the side effects. This project has clear clinical relevance and is translational. The drugs we are exploring are used in controlling breast, lung, ovarian cancer and leukemia. An individual involved in this project will investigate cellular changes in toxicity, specifically we want to explore changes in the mitochondria as well as post-translational modifications of proteins caused by exposure to cancer chemotherapy drugs including doxorubicin, cisplatin camptothecin or irinotecan.

Project #2. Potential role of e-vape flavoring agents in renal impairment. Vaping products have a complex series of flavoring agents. Recent studies have shown alterations in genetic expression in the lung, kidney, brain and liver following vaping. We will examine changes mediated by flavoring aldehydes on human renal proximal tubules. This project will examine the impact of flavoring aldehydes on mitochondrial proteins critical in generation of ATP.

Project #3. Examination of the mechanism of renal damage by an antiviral agent used in in treating HIV and hepatitis B patients. Patients with HIV or hepatitis B must take antiviral agents to slow the progression of their disease. These drugs are taken for very long period of time even years. Side effects often occur after someone takes an antiviral agent for over 1 year. We are examining the mechanism of damage to the kidney by a commonly used antiviral agent. We are using a normal human proximal tubular epithelial cell culture model for this study. We have preliminary results to suggest certain agents can reduce the side effects of the antiviral agents but would not impact the pharmacologic activity.

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1. **Determining how obesity contributes to initiation and progression of myelodysplastic syndromes (MDS), which are blood and bone marrow cancers.** MDS are blood and bone marrow cancers that are often caused in part by overactive inflammation in hematopoietic stem and progenitor cells. Obesity has been linked to MDS and acute myeloid leukemia (AML) but has not been studied in our double knockout (Tifab and miR-146a KO) mouse model of MDS/AML. We will perform studies such that MDS susceptible mice are subjected to control and Western diet to determine mechanisms by which obesity contributes to initiation and progression of disease. We anticipate mechanisms to involve diet/obesity-driven changes in hematopoietic stem and progenitor cell differentiation. We will also plan to determine if this can exist as an epigenetic effect (Do offspring of parents on poor diet have increased susceptibility to MDS initiation and/or progression?)
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2. **Defining the mechanisms by which obese individuals are more susceptible to infection and have lowered vaccine efficacy.** We hypothesize that this occurs through dietary and obesity-driven effects on hematopoietic stem cells, which we have found to be important in providing immune protection against pathogenic threats. We are investigating multiple vaccines in this study. We will also determine if this can exist as an epigenetic effect (Do offspring of parents on poor diet have decreased vaccine-induced immune protection against infectious disease?).
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https://pubmed.ncbi.nlm.nih.gov/30405604/

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Vascular disease is one of the major complications of diabetes and hypertension in the United States. Extracellular vesicles (EVs), including exosomes (EXs) and microvesicles (MVs), are emerging as a novel mechanism of intercellular communication. Increasing evidence suggests that EVs could convey proteins/genetic materials to recipient cells/tissue/organs in both physiological and pathological conditions. The Wang Lab at Marshall University majorly focuses on understanding the pathophysiological roles of EXs and their potential therapeutical applications in diabetes/hypertension-associated vascular diseases, including ischemic stroke and vascular dementia. Our long-term goal is to establish an EX-based therapy for treating vascular diseases.

Ongoing projects for students to be involved in:

Project 1: Role of circulating extracellular vesicles in hypertension-related cognitive impairment. Hypertension is one of the leading risk factors for cognitive impairment, with vascular dementia being the second most common dementia-related disease globally. However, there is no effective treatment plan other than controlling the risk factors. The molecular mechanisms underlying the onset and progression of hypertension-related cognitive dysfunction are unclear. Growing studies indicate that cerebrovascular pathology precedes cognitive dysfunction. Increasing evidence shows the potential of circulating EVs (cEVs) in neurological diseases. We are interested in investigating the possible roles of cEVs in hypertension-related cognitive dysfunction and developing novel strategies for preventing, delaying, and treating cognitive impairment in older adults with hypertension.

Project 2: Role of exercise-intervened exosomes in ischemic stroke. We have previously demonstrated that exercise intervention could modulate the release of EXs. Our recent data shows that the exosomal-mediated communications between endothelial progenitor cells (EPCs) and brain cells, such as endothelial cells and neurons, are compromised in hypertension conditions. Exercise is a well-known nonpharmaceutical approach for cerebrovascular disease and has been shown to modulate the function of EPCs. Given that EX function varies on cellular status and origin, we speculate that exercise intervention can modulate EPC-derived EX (EPC-EX)-mediated intercellular communication in the ischemic stroke brain. In this project, we aim to investigate exercise-regulated exosomes' effects and underlying mechanisms in protecting the brain from ischemic stroke.

Project 3: The potential application of angiotensin-converting enzyme 2 (ACE2)-primed EXs in hypertension-related ischemic stroke. Accumulating evidence provided by others and our group has suggested that EPCs and EPC-EXs have a promising therapeutic application for cerebrovascular diseases. Angiotensin-converting enzyme 2 (ACE2), a negative regulator of the renin-angiotensin system, plays a critical role in hypertension-related cerebrovascular diseases. We have recently reported that EPC-EXs can convey ACE2 to protect vascular endothelial cells. The goal of this project is to investigate the potential effects of combining ACE2 and EPC-EXs for treating hypertension-related ischemic stroke.

Project 4: Role of perivascular adipose tissue-EVs in diabetes-associated vascular dysfunction. Perivascular adipose tissue (PVAT), long assumed to be vessel-supporting connective tissue, is now recognized as the sixth "man" of the vascular system. Our group recently revealed that exercise intervention could improve endothelial function associated with alleviated inflammation and oxidative stress of PVAT in type 2 diabetic mice. The objective of this study is to further explore the role of PVAT-EVs in vascular dysfunction in diabetes. The overall goal is to identify a therapeutic target and develop a new therapeutic approach such as EV-based therapy for diabetes-associated vascular diseases.

Techniques that are routinely used in our study:
1) Cell culture and cell assays: cell proliferation and function assays, protein/RNA extraction, Western blot, qRT-PCR, etc.
2) Animal study: exosome and/or stem cell-based therapy for mice models; small animal microscopic surgeries, including telemetric probe implantation, tail vein injection, stereotactic microinjection; exercise training and behavior studies; vascular function study (pressure myography); cerebral blood flow measurement, etc.
3) Exosome-related assays: Nanoparticle tracking analysis (NTA), exosome labeling, co-culture assays.
4) Histology study: tissue sectioning (cryostat, paraffin embedding, and section), staining, immunohistochemistry, immunofluorescence, etc.

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My research focuses on bacterial biofilms, lung infections and gut microbiota. Four projects are ongoing in the Yu lab.

Project #1: Cystic Fibrosis Biofilms. Individuals afflicted with cystic fibrosis (CF) are susceptible to recurrent lung infections with a bacterium called *Pseudomonas aeruginosa*. During the infection in CF, this bacterium forms a capsule (biofilms) by producing a polysaccharide called alginate. Alginate is a virulence factor that allows greater adhesion to lung epithelial cells, as well as protection from antibiotics and the host's immune system. We study how alginate production is regulated. Elucidation of the alginate pathways will lead to better understand the pathogenesis, and development of novel therapeutics for treatment in CF.

Project #2: Testing Antimicrobials. Most of bacterial lung infections starts with the colonization of upper respiratory tract. Aspiration of oropharyngeal secretions containing colonizing bacteria deep into the lung allows for the establishment of lower respiratory tract infections. We are using an inhalation exposure system to introduce bacteria into the distal airways of the mouse lungs, causing the development of pneumonia. This model is being utilized to test the safety and efficacy of novel antimicrobials against the multiple drug-resistant (MDR) lung infection. The goal of this project is to develop novel therapeutics against the MDR Lung infections and pneumonia.

Project #3: SFB Probiotics. Gut microbiota, a bacterial community made up of 1,000 different species, are important to human health. Among all the species, there is a morphologically-distinct symbiotic member known as segmented filamentous bacteria (SFB). The SFB belongs to a group of clostridia bacteria, which cannot be grown *in vitro*. However, the SFB play a vital role in the development of the immune system in mice. More specifically, SFB have been shown to attach to the apical epithelium of the small intestine to induce the interleukin-17-producing T helper (TH_{17}) cells. TH_{17} cells are important for the protection against intestinal pathogens as well as in maintaining gut homeostasis. In this project, we will examine possibilities of how to develop the SFB into a novel probiotic to prevent and control the gastrointestinal diseases in children.

Project #4: New Biopolymer Development. Through removal of major pathogenicity genes from genome and validation via the genome resequencing, we created a non-pathogenic strain of *P. aeruginosa* that produces large amounts of alginate. Alginate is a polysaccharide widely used in biomedical applications. It consists of an unbranched linear biopolymer comprised of two sugar monomers, β-D-mannuronate and its C5 epimer α-L-guluronate. Through introduction of changes of genetic codes for the alginate biosynthetic enzymes, we hope that we may be able to use the non-pathogenic strain to produce alginate with custom compositions.

