

# **WV-INBRE FUNDED PARTNER INSTITUTIONS MENTORS DIRECTORY**

FOR

**2011-2012 HSTA GRADUATE AND  
2012 SUMMER RESEARCH INTERNSHIP AND  
FELLOWSHIP PROGRAM**

Offered by the

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

to be held at the following Institutions:

**Alderson-Broaddus College  
Bluefield State College  
University of Charleston  
Shepherd University  
West Liberty University  
West Virginia State University  
West Virginia Wesleyan College  
Wheeling Jesuit University**

## Introduction

The WV-INBRE is pleased to offer summer research internships and fellowships to students, high school science educators, and faculty from colleges and universities and high schools participating in the WV-INBRE program. In 2011, the internships to HSTA graduates at the PUIs will be from October 1, 2011 through September 30, 2012 through the academic year. The summer internship/fellowship period will be from June 11 through August 11, 2012 with the Summer Research Symposium to be held on July 28th at West Virginia University. Listed in this directory are WV-INBRE funded faculty members at our partner institutions who have agreed to participate as mentors to the HSTA graduates at the PUIs and in the summer internship/fellowship program. Each mentor has submitted a description of the project(s) that is (are) available to interns and fellows in his/her laboratory. Please review these carefully so that you are aware of what is available for academic year and summer projects. Some descriptions are more comprehensive than others; therefore, you may want to contact certain mentors for more detail or to ask for clarifications about the opportunities in their labs. In any case, it is a good idea to speak with potential mentors to be sure you understand what will be expected if you work in his/her lab for the summer.

A listing of mentors with a description of their research and the general area of research is presented on page 3. Mentors and project descriptions begin on page 4. Listed for each mentor is an e-mail address, phone number and, where available, a home-page address. The home-page addresses will allow you to learn about the mentors and their research programs.

Separate application forms for high school science educators are available on the WV-INBRE web site (<http://www.wv-inbre.net>) at a link under **2012 Summer Program**. **Direct electronic submission is now available and is the preferred method of application. Applications may also be submitted by mail or e-mail.**

For general questions about the internships available to HSTA graduates and the summer internship and fellowship program, or if you have difficulty reaching a mentor, please contact one of the following individuals who are serving as research program coordinators.

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## Mentors at WV-INBRE-Partner Institutions with INBRE-Funding

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## MENTORS AT PARTNER INSTITUTIONS FOR THE 2010-2011 ACADEMIC SCHOOL YEAR AND SUMMER INTERNSHIP PROGRAM FOR HIGH SCHOOL SCIENCE EDUCATORS AND FELLOWS

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### **Molecular Modeling of Cytochrome P450 2C9 and Substrate - Effector Binding.**

The research being conducted in this lab incorporates molecular modeling and kinetic analysis of cytochrome P450. The modeling studies allow us to look at the enzyme that metabolizes various drugs, with and without drugs in the active site. The cytochrome P450 is one of the major enzymes in drug metabolism within the liver. It has been shown that dapsone increases the metabolism of flurbiprofen, this is referred to as atypical kinetics. We are researching the hypothesis that the metabolism of the drug in the enzyme may be controlled by a second drug called an effector within the same active site. Studies using distances and hydrogen bonding of key amino acids as indicators along with correlation with kinetic data will provide us with a clearer picture of the mechanism of action of this enzyme as related to atypical kinetics.

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### **Research projects at Bluefield State College**

#### **Overview**

West Virginia–IDeA Network of Biomedical Research Excellence (WV-INBRE) has partnership of science research program at Bluefield State College (BSC). Dr. Belay's laboratory research at BSC focuses on stress, immune system and infection. Another research focus in Dr. Belay's laboratory is investigating *Pseudomonas aeruginosa* adaptation to environmental stress.

