

**WASHINGTON STATE UNIVERSITY
COLLEGE OF PHARMACY AND PHARMACEUTICAL SCIENCES
SURF/SRF PROJECTS 2023**

FACULTY MENTOR:

Salah-uddin Ahmed, Ph.D., Professor, *ASPET member*

RESEARCH PROJECT:

Identifying novel therapeutic targets in the pathogenesis of rheumatic diseases

DESCRIPTION OF PROJECT:

The Ahmed lab is involved in translational studies aimed at identifying the unknown mechanisms that govern pathogenesis of rheumatic diseases such as rheumatoid arthritis, osteoarthritis, and gout. In particular, our group has been investigating the posttranslational and epigenetic mechanisms that facilitate rheumatic disease propagation. Using synovial cells, tissues, and serum samples from rheumatoid arthritis patient population, we conduct genomics and metabolomics studies to identify some key mediators that promote chronic inflammation and tissue destruction in rheumatoid arthritis. Based on this information, our group in collaboration at international level with computational biologists and medicinal chemists has been designing novel compounds to be tested for their efficacy in vitro and in pre-clinical models of rheumatoid arthritis and other autoimmune diseases.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

The SURF student is assigned a role and/or participation in some ongoing or new projects where they not only get the chance to learn about the inflammatory signal transduction pathways that promote rheumatic diseases, but also get a chance to sharpen their technical skills on running independently procedures such as ELISA, Western blotting, and In-gel zymography. After the training, the SURF fellow will be capable of summarizing his/her progress in the form of poster presentation. The work will be further expanded and presented at one of the national scientific meetings in which SURF students typically serve as co-authors. These studies have also resulted in the publication of manuscripts in the past.

FACULTY MENTOR:

John D. Clarke, Ph.D., Assistant Professor

RESEARCH PROJECT:

Precision medicine: natural product inhibition of drug transporters

DESCRIPTION OF PROJECT:

Toxicities from drugs and natural products are a significant burden on human health. This precision medicine project investigates inhibition of drug transporters by the natural product kratom. Kratom has stimulant and opioid-like properties and can be purchased at convenience stores, head shops, and many other retailers throughout the United States. The popularity of kratom has increased as people with opioid use disorder seek ways to overcome their addiction. If people take kratom with other prescription drugs, the phytoconstituents in kratom may inhibit drug transporters in the intestine and liver and alter drug exposures and toxicities. We will investigate the effects of kratom constituents on drug transporter function using cell culture and rodent models.

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DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

After initial training, the fellow will be expected to work in an independent manner on all standard laboratory techniques (cell culture, Western blotting, etc.). The fellow will have the opportunity to work with lab staff on non-routine procedures (animal experiments, small molecule analysis, etc.) with the expectation that the fellow will be able to work in a semi-independent manner towards the goals of the project. The fellow will participate in the weekly lab meetings and meet with Dr. Clarke individually as needed, but no less than once every two weeks.

FACULTY MENTOR:

Travis Denton, Ph.D., Assistant Professor

DESCRIPTION OF PROJECT:

Projects in the Denton lab focus on small molecule, organic synthesis-based, medicinal chemistry. We also focus on the analytical determination of small molecules in biological matrices (analytical biochemistry). Projects currently ongoing in the lab are multiple, and all focus on trying to make life easier for people who are tormented by devastating neurological diseases.

Project 1: Treatments for ALS, Alzheimer's disease, Parkinson's disease, Huntington's disease, Multiple Sclerosis and other neurological disorders. In this project, we are developing small molecules to enhance cellular autophagy in an effort to fight neurodegenerative diseases. We use medicinal chemistry, human cell culture, primary human cells, *Drosophila melanogaster* (fruit flies), mice and rats and analytical techniques such as NMR, UPLC-HRMS/MS, X-ray crystallography and Western blotting, qPCR, HRMS based proteomics, stable isotope synthesis and analysis and more...