II. Mentors At West Virginia University

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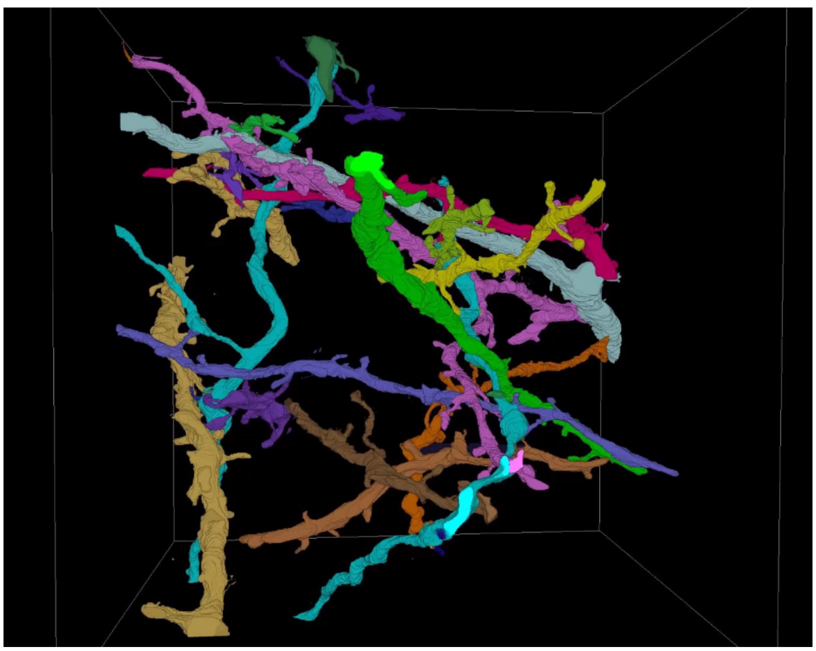
Three-dimensional reconstruction of neuronal networks in the brain

The seemingly miraculous abilities of the human brain are the outcome of billions of neurons constantly communicating with each other via myriads of synaptic connections; however, we still lack a detailed knowledge of who is connected with whom and how - i.e., of the "wiring diagram" of the brain. In the Agmon lab we are studying the cerebral cortex, the site of our sensory perceptions, our motor plans, and our higher faculties of cognition, thought and consciousness itself. We are beginning to make some headway into deciphering the wiring diagram of the cortical network by reconstructing in 3D, from serial electron-microscope images, the detailed structures of identified neurons, together with their detailed input and output synaptic connections. This is labor-intensive work, but rewards its practitioners with eye-opening insights into the structure and function of the brain. This work can be done by motivated undergraduate students on our lab computers, after an initial training by the PI, and is especially suitable as a summer internship project. No prior skills are required, but previous exposure to basic concepts in neuroscience may be helpful.



A 3-dimensional reconstruction, from serial electron-micrographs, of a thalamocortical axonal branch (two light blue segments), together with all its postsynaptic elements. Most postsynaptic elements are spiny dendrites of excitatory cells, but at least one (gray segment) is a smooth dendrite of an inhibitory basket cell. (Unpublished results from the Agmon lab.)

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Exploring the biosynthesis regulation of the antimalarial medicine, artemisinin in *Artemisia annua* L. through genomics, transcriptomics, and metabolomics studies

Introduction and rationale: *Artemisia annua* L. (Asteraceae family) is a prized medicinal herb native to temperate Asia and the main commercial source of the *anti-malarial drug* ***artemisinin***. This sesquiterpene lactone compound is used in standard treatments worldwide. Given its complex chemical structure, artemisinin is difficult to synthesize *ex vivo*, which leads to high costs and makes it amenable to global shortages due to unfavorable climate variables. Therefore, to improve artemisinin yields, it is essential to understand the physiology and regulation of its biosynthesis. **Research goal and objectives:** *Our research aims to study the genetic regulation of the artemisinin biosynthesis pathway*. At WVU, we have a unique germplasm collection of accessions that differ in the yields of artemisinin and related terpene compounds. We *hypothesize that artemisinin accumulation is a factor of carbon drain strength of sesquiterpenes towards the committed pathway, competitive enzymology, and the final step conversion into the final product***.** **Methods:** Artemisinin and related compounds are quantified through gas chromatography followed by mass spectrometry (GC-MS) and ultra-high performance liquid chromatography (UHPLC). Biochemical and physiological parameters and the expression levels of known terpenoid biosynthesis genes are being analyzed to understand the variance in artemisinin accumulation in the different accessions. We are also taking full advantage of the genome sequence of *Artemisia annua* to unveil the natural genetic variation and genomic rearrangements leading to increased artemisinin biosynthesis and accumulation. *This research is ideal for students who want to learn about the metabolic flux of biochemical pathways, biosynthesis of natural compounds of pharmaceutical interest, functional genomics, and chromatography of fine chemicals.*

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Project 1: Precise intracellular delivery of adjuvants for vaccine applications
The activation of intracellular toll-like receptors (TLRs) TLR7, TLR8, TLR9, and TLR4 found in lysosomes, as well as NLRs and stimulator of interferon genes (STING), found in the cytoplasm of antigen-presenting cells (APCs), is considered a challenging task. This is because adjuvants should reach APC intracellular compartments at activatable quantities without degradation after *in vivo* administration. These issues can be addressed by adopting novel pH-responsive nanoparticulate delivery technologies, which enhance adjuvant solubility and improve pharmacokinetic properties and cellular delivery of adjuvants. APCs frequently phagocytose nanoparticles, localizing in the endolysosome and releasing adjuvant payloads in both endosomes and the cytoplasm. Furthermore, nanoparticles allow simultaneous encapsulation and cellular delivery of two or more adjuvants. The proposed project is a new strategy to deliver adjuvants into intracellular compartments effectively. Of note, optimized encapsulation and intracellular delivery of adjuvants will be of great interest in developing vaccine strategies against intracellular pathogens and cancers.

Project 2: Nucleic acid/small molecule treatment strategies for B-cell acute lymphoblastic leukemia
B-cell acute lymphoblastic leukemia (ALL) accounts for 75% of all ALL diagnoses. Unfortunately, conventional chemotherapies for B-cell ALL exhibit high relapse rates, with estimates of 40-50% and 15-20% in adult and pediatric populations, respectively. RNAi therapeutics, e.g., siRNA can efficiently knock down the expression of target genes in a sequence-specific way by mediating targeted mRNA degradation. However, significant progress has not been made in the development of siRNA therapeutics for ALL treatments. From a pharmaceutical standpoint, the delivery of two therapeutics (siRNA and small molecules) intracellularly with diverse physicochemical properties is challenging. For example, siRNA is a negatively charged hydrophilic nucleic acid with modest stability *in vitro* and *in vivo*, while the majority of chemotherapeutics exhibit poor solubility in aqueous solvents, which makes it a tough candidate to formulate and administer to patients. These challenges necessitate a significant need to develop a suitable delivery system that can encapsulate and deliver both of these therapeutics precisely to cancer cells.

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Adverse reproductive outcomes, such as miscarriages, are common in pregnant women working in occupational settings. These women are exposed to toxicants such as, nano-titanium dioxide (nano-TIO_2) or electronic cigarettes (e-cig) via inhalation. One likely, but uninvestigated, way that inhaled toxicants may mediate these poor outcomes is by decreasing critical pregnancy hormones such as estradiol (E_2) or perturbations in reactive oxygen species. Currently, our lab is focused on linking E_2 and adverse reproductive outcomes due to maternal inhalation exposure, as well as understanding the role xanthine oxidase (XO) plays post-exposure. We aim to identify the roles of E_2 and XO (along with their activators/inhibitors) across timepoints in gestation on placental function and fetal health following maternal exposure and determining the impact of maternal inhalation exposure on reproductive health of F1 female progeny. Ultimately, we are working to elucidate the roles of E_2 and XO in regulating a healthy gestational environment for fetal development via uterine and placental vascular function, oxidant stress, and reproductive hormones during maternal inhalation exposure. This will be accomplished through serial blood sampling, *in vitro* vessel preparations and experimentation, as well as hormone and immunohistochemical assays. Students will be able to work with rodents as well as learn surgical and procedures and techniques.

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https://scholar.google.com/citations?hl=en&user=J9KaXY0AAAAJ&view_op=list_works&sortby=pubdate

Stress related to difficult personal encounters is very pervasive yet poorly understood. Such stress over time can lead to physical (e.g. cardiovascular) and mental health (e.g. depression/anxiety) issues. Compassion training is a simple and inexpensive way to help decrease the stressful reactions from difficult personal encounters. The project for the summer will be to examine brain responses (using PET (Positron Emission Tomography)) and heart rate variability to a stressful social situation. The physiological and neural responses to stressful stimuli will be measured before and after compassion meditation or relaxing nature sounds (active control).