#### **Research project #1: How cold-induced stress increases susceptibility to chlamydia genital infection.**

Sexually transmitted diseases are of major medical and social importance globally. Chlamydia genital infection is the most common bacterial STD that may cause severe irreversible complications particularly in women. The research area of Dr. Belay's lab therefore focuses on the association of stress to chlamydia genital infection. Current research work in the lab is

examining the effect of stress on the pathogenesis of *Chlamydia trachomatis*. Data show, exposure of mice to cold water stress resulted in increased stress hormone production and decreased resistance to chlamydia genital infection during primary infection. Moreover, our results demonstrated that exposure of mice to cold water or restraint stress leads to an increase in the production of proinflammatory cytokines and nitric oxide or interferon gamma by splenic T cells.

Current and future studies are a) to elucidate the mechanisms of lymphocyte recruitment into infected reproductive tract tissues and assess the effect of stress in the recruitment; b) to analyze the histopathology changes in the genital tract during ascending chlamydia genital infection of the stress mouse model. We hypothesize that cold water-induced chronic stress increases the severity of genital chlamydial infection and tissue pathology by modulating the immune response against Chlamydia.

### **Research project #2: Survival of *Pseudomonas aeruginosa* in starved conditions**

*Pseudomonas aeruginosa* is well adapted for growth in low nutrient environments, however its ability to survive in these environments is not well investigated. During space flight the immune system is affected and organisms such as *P.aeruginosa* pose a health risk. We recently initiated investigating the viability of *P. aeruginosa* growing in water without nutrients and have observed distinct changes in the morphology or visual appearance of the organisms. Our hypothesis is: Starvation adaptation of *P. aeruginosa* in water results in expression of stress proteins that may enhance long-term existence of the pathogen under nutrition-limited conditions.

Variation in frequencies & intensities of protein bands was observed in response to starvation in water and further characterization of the total profiles in starved and non-starved cells of *Pseudomonas aeruginosa* is underway by Protea Biosciences Inc (Morgantown) using iTARAQ labeling, mass spectrometry and Protein Pilot 3.0 software. The identification of proteins will allow further experiments and develop new hypotheses.

### **Involvement of undergraduate students in research**

Student training includes biosafety, keeping records of laboratory supplies and inventory, animal handling and usage for research, basic microbiological methods, tissue culture, basic molecular biology methods (RNA/DNA isolation, regular/quantitative PCR, gel electrophoresis), immunoassays (ELISA) development, and maintaining data in computers. Successful establishment of standard tissue culture for *Chlamydia* inoculation and detection methods in the lab has elevated our capacity for educating and training students in biomedical research. After training, the students are involved in performing experiments by developing hypotheses of their own. Several students have presented posters in several Annual Summer Research Symposiums of West Virginia INBRE, Research Day at the Capitol, in the Annual Biomedical Conference for Minority Students (ABRCMS) (Austin, TX, 2007, Orlando, FL, 2008), and in the American Society for Microbiology General Meetings, Philadelphia, PA, June, 2009 and San Diego, CA, May 23-27, 2010.

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**Title: Molecular and biochemical mechanisms of the effect of plant derived compounds on cancer angiogenesis and growth**

The main focus of our group is to understand the effect of chemicals and antioxidants found in plants on the growth of the blood vessels (termed as angiogenesis) and tumor in cancers that affect humans. Participants in the INBRE program will work along with faculty and other students to contribute to this INBRE and NIH funded research. Projects range from studying the signal transduction and role of genes, gene knockdown and activation to see their effects on angiogenesis and tumor growth. Our studies are of importance in understanding the role of these genes in signal transduction and in developing novel therapeutic drugs in treating cancers.

Participants will join with an active team of researchers to work with a variety of flavonoids, cancer cells and animal models that mimic the diseases. They will also perform techniques depending on skill levels and interest. On-the-site training will be provided. Techniques include cell growth, cytotoxicity, apoptosis assays, gene transfection and expression, electrophoresis, immunofluorescence microscopy, luciferase reporter assay, RT-qPCR, ELISA, Western Blotting, tube formation assays and CAM assays.