Project 2: Treatments for glutamine addicted cancers. In this project, we are developing phosphonic acid bioisosteres of fundamental α -ketoacids and many other small molecules to inhibit newly identified pathways (including the glutaminase II pathway) important for the progression of devastating forms of cancer such as triple-negative breast, castration resistant prostate, non-small cell lung, kidney and more... We use medicinal chemistry, human cell culture, lenti-CRISPR-Cas9 knockout, lentivirally transduced shRNA, protein overexpression in bacteria, protein isolation and assay development, protein crystallization, X-ray crystallography and more...

Project 3: Treatment of Alzheimer's disease by targeting the $\alpha 7$ nicotinic acetylcholine receptor (nAChR). In this project, we are synthesizing small molecules to target the $\alpha 7$ -nAChR, the only nicotinic receptor in the brain of Alzheimer's patients that is not down regulated. Through this approach, we hope to enhance the cognitive function of people with this disease. All techniques mentioned in **Projects 1** and **2** are utilized in **Project 3**.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

For Projects 1-3, the SURF student will synthesize, purify, and characterize new molecules that will be tested for biological activity by our collaborators. For Project 4, the undergraduate researcher will prepare the compounds using organic chemistry techniques as well as aiding in the development of extraction and ultra-performance liquid chromatography tandem mass spectrometry techniques for the quantification of these biomarkers. The SURF fellow will receive training from members of the laboratory so that they can perform procedures independently by the end of the summer. Results will be discussed with the mentor and presented in a poster at the end of the training period.

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FACULTY MENTOR:

Darrell A. Jackson, Ph.D., Associate Professor

DESCRIPTION OF PROJECT:

Projects in the Jackson lab focus on the intracellular trafficking of neurotransmitter receptors following neuronal injury. Current research projects that are ongoing in the lab are focused in determining the underlying mechanisms responsible for leading to injury and death of neuronal cells following ischemic stroke injury. The overall goals of these projects are to identify critical signaling pathways that ultimately lead to neuronal death following stroke injury in order to develop more focused therapeutic intervention.

Stroke is prevalent in elderly individuals and greater than 70% of all strokes occur above the age of 65. Ischemic stroke, the most prevalent form of stroke, results in delayed neuronal death (DND) in vulnerable brain areas, such as the hippocampus, and is mediated by α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors lacking the GluA2-subunit. GluA2-lacking AMPARs are Ca^{2+} -permeable and are known to contribute in DND. Most AMPARs expressed on adult hippocampal pyramidal neurons contain the edited form of GluA2 (Q607R) and are thus impermeable to Ca^{2+} entry. These receptors undergo an unusual subunit composition switch in response to ischemic/reperfusion (I/R), changing from a GluA2-containing Ca^{2+} -impermeable AMPAR to a GluA2-lacking Ca^{2+} -permeable AMPAR.

Project 1: To determine whether activation of estrogen receptors prevent post-ischemic induced internalization and degradation of GluA2-containing AMPARs. We will utilize immortalized neuronal cell line transfected with fluorescence tagged neurotransmitter receptors (AMPA GluA1 and GluA2 subunit), Rab proteins (use to examine intracellular trafficking of receptors), and estrogen receptors (ER alpha, ER beta, and G-protein coupled estrogen receptor; GPER) to examine whether estrogen signaling pathway can suppress the post-ischemic induced internalization and trafficking of GluA2-containing AMPARs toward degradative pathways.

Project 2: To determine whether activation of estrogen receptors suppress the oxidative stress signaling pathway responsible for mediating the internalization and degradation of GluA2-containing AMPARs. We will utilize immortalized neuronal cell line transiently transfected with fluorescence tagged neurotransmitter receptors (AMPA GluA1 and GluA2 subunit), Rab proteins (use to examine intracellular trafficking of receptors), and estrogen receptors (ER alpha, ER beta, and G-protein coupled estrogen receptor; GPER). Cell cultures will be treated with an oxidant (hydrogen peroxide) to examine whether estrogen receptor activation suppresses oxidative stress mediated internalization and trafficking of GluA2-containing AMPARs toward degradative pathways.