This project will provide opportunities to learn:
- how to work with human participants,
- how to do measure neural response to stimuli using PET
- how to combine physiological, behavioral, and neural measures.

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Description of research:
The Bridi Lab's primary goal is to investigate how the brain's circuits and synapses (especially those made by inhibitory neurons) develop and change typical and atypical/aversive/challenging conditions. We study sensory and stress circuitry in the context of neurodevelopmental conditions, stroke, and stress.

Bridi laboratory activities:
How does the neuroendocrine stress response impact sensory perception and behavior, and vice versa? How does an ischemic injury like stoke relate to the body's stress response? How does lifetime experience affect the hypothalamic circuits that mediate the stress response? What can changes in sensory perception tell us about neurodevelopmental disorders and their root causes? What are the potential loci for these changes and relationships? Our lab uses a combination of *in vivo* imaging techniques, animal behavior, *ex vivo* physiology in brain slices, and biochemical assays to study the brain circuits involved in the perception of sensory information and stress, and how these phenomena may relate. We are especially interested in the development of inhibitory neurons and circuits that are important for shaping sensory perception in the cortex and regulating stress-responsive neuronal activity in the hypothalamus, and the ways that this inhibitory regulation may be affected by neuronal injury like stroke, adverse experience, and neurodevelopmental disorders.

Potential projects include:
- What do sensory circuits tell us about neurodevelopmental conditions? Using sensory assays, *ex vivo* electrophysiology, and *in vivo* multiphoton imaging to investigate how visual and auditory processing are altered in conditions like Autism Spectrum Disorders using transgenic mouse models.
- How do stress-control circuits develop under typical and atypical conditions? Using behavior, in vivo fiber photometry, and ex vivo electrophysiology to investigate how hypothalamic stress-control circuits develop and change in response to adverse experience and during neurodevelopmental challenges.
- How are sensory perception and the perception of stress linked?
- What are the implications of potentiated stress response after ischemic injury? Combining biochemical assays, immunohistochemical staining, behavior, chemogenetics, and in vivo fiber photometry, we are studying how persistent activation of the body's stress response systems after stroke impact recovery and outcomes, and exploring new avenues for treatment.

Techniques:
- Patch-clamp electrophysiology
- *In vivo* fiber photometry
- Animal behavior
- *In vivo* multiphoton imaging

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Cardiovascular responses to disease states

INBRE program participants will work in conjunction with laboratory personnel on current projects. Current pre-clinical research is examining: 1) how chronic stress impacts cerebrovascular function and health as a pathway to dementia; 2) how adipose tissue regulates vascular dysfunction; and 3) how E-Cig exposure affects vascular dysfunction. INBRE participants will interact with graduate students and staff members to answer research questions, using both invasive and non-invasive approaches to examining cardiovascular function.

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Gene therapy to treat inherited retinal disorders

Inherited retinal diseases are a clinically and genetically heterogeneous group of disorders characterized by photoreceptor degeneration or dysfunction. These disorders typically present with severe vision loss that can be progressive, with disease onset ranging from congenital to late adulthood. Our lab studies diseases affecting cone photoreceptors which are responsible for our daylight vision, visual acuity, and color vision. We investigate molecular mechanisms behind cone photoreceptor degeneration and perform gene therapy to restore the function and structure of these cells using animal models resembling patients carrying the corresponding mutations. The commonly used techniques in the lab include: molecular cloning, gene editing by CRISPR/Cas9, histology, immunohistochemistry, immunofluorescent microscopy, genotyping by polymerase chain reaction (PCR), Western blot analysis, real-time PCR, transmission electron microscope, etc.

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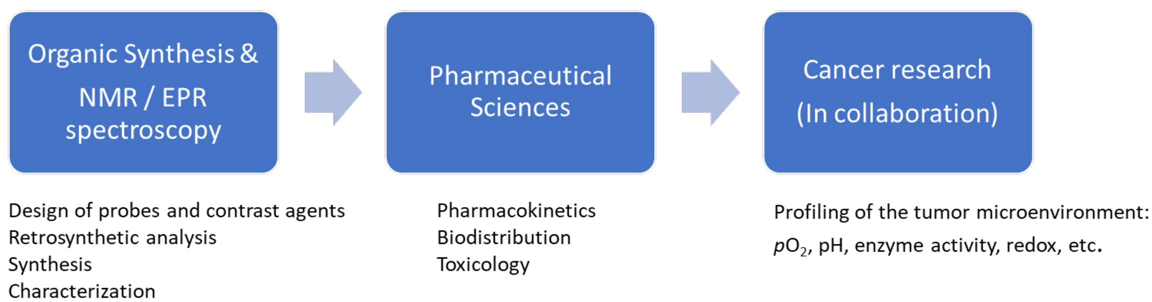
Synthesis of imaging probes for biomedical magnetic resonance applications.
Magnetic Resonance Imaging is a non-invasive medical imaging technique that uses powerful magnets and radio waves to create detailed images of the body's internal structures, helping diagnose and monitor a wide range of medical conditions. Our lab focuses on the development of imaging probes and contrast agents for two types of magnetic resonance modalities, namely MRI and electron MRI (eMRI or EPR). The goal of the project is to synthesize stable organic radicals of type triarylmethyl radicals for application in biomedical imaging.

Lab Workflow:



INBRE participants in our laboratory will have the opportunity for hands-on experiments in organic synthesis (synthesis and purification of small organic molecules), NMR and EPR spectroscopy, and HPLC. INBRE summer participation in our lab is **best suited for students enrolled in a chemistry program**.
For current funding, https://reporter.nih.gov/search/aeMdWHabG0ySAtMt7s6OHQ/projects
For publications, https://immr-probes.com/publications/

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The role of nutrient availability in retinal health

Like individual, different retinal cells have specific preferences for nutrients. We have reported that retinal pigment epithelium (RPE) prefers proline, lysine, branched-chain amino acids and choline. RPE is important to support retinal health and the dysfunction of RPE can cause age-related macular degeneration (AMD), the leading cause of blindness in the elderly. The InBRE summer students will test the importance of these nutrients in retinal metabolism, visual function and retinal morphology. The students will feed mice with nutrient-deficient diets and analyze retinal metabolism with targeted metabolomics, visual function with electroretinogram, and retinal cell morphology with optical coherence tomography (OCT). The INBRE students will also use stable-isotope labeled nutrient tracers to trace the metabolic flux of different cells in mouse retinas in vivo and in vitro. The completion of this project will provide important information about the roles of these nutrients in retinal function and disease.

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Investigating the "protective" immune response against SARS-CoV-2

SARS-CoV-2, the causative agent of COVID-19, has claimed more than 15 million lives worldwide, yet many healthy individuals remain disease free. To date, systemic vaccines containing the viral spike protein have effectively reduced disease fatality, but they have failed to block virus transmission, as breakthrough infections are a common occurrence. We have observed that antibodies against SARS-CoV-2 proteins contained in these vaccines are present in the saliva of "healthy" individuals and likely represent the "protective" immune response against this virus. Such antibodies are absent in saliva collected prior to November 2019, suggesting recent exposure to the virus in our subjects, irrespective of RT-PCR test results and vaccination status. They also demonstrate that vaccination fails to induce/boost virus-specific mucosal IgA and may therefore be unable to protect against virus transmission, a likely reason for breakthrough infection. Ongoing studies focus on mechanism of viral clearance in asymptomatic infection with a goal to develop appropriate therapeutics against SARS-CoV-2, its future variants and other respiratory viruses. Students working on summer projects in the lab will learn techniques such as Immunoassays, western blots, molecular cloning, flow cytometry etc. used in experiments in ongoing studies.

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Discovery of compounds to treat Parkinson's disease

Parkinson's disease is an age-related neurodegenerative disease which affects the motor skills of patients. Unfortunately, no drugs are currently available to slow down the disease progression, and there is a great need to discover these types of compounds. In this study we will be screening a library of compounds which consist of FDA approved and novel compounds identified through computer aided drug design techniques in several enzyme and C*. elegans* models. Once the compounds are identified which show promise, we will test them for neuroprotective activity. During the period of the study, students will learn how to screen compounds for biological activity in a high throughput manner as well as how to utilize models of Parkinson’s disease to screen for phenotypic improvement afforded by the compounds. The student will learn more about the drug discovery process and how new drugs are found and characterized.

Delivery of therapeutic proteins using nanoparticles

Parkinson's disease is an age-related neurodegenerative disease which affects the motor skills of patients. Unfortunately, no drugs are currently available to slow down the disease progression, and there is a great need to discover these types of compounds. In Parkinson’s disease there are some mitochondrial proteins which we have found can be used to restore the damaged mitochondria seen in the disease. In this project we will work on developing a nanoparticle drug delivery system to deliver a therapeutic protein to the brain using several cell culture models. This project will introduce the student to the art of nanoparticle drug delivery formulation using biological therapeutic proteins as disease modifiers.