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**Sex Steroid Hormones and Epigenetics in Meningiomas**

**Abstract**

Meningiomas comprise approximately 30% of primary central nervous system tumors in the United States, however their pathobiology is poorly understood. Over 90% of meningiomas are benign while 5% are atypical and 3-5% are malignant. Complete surgical resection is the treatment of choice for benign meningiomas. Surgical resection is often difficult since approximately one-half of benign intracranial meningiomas arise in the skull base. For skull base meningiomas the surgical complication rate can be as high as 30 to 40% even in expert hands. The female to male incidence ratio in adults is 2:1 for intracranial tumors and 10:1 for spinal tumors, while no such sex difference exists for meningiomas in children. Therefore, the female sex steroid hormones progesterone and  $\beta$ -estradiol are suspected factors in meningioma

tumorigenesis. However, no mechanisms have been demonstrated for female sex hormones in meningioma formation or progression. We recently reported evidence that a steroid responsive gene, deleted in liver cancer-1 (DLC1), may function as a tumor suppressor in meningiomas. Our microarray data indicate that a number of steroid responsive genes are differentially expressed between meningiomas and normal meninges. We also found that steroid hormones and their antagonists can alter the growth of meningioma cells and that histone deacetylase inhibitors induce a decrease of meningioma cell growth in culture. Our long-term goal is to develop strategies to prevent or slow meningioma tumor growth that can serve as alternatives or adjuncts to surgery. The central hypotheses of this study are 1) that meningioma tumorigenesis is driven in part by actions of female steroid hormones and 2) that the tumorigenesis may be mediated in part by progesterone and estrogen receptor containing chromatin-modifying complexes. We are testing our hypothesis by pursuing the following three specific aims:

- 1) To treat meningioma cells with progesterone or 17 $\beta$ -estradiol and assess the expression of several genes that are differentially regulated between meningiomas and normal meninges.
- 2) To evaluate the effects of inhibitors of DNA methylation or histone de-acetylation on the growth of meningioma cells *in vitro* and on expression of genes that are differentially expressed between meningiomas and normal meninges.
- 3) To determine whether the promoters of the differentially regulated genes in specific aim 1 and 2 are bound by progesterone receptor, estrogen receptor, ETS2, or the histone acetyltransferase p300.

#### **Proposed project for HSTA participant**

One characteristic of some tumors is calcification. Meningiomas are typically calcified as are breast tumors (The first detectable sign of a breast tumor on a mammogram is often clusters of small dots of calcification.). Our work indicates that the *SLC20A2* gene, which codes for a cell surface receptor that transports phosphate into the cell, is over-expressed in meningiomas compared to normal meninges. This over-expression could play a role in both the tumor cell metabolism and the tissue calcification seen in tumors. The HSTA participant would grow tumor cells and evaluate whether levels of the *SLC20A2* gene responds to female steroid hormones and inhibitors of DNA methylation or histone de-acetylation in the tumor cells.

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#### **Mechanotransduction, Intracellular Signaling, and Vascular Cell Biology**

My research group is interested in how cells are able to sense and respond to changes in their environment. We are especially concerned with how smooth muscle cells in blood vessels perform their important functions. Cells of the cardiovascular system are continuously exposed to the effects of mechanical forces such as stretching and fluid shear stress. These forces, which are created by the pulsatile nature of blood flow when the heart contracts and

relaxes, have a marked influence on cell structure and function. Our primary concern is in how these cells are able to detect external forces and then to transmit the appropriate signal through chemical pathways inside the cell so that it can respond. We hope to not only work out what signaling pathways are involved but also to see if we can manipulate these signals to perhaps alter how the cell responds. The adaptations of these cells, which include enhanced growth and migration, seem to be important in the pathological conditions that accompany cardiovascular diseases such as atherosclerosis and hypertension.