Projects 1 and 2 will utilize the following techniques:

1. Cell culture
2. Oxygen-glucose-deprivation (OGD)/reperfusion (simulated ischemic stroke)
3. Transfection; recombinant DNA expression
4. Confocal fluorescence microscopy
5. Western blot analysis
6. Drug administration (estrogen and estrogen antagonists); dose response
7. Maxi-prep; recombinant DNA plasmid isolation

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FACULTY MENTOR:

Philip Lazarus, Ph.D., Professor and Chair of Pharmaceutical Sciences, *ASPET member*

RESEARCH PROJECT:

The overlapping metabolism of opioids and cannabis: Potential consequences for toxicity and withdrawal among opioids users.

DESCRIPTION OF PROJECT:

As the U.S. population struggles with the ever-increasing burden of the ballooning opioid crisis, the interaction of cannabis and opioids is becoming an emerging area of research. Cannabis and opioids are metabolized by many of the same phase I and phase II enzymes in the liver, which could potentially influence the effects of either or both of these compounds. Compounds in both classes undergo rapid hydroxylation or oxidation by hepatic cytochrome P450 (CYP450) enzymes, followed by glucuronidation via the uridine 5'- diphospho-glucuronosyltransferase (UGT) class of enzymes, and finally excretion. The inhibition of these shared enzymes by cannabis and its metabolites has the potential to greatly influence opioid plasma levels in patients currently taking opioids as part of a pain management strategy. Preliminary research in our lab indicates that the cannabinoid (-)-trans- Δ^9 - tetrahydrocannabinol (THC) and three of its major metabolites (11-OH-THC, THC-COOH and THC-COO-glucuronide) are indeed inhibiting a number of CYP and UGT enzymes. Of particular interest is the inhibition of CYP2D6 and UGT2B7, which act to metabolize codeine, morphine and hydrocodone. **The goal of the present proposal will be to examine this inhibition in vivo in up to 30 people co-administered hydrocodone and a marijuana cigarette.** Given the public perceptions and misconceptions surrounding the efficacy of cannabis, and its potential uses in pain management, opioid withdrawal, and as a part of many patients' general health practices, a better understanding of how these bioactive compounds are influencing metabolic systems within the human liver are necessary. This knowledge will aid in understanding how cannabis fits into the public health crisis surrounding opioid overdose and opioid use disorder, provide a baseline understanding of how cannabis is metabolized, and how those active metabolites are influencing liver function in patients currently taking opioids.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

Students will be trained in the enzyme activity assays and analytical techniques needed to accomplish the experiments described above. The student will work directly with a graduate student trainee on the lab techniques for hydrocodone metabolite assessment, and a research nurse on the clinical aspects of the study. They will have bi-weekly meetings with the mentor as well as attending bi-weekly laboratory meetings. Once trained, the student will be able to work fairly independently, and will report on progress at meetings independently.

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FACULTY MENTOR:

Senthil Natesan, Ph.D., Assistant Professor

RESEARCH PROJECT:

Membrane-facilitated allosteric modulation of GPCRs

DESCRIPTION OF PROJECT:

Recent advances in structural biology of GPCRs, along with biophysical and computational studies suggest that amphiphilic and lipophilic molecules may gain access to binding sites of these receptors by first partitioning into the membrane and then reach the sites via lateral diffusion through the lipid bilayer. The highly ordered structure of the lipid bilayer may guide a lipophilic or amphiphilic drug molecule to a specific depth within the bilayer, affecting its local concentration near the binding site, and influencing its binding kinetics. Additionally, the constraints of the lipid bilayer including its composition and biophysical properties might play a critical role in “pre-organizing” ligand molecules in appropriate orientation and conformation to facilitate receptor binding. Despite its conspicuous involvement in molecular recognition processes, the critical role of membrane in ligand-binding mechanism to lipid-exposed transmembrane binding sites remains poorly understood. This project aims to gain mechanistic understanding of the structure-membrane interactions relationship (SMIR) of several approved drugs. Knowledge of SMIR will not only provide useful insights to receptor binding kinetics, but also enhance our ability to take advantage of the apparent membrane contributions in rational design of drugs targeting transmembrane proteins with improved efficacy and safety.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