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Development of novel therapeutic strategies for tumors that reside or home to bone

Background: Our laboratory has identified a novel target called Ero1-alpha for the treatment of lung cancer. Our data indicate that depleting Ero1-alpha using Crispr technologies inhibits the growth of lung cancer using cell culture as well as *in vivo* models. Ero1-alpha is critical for protein folding of secretory and membrane proteins. Our working hypothesis is that Ero1-alpha is critical for maintaining the tumor based secretome which drives growth, metastasis and immune suppression.

Project: The incoming INBRE student would work with graduate students in identifying secretory matrix proteins that confer the observed phenotype in Ero1-alpha depleted cell lines.
Students that joined our laboratory will learn cell culture, RT-PCR, standard genetic and pharmacological approaches for inhibiting Ero1-alpha as well as exposure to analyzing microarray and proteomic data.

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Enzyme Inhibition: Using Microscale Separations to Screen and Quantify the Inhibition of Viral Neuraminidase

Project description

Both the economic and disease burden of viral infections in the United States are high, costing $11 billion for influenza in 2018 with 800,000 hospitalizations. Among the different biochemical targets, glycosylation is a powerful post-translational modification that plays a pivotal role in many viral infections. For example, receptor binding frequently involves sialic acid residues on cell surfaces. Moreover, the release of virions replicated inside of a host cell is often enhanced by viral enzymes that cleave sialic acids on the cell surface which subsequently accelerates the infection of other cells. While sialylation is a promising target to intercept viral infections, the quantification of neuraminidase inhibition remains a challenge with current assays. The objective of this research is to create enabling bioanalytical tools to rapidly quantify the interaction between sialylated compounds and neuraminidase enzymes in the presence of small molecule inhibitors. Nanogels are used to create nanoliter reactions zones to interrogate neuraminidase activity in seconds. This is possible because the viscosity of nanogel is thermally dependent and thermally reversible. At temperatures below 22°C nanogels have liquid-like viscosity. At higher temperatures nanogels have a gel-like viscosity. This property makes it easy to fill and pattern nanogels in narrow-bore capillaries at low temperatures using an automated capillary electrophoresis instrument. Once the nanogel is loaded into the capillary, the fluids are then locked in place by raising the temperature to gel the material. This enables the precise placement of 2-5 nanoliter enzyme reaction zones at the beginning of a capillary with a total liquid volume less than 1 microliter. Enzyme reactors of this low volume are mixed electrophoretically and then the substrate and products, or products, are separated, detected, and quantified. This approach is automated and reduces the time for enzymatic conversion from hours to minutes. The analyte resolution of biomolecules separated in nanogel yields efficient separation. This work is significant to separations because it transforms standard electrophoresis methods into sophisticated multifunctional separations that are programmed, erased, and repeatedly run.

Experimental/theoretical methods

- capillary electrophoresis
- enzyme inhibition measurements

Location of the project

353, 353 Chemistry Research Labs (primary location)

Key reference for further reading

Casto-Boggess, L.D., L.A. Holland, P.A. Lawer-Yolar, J.A. Lucas, and J.R. Guerrette, Microscale Quantification of the Inhibition of Neuraminidase Using Capillary Nanogel Electrophoresis. Analytical Chemistry, 2022. 94(6): p. 16151–16159.

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Cardiovascular Research (This project is appropriate for faculty and/or students)

INBRE program participants will work in conjunction with laboratory personnel on projects examining metabolic aspects of cardiac diseases. Projects in the laboratory focus specifically on understanding the role played by proteins thought to be protective against the development of heart failure during diabetes mellitus as well as the genetic regulation of these proteins. Our studies have a tremendous impact on Appalachia due to the high incidence rate of diabetes mellitus and obesity. The goal of these studies is to provide insight into the mechanism of action of these proteins and genes, with the goal of designing therapeutics to treat cardiac disease states. Our experimentation involves both basic research and analyses in patient populations.

INBRE participants will interact with graduate students and staff members to answer research questions, using a multidisciplinary approach that includes genetic modification of the heart in both cell and animal models as well as analyses in patient samples. Training will be provided to the participants, which includes molecular cloning, whole heart physiology, RNA, DNA, and protein manipulation, bioinformatics as well as biochemical analyses.

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Bioinformatics, AI (Large Language Models), Data Science, and Epigenome Biology

Dr. Hu's laboratory exemplifies the effortless merger of Large Language Models (LLMs), such as ChatGPT, with bioinformatics education and research. This commitment has birthed 'Prompt Bioinformatics', an innovative concept to harness the profound potential of natural language in guiding the nuanced processes of bioinformatics data analysis. In addition, our investigative efforts extend into the innovative use of LLMs across a vast spectrum of clinical environments.

Simultaneously, we deeply immerse ourselves in another cornerstone of our research - decoding the cryptic epigenetic mechanisms that breed drug resistance in hematological malignancies. Equipped with a comprehensive collection of epigenetic assays such as RNA-Seq, ChIP-Seq, ATAC-Seq, and single cell Multiome, our commitment remains strong to dissect these intricate processes, consequently setting the stage for the identification of new drug targets.

Projects: 1) Innovative use of ChatGPT in processing medical texts and images for improving diagnosis. 2) decoding cellular heterogenetic underlying drug resistance of multiple myeloma using single-cell epigenetic assays. During the internship, the trainees will learn prompt engineering skills for effective communication with chatbots, basic concepts on the biology of epigenetic regulation and receive hands-on experience on the processing and integrating high-throughput genomic sequencing data.

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Lung diseases are among top five causes of global mortality (WHO). Almost 15% of the US population suffer from lung inflammatory diseases e.g., asthma effects 1-18% of global population (approximately 300 million individuals), including over 25 million people in the United States. Global asthma patient number is expected to reach 400 million by 2020 and asthma is implicated in one of every 250 deaths worldwide. Environmental and occupational agents (ground level ozone, particulate matter, nanomaterials) significantly impact the development as well as exacerbations of the respiratory disorders including asthma and pulmonary fibrosis. The over-arching goal of our research is to identify novel therapeutic targets to treat pulmonary disorders. We elucidate cellular and molecular pathways implicated in pulmonary disease susceptibility by studying patient samples, in vitro and in vivo models of pulmonary disease and primary airway an/alveolar organoids.

Techniques:
- In vitro Organoid Cultures (air-Liquid Interface cultures, 3D Alveolar cultures)
- Lung Physiology Measurements (state of the art lung function measurements)
- Translational Studies (human clinical samples)
- Rodent Models (disease, transgenic, cell type specific gene deletions).

Available Projects:
- Role of Alveolar Progenitor/Stem cells in Lung regeneration after Acute Lung Injury
- Innate immune responses in pulmonary disease susceptibility
- Early life/Childhood Asthma (Environmental Exposures x susceptible gene interactions)

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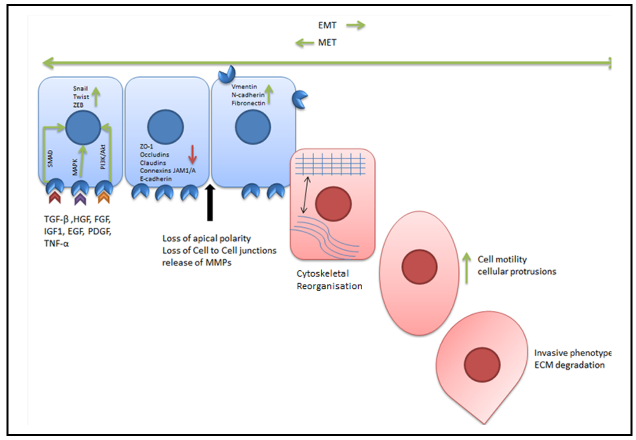
Description of Research

Vast majority of human tumors are of epithelial origin, e.g. they derive from cells highly organized in specialized epithelial layers. At the same time, most cancer-related deaths occur due to tumor recurrence and spread to distant organs (metastasis), which are tightly linked to acquisition by cancer cells of mesenchymal properties such as increased motility, invasion and resistance to chemotherapy. The metastasis stage of cancer is associated with the epithelial-to-mesenchymal transition (EMT). Normally acting only during early embryonic development, the EMT program is highjacked by cancer cells during evolution of individual tumors. EMT is activated by a handful or transcription factors referred to as the EMT master regulators, such as Snail and ZEB.



The goals of our research are to identify transcriptional network involved in activation of EMT during cancer metastasis. This knowledge will help to develop future therapeutic approaches in treating cancer and prevention of metastasis.

Available projects:

1. **Negative control of EMT by epithelial-specific transcription factors.**

EMT promotes cancer cell invasion, metastasis and drug resistance. Primary breast tumors largely maintain inherent epithelial status. However, cancer cells on the tumor periphery are believed to undergo partial EMT and disseminate to distant organs. The goal of this project is to define the roles of several transcription factors including OVOL, GRHL and FOXA1 responsible for the maintenance of the epithelial state in suppression of EMT.