## **Research Summary**

Cardiovascular disease remains a major cause of morbidity and mortality in the US and the economic and human costs associated with pathologies such as atherosclerosis, hypertension and restenosis are enormous. This has resulted in an intense research interest in the mechanisms which regulate contraction, migration and growth of vascular smooth muscle cell (VSMC). While it is now clear that mechanical forces imposed on VSMC in the vessel wall are important factors in the initiation and progression of these changes, the molecular mechanisms involved in these adaptations are not fully understood. In addition, it is now clear that the basic mechanism of smooth muscle contraction can only be explained in light of extensive remodeling of the cytoskeleton within cells. However, the exact nature of cytoskeletal reorganization and the mechanisms regulating these changes are not well known. The main goal of this project is to elucidate the acute response in cytoskeletal reorganization and intracellular signaling and during mechanical stress of VSMC. Utilizing molecular approaches combined with fluorescence microscopy, my lab evaluates the role that various cytoskeletal structures play in the response of VSMC to stretch. We are attempting to make a systematic determination of effects of various types of mechanical stress on activation of cell signaling molecules. In addition we are working to evaluate the effects of resveratrol, a purported cardioprotective molecule for its potential effects on stretch-induced cell signaling and receptor mediated cellular hypertrophy. The knowledge gained may be useful in the development of therapeutic agents regulating mechanotransduction mechanisms contributing to cardiovascular pathologies.

## **Student/ Teacher Involvement**

There are currently two on-going research projects in my lab:

### **Study 1: Determine the effects of static stretch, cyclic unidirectional stretch, cyclic multidirectional stretch, and fluid shear stress on activation of cell signaling molecules.**

Cells in the blood vascular system are subjected to a three main types of flow-related forces: fluid shear stress caused by friction between blood and the vessel wall, static stretch created by hydrostatic pressure, and cyclic stretch which is due to the pulsatile nature of blood flow. Perhaps due to the fact that VSMC normally are protected from the effects of shear, very little data is available. However, vascular interventions such as balloon angioplasty and stent implantation denude the endothelium and thus expose underlying SMC to effects of blood shear stress.

To our knowledge, there has been no systematic investigation of the effects of different types of mechanical stress in the same type of cell. Accordingly this study involves a systematic study of four major types of mechanical stress in the same cell line and carefully controlled

experiments using the same techniques of sample collection, analysis, and cell passage number.

**Study 2: Determine the effect of different levels of resveratrol on cell signaling associated with stretch induced changes in morphology and chemically induced hypertrophy.**

Resveratrol (RV) is a natural compound that is associated with several positive health benefits including protection from cardiovascular disease. RV is found in high concentrations in the skin of red grapes and is a constituent of red wine. The consumption of red wine in France has been suggested to account for the so-called “French paradox”, the observation that many French citizens suffer a relatively low incidence of coronary heart disease, despite having a diet relatively high in saturated fats. It is clear that RV has a number of effects on cell biochemistry that is of particular relevance to vascular smooth muscle. Accordingly, this study focuses on the dose-dependent, time-dependent effect of RV on stretch-induced changes in cell morphology and receptor mediated hypertrophy.

**Laboratory Techniques**

The research projects in my lab utilize several techniques including basic animal cell culture, mechanical stretching of cells, RNA isolation, cDNA synthesis, microarray analysis of gene expression, and real time PCR. In addition we use immunocytochemistry and fluorescence microscopy to visualize the cytoskeleton and other structural components within individual cells. Participants will be afforded the opportunity to learn these techniques.

**Recent Undergraduate Projects**

Effect of Unidirectional Stretch on Vascular Smooth Muscle Cell Structure and Function. Brent Pressman. SURE Research Symposium. WVSU. July 2009.

Gene Expression Profiling in Mechanically Stressed Smooth Muscle Cells. Phillip R. Jones. 14<sup>th</sup> Annual WVSU Research Symposium April 2008.

Response of Smooth Muscle Cells to Cyclic Versus Static Stretch. Niki Davis. 14<sup>th</sup> Annual WVSU Research Symposium April 2008.