The SURF fellow will learn basic to intermediate level computational techniques including building 3D structures of small molecules, geometry optimization, charge calculations, preparation of protein-ligand complex structures, performing MD simulations using NAMD, calculating binding free energy etc. As these techniques require extensive initial training, the fellow will work very closely with Dr. Natesan and his postdocs in the beginning and will become semi-independent towards the completion of the project. The student will have plenty of opportunity to interact with the mentor and his group members to discuss the progress and future directions.

FACULTY MENTOR:

Mary Paine, R.Ph., Ph.D., Professor, *ASPET member*

RESEARCH PROJECT:

Natural product-drug interactions: elucidating mechanisms and causative ingredients using translational research methods

DESCRIPTION OF PROJECT:

Patients often supplement their prescribed drug regimens with botanical and other natural products, which are readily available and often perceived as safe. In parallel, patients may not inform their health care providers, and health care providers may not query their patients, about their use of natural products. These practices raise concern for potentially adverse interactions between natural products and pharmaceutical drugs. Mechanisms underlying these understudied natural product-drug interactions can be pharmacokinetic (what the body does to the drug or

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natural product), pharmacodynamic (what the drug or natural product does to the body), or both. Common pharmacokinetic mechanisms include inhibition of drug metabolizing enzymes and transport proteins by the natural product, leading to enhanced or reduced systemic concentrations of the object drug and potentially, harmful effects or reduced efficacy of the drug. Projects involve (1) in vitro experiments using human-derived tissue to recover key kinetic or binding parameters of constituents in a given natural product, (2) applying these parameters to mathematical models to make in vivo predictions, (3) determining the pharmacokinetics of natural product constituents and object drugs using plasma and urine obtained from a clinical interaction study, and/or (4) assisting with an ongoing clinical natural product-drug interaction study involving human participants.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

Dr. Paine will work with the fellow to develop a project that includes one or more of the four aforementioned facets of our translational approach based on the fellow's interests and skillset, and an experienced member of the research team will work with the fellow directly. The fellow will meet with Dr. Paine regularly to monitor progress. In addition to participating in various SURF activities, the fellow will interact daily with group members and participate in weekly group meetings. The fellow is expected to become semi- or fully independent.

FACULTY MENTOR:

Bhagwat Prasad, Ph.D., Associate Professor

RESEARCH PROJECT:

Interindividual differences in drug disposition and response

DESCRIPTION OF PROJECT:

The research in the laboratory of Bhagwat Prasad focuses on the prediction of inter-individual differences in drug disposition and response. The Prasad lab utilizes quantitative mass spectrometry (targeted and untargeted proteomics and metabolomics) to quantify drug metabolizing enzymes, transporters and endogenous metabolites in human tissues and biofluids. These data along with genomics information and in vitro data are integrated into physiologically-based pharmacokinetic (PBPK) models to predict variability in drug and endobiotic disposition. The integrated genomics-proteomics-metabolomics approach is further applied to discover specific biomarkers of drug metabolizing enzymes and transporters for predicting disposition and response of xeno- and endo-biotics in special populations such as pediatrics where clinical data are not generally available.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

The SURF fellow will work under the mentorship of Dr. Prasad and a senior postdoc in Prasad lab on the proposed project. The SURF fellow will receive training on mass spectrometry and in vitro and in silico drug metabolism. The fellow will attend Prasad lab meetings weekly and present his/her research work twice in the lab meetings. The fellow will be encouraged to present the research findings as a poster presentation in scientific meetings.