2. **Role of the TGF-beta pathway in partial EMT and drug resistance of triple-negative breast cancer.**

Transforming growth factor beta (TGF-beta) acts as a tumor suppressor at the early stages of cancer development. Cancer cells evolve various mechanisms to overcome TGF-beta inhibitory effects, including silencing and mutation of TGF-beta receptors or silencing and deletion of TGF-beta target genes involved in growth suppression. The latter mechanism is often observed in triple-negative breast cancer (TNBC). TNBC cells show increased TGF-beta signaling leading to partial EMT and resistance to certain drug therapies. The goal of this project is to investigate if pharmacological inhibition of the TGF-beta pathway combined with standard cancer therapy will improve drug response in vitro.

3. **Identification and characterization of prognostic markers for lung cancer.**

Lung cancer is the leading cause of cancer related deaths. Previously, we have identified several biomarkers, including gene ZNF71, which can predict lung cancer patient response to chemotherapy. The goal of this project is to characterize the molecular mechanisms of ZNF71 function in lung cancer metastasis and EMT.

Former WV-INBRE summer research interns in the lab

Year	Name & College	Current position
2021	Linh Nguyen, Concord University	in PhD program at UT Austin
2019	Jessica Johnson, Fairmont State University	TBD
2018	Emily Means, WV Wesleyan College	earned MD at Lake Erie College of Osteopathic Medicine
2015	Brandon Trinh, Bethany College	environmental hazardous waste management

2013	Morgan Johnson, Shepherd University	earned MD at WVU School of Medicine
2012	Anna Alappat, Shepherd University	earned MD at WVU School of Medicine
2009	Icel Cavis, Shepherd University	FBI Forensics Lab, Quantico, VA

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The molecular mechanism behind nuclear-specific NAD+ role in retinal neurogenesis.
Nicotinamide adenine dinucleotide (NAD^+) is an essential molecule required for a variety of biological processes including cell survival, differentiation, senescence, and genomic integrity. The mammalian retina is exceptionally reliant on proper NAD+ homeostasis for health and function, however, the specific roles of subcellular NAD+ pools in retinal development, maintenance, and diseases remain unclear. Previous findings including ours demonstrate that the loss of nuclear specific NAD+ synthase “nicotinamide mononucleotide adenylyltransferase-1” (NMNAT1) linked to blinding disease through yet-unclear mechanisms leads to early and severe defects in retinal development. The goal of this study is to investigate the molecular mechanisms behind NMNAT1 in the developing murine retina, where its deficiency causes early and severe degeneration of outer and inner nuclear layers (ONL and INL). During the course of this investigation, students will investigate the link between nuclear-specific NAD^+ in retinal neurogenesis, and the differentiation of photoreceptors and bipolar neurons. Summer students will learn more about animal handling, retinal tissue preparation, immunocytochemistry, Western blotting, mammalian cell culture studies, confocal imaging, etc. In addition to this, students will be encouraged to present their research in the vision group research meetings at WVU. Finally, the data produced will be included in the manuscript and granted authorship.

Decipher the importance of Na, K-ATPase in the retinal function, survival, and neural circuitry maintenance. The photocurrent is an ion gradient that photoreceptors rely on for the conversion of light into electrical impulses. This gradient is largely mediated by the Na^+/K^+-ATPase (NKA), which is localized to the inner segment (IS), where it exists as a heterodimer, comprised of a catalytic α3- (ATP1A3) and a non-catalytic β2- (ATP1B2) subunits. Previous studies show that loss of ATP1B2 from the mouse retina results in rapid degeneration of photoreceptors, but the specific role of ATP1B2 remains unclear. In addition to that NKA interaction with retinoschisis-1 (RS-1), a protein linked with X-linked juvenile macular degeneration remains not clearly understood. Overall, the goal of this study is to elucidate the role of NKA in the retinal function, survival, and maintenance of neural circuity. Summer interns will have many opportunities to assist with this project in the lab including, physiological recording using electroretinogram (ERG), immunolocalization studies, confocal imaging, Western blotting to evaluate proteins in the retinal tissues, PCR, and quantitative RT-PCR and agarose gel electrophoresis.

Study the mechanism behind PRCD, a protein linked with retinitis pigmentosa associated with RPE disfunction. Our recent findings show that loss of progressive rod-cone degeneration (PRCD) protein in mice leads to defects in retinal pigment epithelium (RPE) function and survival through unknow mechanism. To understand further, using desired animal models either lacking PRCD or patient mutation, we will study using novel techniques including molecular, biochemical, and metabolomic approaches. Any data produced in this study will be included in the manuscript and granted authorship.

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Coenzyme A (CoA) is an essential and universally distributed cofactor that acts as the major acyl group carrier in the cell. Free CoA and acyl-CoAs are involved in hundreds of metabolic reactions and are among a selected number of small molecules that have the ability to act as global regulators of cellular metabolism. Consistent with this key function, CoA levels are at the same time tightly regulated and flexible, so that the available supply is sufficiently adaptive to metabolic challenges such as fasting or a high fat diet. Regulation of CoA levels occurs through coordination of synthesis and degradation. In the liver, modulation of the amount of CoA contributes to the metabolic flexibility of this organ and to its ability to maintain glucose homeostasis during a fast. Conversely, in diabetic mice, hepatic CoA levels are abnormally high and unresponsive to changes in the nutritional state.
Not much is known about CoA degradation. The goal of our research is to establish the importance of CoA-degrading enzymes in the regulation of CoA levels and energy metabolism. In particular, we are interested in studying these enzymes in the context of diabetes, obesity and other metabolic diseases using a combination of biochemistry, animal studies and metabolomics.

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Fibrosis is the excess accumulation of extracellular matrix proteins such as collagen that adversely effects organ function. In the heart, fibrosis stiffens the heart, compromising its ability to fill properly. This ultimately can lead to heart failure with preserved ejection fraction (HFpEF). Currently, there are no specific treatments for HFpEF or the underlying cardiac fibrosis. Using mouse models of diabetes and hypertension, we have identified that the neurokinin-1 (NK-1R) is anti-fibrotic when activated by specific metabolites of the neuropeptide substance P. We now are trying to elucidate the mechanisms by which the NK-1R exerts anti-fibrotic effects. This includes the specific cell types involved (e.g. mast cells, macrophages, fibroblasts), and signaling pathways activated and/or inhibited by the NK-1R. Students involved in this project will learn multiple techniques including: 1) assays to assess gene expression and protein levels; 2) cell culture; 3) histological assessment of fibrosis and inflammatory cell infiltration; and 4) assessment of cardiac function.

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Description of Research (2024-2025): Neuroimaging of human brain function in perception.

Lewis laboratory activities:
Our lab studies a diverse range of human brain functions regarding sensory and pain perception, plus language perception. We use various neuroimaging techniques, including functional magnetic resonance imaging (fMRI), electroencephalography (EEG), and also transcranial magnetic stimulation (TMS) to modulate pain perception (in a patient population). Recent research projects include:

1. Using EEG together with 3D-printing technology to objectively characterize signatures of chronic headache pain.
2. Using TMS to alleviate chronic headache pain (in patients)
3. Analyzing a completed fMRI dataset to characterize language-specific mechanisms of perception of spoken phrases in Chinese/English bilinguals.
4. Mapping morphology changes in the adult human brain over time (two or so decades), analyzing MRI data collected from two long-term participants.
5. Exploring the brains of participants with autism spectrum disorder (ASD) using collected resting-state functional connectivity (rsfMRI) data.

We primarily use computational approaches and methods for studying brain function, and thus applicants with a solid background in computer sciences or engineering are preferred.

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Molecular motors are fascinating biological machines that power much of the movement performed by living organisms. They utilize chemical energy in cells to generate mechanical force and motion, and play essential roles in diverse cellular and developmental processes. Numerous human diseases owe their origins to defects in molecular motor proteins, including cancer, neurogenerative disorders, as well as hearing and vision losses.

The overarching goal of our lab is to understand how motor proteins function at the molecular level, with an emphasis on their roles in neurosensory cells (auditory hair cells and photoreceptor cells). To accomplish this, we use a bottom-up reconstitution approach to “reconstruct” a sub-fraction of the cellular network with purified components and to quantitatively study individual molecular behaviors using advanced microscopy. Combined with structural biology and live-cell imaging, these studies provide essential information across scales on the mechanisms by which molecular motors power development and self-organization of neurosensory cells.