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**D-Cycloserine Transdermal Gel Formulation Development**

D-Cycloserine (DCS) is an NMDA partial agonist with demonstrated enhancing effects of exposure therapy for specific phobias (and other anxiety disorders). In our previous WV-INBRE funded grant we demonstrated a significant degradation of DCS as soon as it comes in contact with low pH. If taken by oral route this drug should be protected from the acidic environment of the stomach. One solution can be an enteric coated form of this drug which can protect it from

the acidic pH of the stomach. The downside of this form is a delay in the release of DCS for at least one hour. The other possible approach would be to administer the drug through the skin and by-pass the stomach. A transdermal patch would allow a more controlled delivery of the drug during exposure therapy. Thus a DCS patch will avoid undue amounts of drug in the body and also the drug effects will be sustained throughout the exposure treatment. The present study is aimed to investigate the development of a gel formulation of DCS for transdermal administration including the following steps: (1) characterization of physicochemical properties of DCS; (2) selection of a suitable solvent system, and (3) formulation of the final transdermal gels. Several screening studies will be done to select the most appropriate excipients. The screened gel formulation will also be subjected to in vitro release studies, in vitro permeability studies, and stability studies.

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**Title of Abstract: Modeling and Simulation of Biochemical Processes using Petri Nets: Steroid Hormone Biosynthesis, Action of Aldosterone, and Disease States**

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## **ABSTRACT**

With the unprecedented growth in the volume of molecular biological system processes data, it is essential to integrate and organize that data into coherent descriptive and representative models. Structured modeling processes of complex systems are important for verification and understanding these systems. Modeling the systems in terms of a Petri net gives better understanding and powerful analytical capabilities as to the specific reactions and the order in which they need to be carried out are clearly stated. Petri Nets can be used to model biological processes on many levels. Qualitative Petri Nets, like a stochastic Petri Net, can answer quite a few questions about biological processes concerning their reachability (key compounds), structure (conflicting transitions), and topology (accumulation of metabolites).

In this research project, two different Petri net simulation tools, i.e., HPSim and YASPER and a stochastic Petri net modeling were used. In steroid biosynthesis the stochastic Petri Net shows several conflicting transitions where precursors are substrates for more than one enzyme (they can take more than one path). In the action of aldosterone, the effect of regulation, degradation,

and disassociation on the control of blood pressure, fluid and electrolyte homeostasis was shown. The major issue in modeling was balancing the complexity with clarity. Using Petri net simulation tools, the Steroid Hormone Biosynthesis (SHB) processes were created to compare the differences between the processes of adrenal glands and gonads of males and females expressing different forms of congenital adrenal hyperplasia (CAH), 5 $\alpha$  Reductase Deficiency, Aromatase Excess, Aromatase Deficiency and a non-expressing state. These models could be utilized in a simplest format as a visual aid for patients and undergraduate students, or in a more advanced mathematical format for further research into disease states.

Applied to SHB, PN modeling allows evaluation of the stages each steroid must pass through before expression and utilization by the body. Referencing hormone levels from blood analysis of males and females not expressing disease states, we establish non-diseased hormone levels as a baseline. Combining this baseline with the clearance rate of the steroids produced by the adrenal glands and gonads yields the minimum production rate. The production rate of each steroid divided by total steroid production yields the production ratio. Since each steroid begins the biosynthesis process as a cholesterol molecule, and manifestation of a steroid implies reaction with specific enzymes, the process directly resembles a Markov chain, analogous to a state machine PN. Adjusting the PN's interaction probabilities to match the production ratios arrived from the blood sample data creates a probability map demonstrating how the steroids are most probably formed in an individual not expressing CAH. Each disease state is defined by a deficiency or excess of a production enzyme, mathematically expressible as an altered probability of interaction with the steroid molecule.