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FACULTY MENTOR:

Boyang (Jason) Wu, Ph.D., Associate Professor

RESEARCH PROJECT:

Novel Role of LMO4 in Lethal Prostate Cancer

DESCRIPTION OF PROJECT:

This laboratory currently centers on prostate cancer research, with particular interests in exploring the molecular mechanisms underlying the development of aggressive behavior, lineage plasticity, and therapy resistance to antiandrogen and chemotherapeutic drugs in advanced prostate cancers. We recently identified aberrant upregulation of transcription cofactor LIM-domain only protein 4 (LMO4) in established enzalutamide (an antiandrogen drug)-resistant prostate cancer cell lines using RNA-seq approach. LMO4 has been originally implicated in regulating embryonic and neural development by interacting with other transcription factors and shown to play roles in certain types of cancers. Using enzalutamide-resistant cancer cell lines, we will elucidate the molecular mechanism by which LMO4 confers the aggressive growth, lineage plasticity and therapy resistance in prostate cancer cells. We will also determine whether disrupting the nuclear interactome of LMO4 could reverse antiandrogen drug resistance and synergize with other therapies for improved treatment efficacy.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

At the beginning of the summer, the fellow will work closely with Dr. Wu's postdocs to learn basic cell and molecular biology techniques necessary to execute the project in the in vitro setting, including cell culture, DNA and RNA extraction, Western blot, RT-qPCR, ELISA, MTS assays, etc. Meanwhile, the fellow will be exposed to reading scientific papers assigned by mentor, which are not only related to the project but also in a broad way to allow the fellow to learn the frontier research in prostate cancer field specifically and cancer field in general. After obtaining required training for animal studies, the fellow is also welcome to participate in observing animal experiments and assisting other lab members with animal experimental procedures, such as measurement of tumor growth and scoring body conditions of mice. After the training, the fellow will work semi-independently on his/her project. The fellow will report research progress and give a research summary presentation to the lab at the end of summer.

FACULTY MENTOR:

Hui Zhang, PhD, Assistant Professor

RESEARCH PROJECT:

iNKT cell-related cancer immunology and immunotherapy

DESCRIPTION OF PROJECT:

The research in the Zhang Lab is focused on Immunology and Cancer Biology, with a specific emphasis on iNKT cell-related cancer immunology and immunotherapy. Cancer immunotherapy, specifically immune checkpoint blockade (ICB)-based immunotherapy, is one of the most effective and promising approaches for cancer treatment. However, clinically only about 20% cancer patients respond to ICB therapy. The major problem is lymphocyte, specifically CD8⁺ T cells, cannot infiltrate into the tumor. CD8 T cell infiltration in the tumor relies on type I

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conventional dendritic cell (cDC1). NK cells play a critical role in cDC1 migration and maturation in tumor. Therefore, finding the factors controlling NK cell infiltration and activation in tumor is the key to improve ICB-based immunotherapy. We recently found that iNKT cells play crucial roles in NK cell filtration in tumor site and shaping tumor microenvironment. Our current project is to dissect the cellular and molecular mechanism of how iNKT cells modulate NK and myeloid cells in the tumor to shape tumor microenvironment and antitumor immunity. To this end, we use mouse model of melanoma and breast cancer to study tumor progression in adoptive cell transfer, gene expression in the tumor, and immune cell function of tumor infiltrating lymphocytes and myeloid cells. Main techniques used in this project include cell culture, animal handling, immune cell isolation, flow cytometry, ELISA, molecular biology (DNA, RNA and protein extraction, RT-PCR, qPCR, western blotting, RNA-Seq, etc.), immunohistochemistry and immunofluorescence.

DEGREE OF INDEPENDENCE EXPECTED OF SUMMER FELLOW:

At the beginning of the summer, the fellow will work closely with Dr. Zhang and his lab technician to learn some basic and specific procedures such as animal handling and molecular and cellular techniques. After the training, the fellow will work semi-independently on his/her project. There will be a lab meeting weekly to discuss progress and future directions.