INBRE summer students will have the opportunity to engage in the following projects and techniques:

Available projects:

- In vitro reconstitution of the microtubule cytoskeleton of auditory sensory epithelial cells
- Single-molecule super-resolution imaging of intraflagellar train (IFT) transport in mammalian primary cilia
- The structure and molecular characterization of Drosophila myosin-15

Techniques:

- Baculovirus/insect cells protein purification system
- Modern molecular biology techniques
- Total Internal Reflection Microscopy (TIRF) single-molecule imaging
- Mammalian cell culture and live cell imaging
- Super-resolution localization microscopy
- Biochemistry and enzymatic assays

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Significance and Translational Relevance

Brain metastases pose a life-threatening problem for women with advanced metastatic breast cancer. Of women who have been diagnosed with disseminated breast cancer, ~10-16% will develop symptomatic brain metastases and at least 20-30% will have micrometastatic lesions present at autopsy. Once lesions are established in the central nervous system, only one in five women survive one year. We have recently shown that chemotherapeutics do not reach effective concentrations in ~90% of CNS metastases. Therefore, our lab is working on ways to prevent the formation of metastases in brain.

Project Information

Our lab uses cutting edge microscopy to identify single breast cancer cells that can invade into brain tissue. Once the cells are found we have techniques that can remove the individual cancer cell. Once the cell is collected the goal of the project is to identify if there is a DNA signature that allows the cancer cell to get into brain (>99% of breast cancer cells do not enter into brain tissue). Once that signature is identified it is hoped we will find a molecular target that can be blocked by a drug, which should reduce penetration of the cancer cells into brain. It is hoped this project will be a first step in the prevention of brain metastases of breast cancer.

Skills and/or experiences the student will be exposed to

1. Cell culture of human and mouse cells
2. Fluorescent microscopy – to potentially include multi-photon imaging
3. Bioluminesence imaging of cancer cells in living animals.
4. Laser micro-dissection of cells in tissue
5. RNA amplification
6. Microarray data

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Using stress as one of the common experiences that brings about neuropsychiatric disease, the Morrison lab works to understand the cellular and molecular mechanisms of experience-dependent resilience and vulnerability to stress, and how sex, developmental stage, and type of experience shape an individual's trajectory. We are particularly interested in women's mental health, as there has been little preclinical work to understand why women are more likely than men to suffer from mood disorders. Working with clinical collaborators, we have developed a translationally relevant mouse model that allows the lab to address how neuropsychiatric disease risk is compounded throughout the life of a woman, a process that is multifaceted and not well understood. The Morrison lab utilizes a variety of approaches, including advanced sequencing techniques, bioinformatics, molecular methods, and behavioral assays.

We have previously examined the hypothesis that female humans and mice would be susceptible to stress during the peripubertal period of development and that long-term outcomes would depend on hormonal state. We found that women exposed to adversity during peripuberty show a disrupted stress response during pregnancy and postpartum, as well as increased postpartum depression symptoms. Our mouse model completely recapitulates this phenotype, such that female mice exposed to stress during puberty demonstrate a disrupted stress response during pregnancy and postpartum. Subsequent work with this translationally-relevant **mouse model** suggests that long-term epigenetic reprogramming of the paraventricular nucleus of the hypothalamus by peripubertal stress may underlie this phenotype.
Projects in the lab revolve around four central questions, and students have some flexibility in contributing to a particular area of their interest:

1. *What is the epigenetic programming enacted by peripubertal stress?*
2. *What about pregnancy permits the expression of the stress dysregulation phenotype?*
3. *What impact does peripubertal stress have on normal maternal behavior, postpartum anxiety-like behavior, and offspring outcomes?*
4. *What are the consequences of a "second hit" stressor during pregnancy for mom and offspring?*

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Circadian Rhythm Disruption and Health

Circadian rhythms are endogenous biological rhythms of about 24 hours and are a fundamental characteristic of life. Although life evolved over the past 3-4 billion years under bright days and dark nights, humans have been able to interrupt this natural light-dark cycle for the past 130 years or so with bright light at night. Our research group studies the effects of these disrupted circadian rhythms on several parameters including immune function, neuroinflammation, metabolism, pain, sleep, and mood. Summer interns and fellows would have the opportunity to assist with current projects in the lab which include: 1) the effects of light at night on metabolism, cognition, and pain sensitivity, and 2) the effects circadian disruption on neuroinflammation associated with cardiac or cancer development and treatments.

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Airborne Particles and Systemic Microvascular Endothelial Dysfunction

Evidence indicates that acute exposure to airborne pollutants such as particulate matter (PM) and nanoparticles increases the risk of pulmonary and cardiovascular morbidity and mortality. This implies that PM affects extra-pulmonary tissues, as evidenced by the occurrence of cardiovascular dysfunction on high pollution days. However, the biological mechanisms by which PM evokes systemic effects remain to be defined. Despite its obvious importance in regulating the delivery of cells and molecules to all tissues, and in the etiology of most cardiovascular diseases, no research has investigated how systemic microvascular function is affected by pulmonary PM exposure. Our preliminary observations in the rat spinotrapezius muscle indicate that endothelium-dependent arteriolar dilation is significantly impaired after pulmonary particle exposure, and this impairment is associated with microvascular oxidative stress. Interestingly, this systemic microvascular effect can occur independent of pulmonary inflammation. My central hypothesis is that acute particle exposure affects peripheral microvascular function, and this effect is achieved by local reactive oxygen species production and/or altered neurogenic input to the systemic microcirculation. A fundamental understanding of these mechanisms is vital in preventing and treating the life-threatening events associated with air pollution. Our studies are further applied to the rapidly growing field of nanotoxicology. Wherein, it is acknowledged that nanotechnology has become a regular component of most every aspect of our daily lives, yet the toxicity of exposure to specific nanoparticles remains to be determined. Exposure to these nanoparticles carries just as much, if not more potential for generating profound effects on microvascular function. The student or faculty member will have the opportunity to develop surgical and experimental techniques associated with animal studies and isolated microvessels, as well as assist in exposing animals to various particle aerosols. These techniques include: inhalation exposure, animal surgery, microsurgery, intravital microscopy, in vivo measurement of oxidative stress and various micropipette-based techniques.

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Decode the mystery of matrix microenvironment behind the rejuvenation of adult stem cells

Adult stem cells are a potential cell source for tissue engineering and regeneration. Cell senescence resulting from ex vivo expansion is a challenge for application of adult stem cells in the treatment of human diseases. Our previous work indicates that matrix microenvironment is a promising approach for rejuvenation of adult stem cells toward a specific lineage differentiation. However, it remains unknown about the molecular mechanisms underlying this rejuvenation. Elucidation of potential mechanisms not only can facilitate to provide a large-quantity of high-quality tissue-specific stem cells for tissue engineering and regeneration but also can promote better understanding of cell-matrix crosstalk.

The project provides an opportunity to learn:

1) Cell and tissue culture
2) Cell proliferation and tri-lineage differentiation
3) Flow cytometry
4) Real-time quantitative PCR
5) Tissue sample process, sectioning, and staining
6) Western blot
7) RNA Sequencing
8) Proteomics

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Molecular mechanisms of breast cancer metastasis and tumor hypoxia

While significant progress has been made in treating breast cancer, there remain substantial problems in metastasis treatment. Tumor cells not successfully eliminated by treatment often remain dormant and later begin to grow and contribute to disease relapse. Our current project aims to define how cancer cells escape dormancy. One of the candidates we have published is AURKA kinase. The summer project will include using mouse tumor models and cultured breast cancer cells to investigate the effects of experimental drugs against tumor metastasis. During this investigation, students will learn about cancer and how to do cell culture, Western blot analysis of proteins, mouse tumor isolation, and confocal microscopy.

The role of NEED9 adaptor protein in metastasis of HER2+ breast cancers

High rates of division and aggressive metastasis characterize HER2+ breast cancers. We have shown that upregulation of NEDD9 protein is often observed in HER2-expressing cells and correlates with poor outcomes. Recently, we developed a mouse model overexpressing HER2 and NEED9 to study its role in tumorigenesis and drug resistance. This project aims to decipher the role of NEDD9 in the resistance of HER2+ cancers to standard-of-care drugs such as trastuzumab. This project already had many data points collected, and the student's role will be to measure metastatic lesions using immunofluorescent histology and digital pathology, cell culture, and drug treatment. The produced findings might be included in the manuscript and granted authorship.

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Inflammatory bowel disease (IBD) includes Crohn's disease and ulcerative colitis. Crohn's disease affects any part of the intestine (small and large intestine). Ulcerative colitis affects only the large intestine/colon. Both Crohn's disease and ulcerative colitis are chronic inflammatory diseases. Ulcerative colitis that affects young adults is most prevalent in developed countries. This laboratory focuses on identifying the cause of the disease and on developing drugs for its prevention and cure.

Molecular Basis of Ulcerative Colitis
Ulcerative colitis occurs as a result of inflammation of the mucosal cells that line the inner surface of the colon. This mucosal inflammation results in a reduced rate of nutrient, electrolyte and water absorption, leading to diarrhea. In addition, inflammation also reduces nutrient metabolism that produces energy needed to maintain healthy mucosal cells. An overactive immune system is one of the major reasons for mucosal inflammation in ulcerative colitis. The overactive immune reaction constantly produces free radicals (reactive oxygen species) that inhibit water absorptive processes and damage the mucosal cells.