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## **The Molecular Actions of Statins**

Ischemic heart disease is the generic designation for a group of closely related syndromes resulting from myocardial ischemia-an imbalance between the supply and demand for oxygenated blood. Up until recently, the disease was believed to be due to reduction in coronary blood flow due to atherosclerotic coronary artery obstruction. Now we know it is much more complicated. The disease of atherosclerosis is now thought to include endothelial cell response to injury and has been compared to chronic inflammation of the vascular wall. It appears that lipid-laden macrophages brought to the vascular wall due to endothelial activation play a role in the stability of the atherosclerotic plaque; rupture of the plaque due to release of matrix metalloproteinases from the macrophages results in exposure of the subintimal space which results in thrombus formation and obstruction (1). Recently, it has been shown that statins, which are used to treat high LDL

levels, also lower inflammation of the vascular wall independently of their lipid lowering effects (2). Our long-range goal is to uncover the molecular mechanisms of endothelial cell and macrophage activation by mildly modified LDL (LDL) and to determine whether statins inhibit this activation. One available project is determining which cytokines (involved in cellular recruitment) are stimulated by LDL and subsequently inhibited by statins. Another available project is looking at the interaction of Human Aortic Endothelial Cells and Macrophages.

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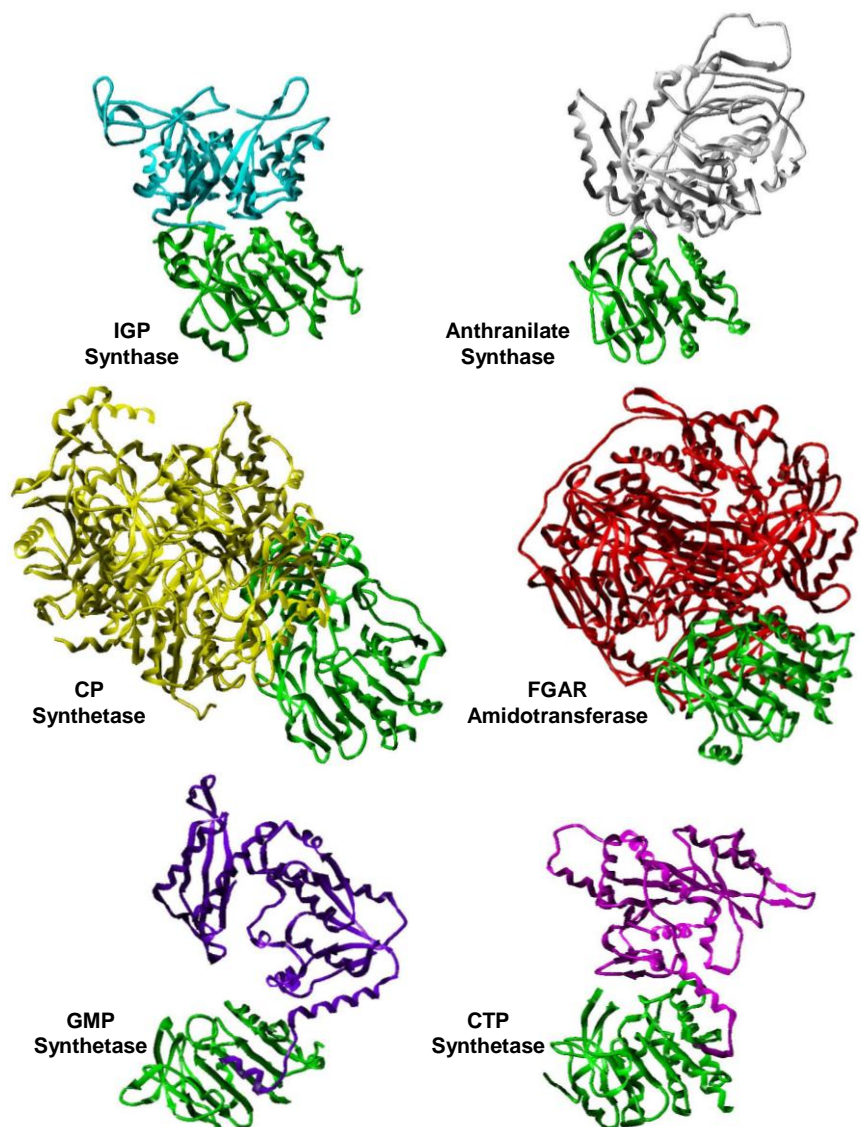
### *Research in the Linger Laboratory*