Approach to Cure and Prevent Ulcerative Colitis
To prevent ulcerative colitis, simultaneous reduction of the overactive immune system and improvement of mucosal cell health are essential. Overactive immune reactions could be controlled by supplying antioxidants. Mucosal health could be improved by supplying butyrate (a short chain fatty acid), which is the major nutrient of colonic mucosal cells. Butyrate is not present in food but is produced from non-absorbed carbohydrates by bacteria present in the colon. Therefore, this laboratory is designing non-digestible carbohydrate (resistant starch) and free radical scavenger derivatives that, upon fermentation, deliver butyrate and an antioxidant right on target at the colon.

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Defects in cilia lead to ciliopathy with male infertility, blindness, deafness, obesity, and hydrocephaly (accumulation of fluid in the brain). Using animal and cell models, we investigate the mechanisms behind ciliopathies, in particular blindness, deafness and hydrocephaly. We are also interested in how glia interacts and support the neurons.

The research group is a mix of technical staff, graduate and undergraduate students. INBRE students will be paired with graduate students. The students will be exposed to diverse experimental strategies, including molecular, cellular, and electrophysiological approaches. The experimental system uses neurons/glia and multiple animal models that phenocopy ciliopathies, blinding diseases and hearing loss.

For details on current research funding and details on our research, click the link below
https://reporter.nih.gov/search/n6yU7RSY50O5NQrObIFxqw/projects?projects=Active

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Structural Biology-Guided Exploration of Flavivirus 5' and 3' UTRs for the Development of Innovative Antiviral Therapeutics

Significance and Translational Relevance:
This research project focuses on unraveling the structural mechanisms of the 5' and 3' untranslated regions (UTRs) of flavivirus genomes, with an emphasis on viruses such as Zika, yellow fever, and dengue. These regions play critical roles in the regulation of viral replication, translation, and host-virus interactions, making them attractive targets for therapeutic interventions. Insights into their structural dynamics could lead to the development of novel therapeutics, including small molecule inhibitors or RNA-based therapeutics designed to disrupt viral life cycles.

Potential Project Outcomes:
Using advanced structural biology techniques, this project will uncover the three-dimensional structures and mechanistic properties of the 5' and 3' UTRs. This knowledge will pave the way for designing targeted antiviral strategies aimed at interfering with RNA-mediated regulatory processes critical to flavivirus replication. Potential outcomes include the identification of key structural motifs for drug targeting, development of novel RNA-based inhibitors, and frameworks for innovative therapeutics with applications to multiple flavivirus-related diseases. This work will provide foundational insights into RNA structural biology and contribute to the global fight against emerging and re-emerging viral diseases.

Skills and/or Experiences Students Will Be Exposed To:

- Molecular biology techniques such as in vitro transcription, mutagenesis, and manipulation of viral RNA.
- RNA biochemistry, including purification, folding, and probing of RNA structures.
- Structural biology methods such as cryo-EM, NMR spectroscopy, and SAXS for analyzing RNA structures.
- Biophysical techniques for studying RNA-ligand interactions, including fluorescence anisotropy and ITC.
- Microbiology approaches, including viral RNA culture systems and replication assays.
- Participation in weekly lab meetings and collaborative brainstorming sessions.
- Individualized training in scientific writing and oral presentation skills.

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Super-resolution fluorescence microscopy of subcellular trafficking events in retinal photoreceptor neurons.

Photoreceptor neurons of the retina are one of the most specialized neuronal cell types in the nervous system, capable of transducing photons of light into a neural signal for visual perception in the brain. To maintain light detection capability for a lifetime, photoreceptors have an extraordinarily complex cell biology with many key subcellular events that remain undiscovered. These represent critical gaps in our knowledge about the visual system since many debilitating visual disorders are caused by disruptions to photoreceptor cellular homeostasis. In our laboratory, we study one of the most fundamental cellular mechanisms in photoreceptors, protein trafficking and turnover, using super-resolution fluorescence microscopies, which are powerful imaging systems that enable us to visualize nanoscale, subcellular events within individual cells. These techniques are capable of localizing individual specific protein targets within photoreceptor subcellular domains, making them effective tools for driving projects into the discovery of new molecular mechanisms that maintain visual processing in photoreceptor neurons. Super-resolution microscopy imaging in our laboratory is performed alongside complementary approaches, including electron microscopy, cell culture, and standard molecular biology techniques.

INBRE participants in our laboratory will have the opportunity for hands-on experiments with super-resolution and electron microscopes, including STORM (stochastic optical reconstruction microscopy) and SIM (structured illumination microscopy) systems. In addition, participants will also learn a wide range of preparative techniques performed in our laboratory, including mouse handling, eye dissection, tissue preparation, and ultramicrotomy. Participants will contribute to projects that aim to 1) discover fundamental cellular mechanisms that maintain photoreceptors in the retina and 2) discover new subcellular pathogenic events in photoreceptors from mutant mice that model the eye disease retinitis pigmentosa, a severe retinal neurodegeneration that leads to blindness.

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Using various strains of mice, we conduct behavioral experiments, neuronal activity labeling experiments, and single-neuron gene expression experiments to better understand the cell types and circuits involved in experiences and behaviors related to substance use disorder (SUD). We also use cultured cells and dissected brain tissue to conduct biochemical and pharmacological experiments to better understand how drugs of abuse affect SUD-relevant neuronal signaling. The impetus for these pursuits is to identify drugs and/or molecular targets that could lead to new medications for SUD.

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Understanding the roles of mitochondria in cardiac function and protection

In one of the major projects, we are currently working on understanding the role of mitochondrial LonP1 in cardiac function and protection. Here, we focus on myocardial Ischemia-reperfusion (IR) injury, which is a significant challenge in treating myocardial infarction (MI), the leading cause of death worldwide. Mitochondrial reactive oxygen species (mtROS) generated by electron transport chain (ETC) Complex-I are the principal mediators of IR injury. Excess mtROS generated during early IR triggers vicious cycles of free radical production promoting cardiomyocyte death. Therefore, understanding the early molecular events of reperfusion will provide new targets for developing novel interventions for limiting cardiac injury. Our published findings show that LonP1- a major mitochondrial stress response protease mitigates oxidative stress-induced damage during early IR; therefore, we believe that LonP1 could be a promising target for attenuating reperfusion injury. Our long-term goal is to leverage the mitochondrial protein quality control (MPQC) mechanisms of LonP1 as a pivotal point in developing therapeutic strategies such as delivering LonP1 to the heart and/or activating LonP1 by small molecules for mitigating IR injury and post-MI- heart failure. In addtion, we are interested in studying what is the physiological role of LonP1 in the heart by employing genetic mouse models that lack or express Lonp1 in the heart.

In another breakthrough project, we are investigating the role of mitochondria in contributing to doxorubicin (DOX) induced cardiomyopathy. DOX is one of the first-line chemotherapeutic agents against various cancers and acts by interfering with DNA replication. But its action on non-replicating cardiomyocytes causing cardiotoxicity is largely unknown. Thus, this lack of understanding limits identifying therapeutic strategies to treat DOX-induced cardiomyopathy. In this project, we hypothesize that DOX accumulates within mitochondria, binds mtDNA, and reduces mitochondrial biogenesis, thereby inducing progressive mitochondrial and cardiac dysfunction. Therefore, understanding the exact mechanisms of DOX-mediated heart failure will help to identify novel strategies to develop therapeutic applications. We are also investigating whether LonP1 plays any role in protecting against DOX-mediated cardiotoxicity. In addition, in this project, we work Cardio oncologists at WVU Heart and Vascular Institute, to potentially develop a novel, affordable, and clinically relevant means to predict patient outcomes before DOX treatment.

We are interested in addressing another critical issue: metabolic diseases, particularly type-II diabetes-induced heart failure. Type-II diabetes significantly affects cardiac metabolism, leading to altered energy substrate utilization and mitochondrial dysfunction. In diabetic hearts, the metabolic shift from glucose oxidation to increased reliance on fatty acid oxidation results in inefficient ATP production, exacerbating stress on cardiac function. This altered metabolic environment contributes to diastolic dysfunction, a hallmark of diabetic cardiomyopathy, which eventually progresses to heart failure. Our research aims to elucidate the underlying mechanisms of how diabetes-induced metabolic dysregulation leads to cardiac dysfunction. One focus is on the mitochondrial LonP1 protein, a key regulator of mitochondrial proteostasis and function. Preliminary data from our studies indicate that LonP1 plays a critical role in maintaining mitochondrial integrity, particularly during the transition from glycolysis to fatty acid oxidation in the heart. By investigating the role of LonP1 in diabetic hearts, we hope to uncover how its dysregulation contributes to cardiomyocyte death and dysfunction. We are testing both the overexpression of LonP1 to confer protection against metabolic stress and its deletion to examine the resulting cardiac outcomes in diabetic models.