Dr. Linger is primarily interested in understanding how enzymes function to interact with small molecules in metabolic pathways or in discreet systems within the body. There are currently two projects ongoing in the Linger laboratory.

#### **GMP Synthetase: A Triad Glutamine Amidotransferase**

We will be investigating the function of the ultimate enzyme in the de novo purine biosynthetic pathway that converts xanthosine 5'-monophosphate to guanosine-5'-monophosphate, GMP synthetase (GMPS) through the incorporation of ammonia created from glutamine hydrolysis. By mechanism of this hydrolytic reaction, GMPS is classified as a member of the triad glutamine amidotransferase family of enzymes.

Triad glutamine amidotransferases share a common protein fold that contains the active site for glutamine hydrolysis. A conserved



**The Six Triad Glutamine Amidotransferases**

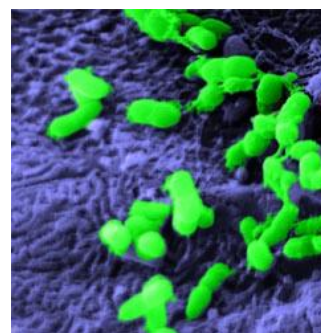
triad of amino acids composed of a cysteine at approximately position 86 residing in a conserved CXGXQ motif, and a histidine and glutamic acid approximately 90 residues downstream catalyzes glutamine hydrolysis. The utilization of a common protein fold in the glutaminase domain has led to the theory that the glutamine amidotransferases have evolved through gene duplication. Ancestral proteins in each subfamily of acceptor domains were likely ammonia-dependent proteins that utilized exogenous ammonia in their synthesis reactions. The functional association of a glutamine hydrolyzing protein then assured a steady source of ammonia, resulting in a greater abundance of essential building blocks for growth and replication. In review of the six triad glutamine amidotransferase structures solved to date, it is apparent that a common face of the amidotransferase domain docks onto the divergent acceptor domains.

The triad amidotransferases have evolved to allow ammonia to be incorporated into a wide variety of substrates including amino acids, nucleotides, amino sugars, and coenzymes. The transfer of ammonia is unique within every triad glutamine amidotransferase protein, but all share the common feature that the resulting ammonia from glutamine hydrolysis is sequestered within the protein, away from the bulk solvent, and shuttled between the two active sites so that the reactivity of  $\text{NH}_3$  is retained. In GMPS, the binding of XMP stimulates glutaminase activity 22,000-fold. This general feature is a property of the other triad amidotransferases, although there are quantitative distinctions as to the degree of the glutaminase regulation.

In previous studies with the triad glutamine amidotransferase imidazole glycerol phosphate synthase, an interdomain salt bridge was identified as a key contact between the glutaminase active site and the acceptor site conferring the binding signal previously described. In GMPS we have identified similar residues making up an interdomain salt bridge and plan to mutate these residues and analyze kinetically changes in the protein's activity and inhibition

### **Enhancing the Virulence of *Burkholderia cenocepacia*: The Action of LlpE**

*B. cenocepacia* is a soil bacterium that has been isolated from the lungs of individuals with decreased pulmonary function, either through granulomatous disease or cystic fibrosis. Infection by *B. cenocepacia* results in lung function deterioration and is highly transmissible between patients. *B. cenocepacia* has been classified as an emerging, highly virulent opportunistic and nosocomial pathogen. *B. cenocepacia* has multiple mechanisms to induce antibiotic resistance.