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The goal of our research laboratory is to understand the role played by lysine acetylation, a post- translational modification shown to regulate function of majority of mitochondrial proteins. INBRE program participants will work on projects examining the role played by acetylation in regulating cardiac mitochondrial metabolism, oxidative stress, mitophagy, mitochondrial dynamics and bioenergetics. Some of the current ongoing projects in the laboratory that a student can work on are: 1) Role of mitochondrial protein acetylation in regulating mitochondria and cardiac function in hearts exposed to carbon black and ozone particles. 2) Mitochondria/ER Crosstalk and its role in healthy aging. 3) Transcriptional regulation of mitochondrial quality control and homeostasis by acetylation. 4) Mechanisms behind unconditioned chronic mild stress specific differences observed in male and female mice.

Participants will closely work and interact with graduate and undergraduate students in the lab and utilize/learn several research techniques, which includes western blotting, immunoprecipitations, quantitative RT-PCR, cell culture studies, protein activity assays, RNA, DNA isolations, and several assays to measure mitochondrial metabolism. During this internship, the goal would be to provide the trainees a supportive research environment where they learn to appreciate research and work towards answering their research questions.

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Area of research: Immunology, Neuroscience, Autoimmune disease, Neuroimmunology, Inflammation

The overall goal of Edwin Wan's neuroimmunology lab is to understand how the immune system impacts the outcomes of neurological diseases, with the current focus on multiple sclerosis and ischemic stroke. We hope our findings will help identify novel therapeutic targets for these diseases.

Project one: cytokine signaling in the pathogenesis of multiple sclerosis (MS)
MS is an autoimmune disease initiated by the activation of central nervous system (CNS)-targeting T cells. CNS is ensheathed by three layers of cell-based meninges and is protected by the so-called blood-meningeal barrier (and a few other barriers) so T cells normally cannot enter the brain. However, during inflammatory events such as MS, T cells entered the meningeal area where they interact with antigen-presenting cells. This interaction is critical for the generation of factors that compromise the blood-meningeal barrier and direct cell trafficking so that immune cells can enter and damage CNS. Our main goal is to use animal models to identify the signals that initiate cell interactions in the meninges, the molecular pathways involved, and the effector molecules that are responsible to control immune cells entering CNS. We developed an exciting live-imaging technique to monitor immune cell interaction in the meninges of live mice to address our scientific questions.

Project two: Immune-glial cell interactions in brain recovery following ischemic stroke
The role of immune cells following ischemic stroke events is very different from their role in MS. Neurons are cells with high energy (i.e., glucose and oxygen) demands. When an ischemic stroke event happens blood flow is blocked in certain areas in the brain that leads to neuronal death and brain damage within minutes. Severe brain damage is hardly reversed due to the limited regeneration capacity of neurons. Thus, physiologically the brain will respond and form a “glial scar” aiming to confine the damage area and develop new blood vessels to compensate the damaged ones. The whole repair process is controlled by precise interactions between microglia (tissue-resident macrophages), astrocytes (CNS-supporting cells), and immune cells entered CNS. Our lab is interested to identify mediators and signaling pathways that control the interactions of cell types involved and investigate how aging affects brain repair following ischemic stroke. We use several innovative approaches in our study, including 3D fluorescence imaging to visualize the interactions between immune cells and vasculature network in the entire mouse brain and “spatial transcriptomic” technique that allows us to not only determine what genes are expressed in the glial scar but also the precise anatomical location within the glial scar where the genes are expressed.

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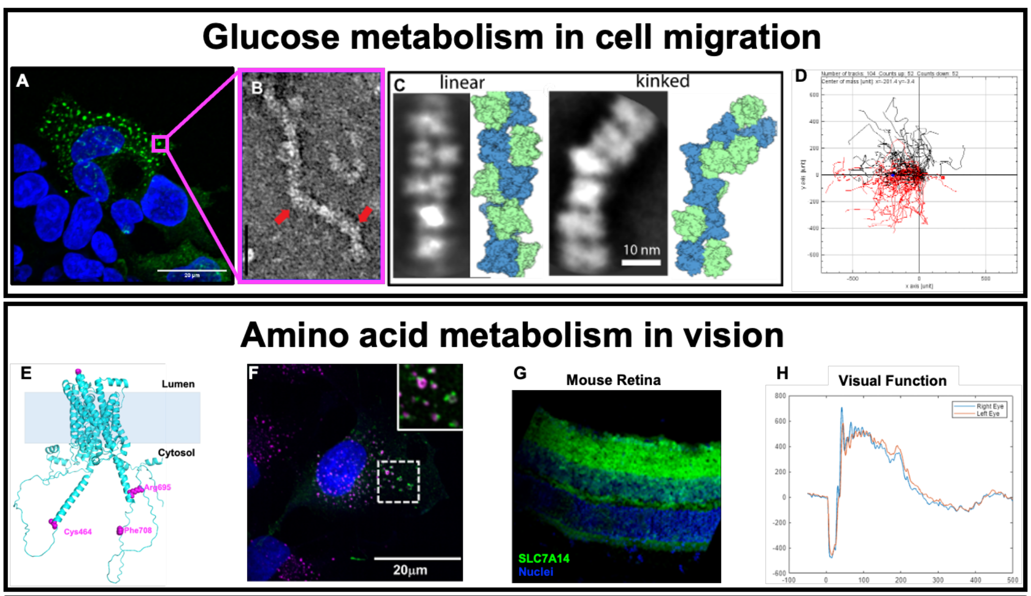
Cell biology and biochemistry of intermediary metabolic enzymes

The enzymes and pathways controlling intermediary metabolism for energy production, nutrient utilization, and biomass synthesis play critical roles in cellular homeostasis. Dysregulated metabolic enzymes and pathways are now considered central to diseases such as cancer, diabetes, and blinding disorders. Despite being studied for half a century, we still have limited knowledge of the spatial and temporal dynamics of metabolic enzymes in cells, which is critical for understanding metabolic flexibility in normal cells and aberrant metabolism in diseases. Webb lab is currently addressing questions regarding the localization, regulation, and structure/function of metabolic enzymes and transporters. Using biochemical, cell biological, and cell imaging techniques, INBRE students will enhance our understanding of the spatial and temporal regulation of metabolic enzymes and how their dysregulation contributes to disease.



Cell biology of metabolic enzymes and transporters.
Glucose metabolism: A) Fixed cell imaging reveals that the glycolytic enzyme phosphofructokinase-1 assembles into higher-order structures in cells. B) Transmission electron micrograph of negatively stained recombinant phosphofructokinase-1 reveals filament formation. C) Filaments are composed of the orderly arrangement of stacked tetramers. D) Spider diagram of cells migrating towards a growth factor. Depletion of phosphofructokinase-1 decreases cell migration.
Amino acid metabolism: E) Structure of the lysosomal amino acid transporter SLC7A14. F,G) Localization of SLC7A14 in cultured retinal pigment epithelial cells (F) and mouse retina (G). H) Electroretinogram of 2-month-old mice reveals a role of SLC7A14 in retinal health.

Publications:

https://www.ncbi.nlm.nih.gov/sites/myncbi/bradley.webb.2/bibliography/46426825/public/?sort=date&direction=descending

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The Effects of Foxe1 Loss of Function in Diabetic Thyroid

FOXE1 (forkhead box protein 1) is part of the forkhead box family of transcription factors that are responsible for cell proliferation and differentiation. The loss of FOXE1 function has been strongly associated with genetic predisposition to congenital hypothyroidism. Moreover, common FOXE1 polymorphic variants have been strongly associated with adult-onset hypothyroidism. Using our newly developed tamoxifen-induced *Foxe1*$^{flox/flox}$*/Cre* mouse model of adult *Foxe1* deficiency, we showed *Foxe1* loss in the adult mouse thyroid led to adverse changes in thyroid structure, including smaller and more condensed follicular architecture, as well as inflammation and fibrosis. This fibrotic response is significant given the estimate that fibrotic disease accounts for ~35% of world-wide deaths. Fibrosis is defined as an excess of extracellular matrix such as collagens in organs that ultimately impairs their functions. Type 2 diabetes mellitus (T2DM) can be classified as one such disease, given that in diabetes-induced interstitial fibrosis in the heart leads to impaired left ventricular compliance and dysfunction, ultimately leading to heart failure. However, T2DM-induced thyroidal fibrosis and the loss of Foxe1 function in diabetes setting remain undetermined. In this project, we aim to determine the extent to which the loss of Foxe1 functions play a role in T2DM-induced thyroidal inflammation and fibrosis using a mouse model of T2DM, Lepr$^{db/db}$. Through well established histological and immunofluorescence staining as well as quantitative real-time PCR, students will learn experimental techniques at the genetic and protein levels and apply the knowledge to understand the impact of T2D in thyroid health.