*B. cenocepacia*

Studies in the laboratory of Dr. Jane L. Burns at the University of Washington identified a highly conserved gene encoding a 286 amino acid protein associated with multi-drug efflux operon in *B. cenocepacia*. The operon was found to exist in several species of *Burkholderia*, both virulent and nonvirulent, but only in the highly virulent species, *B. cenocepacia*, was the gene for LlpE highly conserved. The putative protein is predicted to belong to the family of  $\alpha/\beta$  hydrolase lipases and esterases and has been given the name LlpE (Lipase-like protein, Efflux).

Studies in the laboratory of Dr. John J. Lipuma at the University of Michigan analyzed the ability of *B. cenocepacia* to survive in lung cells. They investigated the fate of both live and heat-killed *B. cenocepacia* after endocytosis. Only the live bacteria were able to escape from the endosomes within the cell through some unknown mechanism and were able to replicate in the cell. The

dead bacteria that remained in the endosomes were degraded by lysosome action. The researchers concluded that the ability of *B. cenocepacia* to escape and replicate within the epithelial cells suggested an enhanced survival strategy for the bacteria and may contribute to the highly virulent nature of this contagion.

We hypothesize that the bacterial mechanism of endosomal escape is a result of LlpE activity. We theorize that LlpE disrupts the endosome membrane through its putative lipase activity, disrupting the integrity of the membrane, allowing the bacteria to escape and relocalize to replicate within the cell, away from host immunological responses and thus conferring enhanced survivability to the bacteria.

Students who choose to work in the Linger Laboratory will gain facility with general protein expression techniques including bacterial culture, protein purification and spectrophotometric analyses.

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### **Kaempferol Inhibits Angiogenesis in Prostate Cancer Cells**

Prostate cancer is the most frequently diagnosed cancer and the 2<sup>nd</sup> leading cause of cancer deaths for men in America. Angiogenesis, the formation of new blood vessels from pre-existing microvasculature, is virtually quiescent in healthy adults but constantly active in prostate cancers, and anti-angiogenesis has been proposed to be a practical strategy for prevention and treatment of prostate cancers. Despite the test and approval of several drugs for anti-angiogenesis treatment in prostate cancer patients, there is an unmet need for safe, effective, cheap, and convenient agent for angio-prevention in prostate cancers, especially at its early stage. Kaempferol, a natural flavonoid widely distributed in fruits and vegetables, has been shown effective for angio-prevention in ovarian cancer cells, and our preliminary data demonstrate that kaempferol also inhibits secretion of vascular endothelial growth factor (VEGF), the most important growth factor for angiogenesis, in prostate cancer cells. In this research project, We propose to check kaempferol's effect on cell proliferation and cytotoxicity in prostate cancer cells, examine VEGF expression by prostate cancer cells under oxidative stress, investigate whether kaempferol inhibits angiogenesis induced by prostate cancer cells in *in vitro* and *in vivo* models, and explore several other genes and signaling molecules to understand the mechanisms for kaempferol's effects. This proposed study will have kaempferol better characterized for angio-prevention in prostate cancer cells, paving the way for further studies in animal models and human trials toward angio-prevention of prostate cancers in general population with a dietary component, kaempferol.

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**Area of research: Cancer**

**Title: Homer2 as a suppressor of filopodia formation and cell invasion.**

Filopodia and lamellipodia are extensions of the plasma membrane that are associated with cell motility, and are implicated in invasion by cancer cells. Their formation involves rearrangement of actin filaments through the interaction of a host of actin-binding proteins. Included in this group is the actin filament associate protein AFAP1, an adaptor protein that links the protein kinase C-alpha and src signaling systems. Although it is generally accepted that AFAP1 is a src activator, the mechanism by which src activation leads to filopodia formation is not fully understood. The regulation of AFAP1's ability to activate src may be a key event in filopodia formation. A yeast two hybrid study to identify binding partners of AFAP1 indicated that Homer2 as a strong binding partner.

Homer2 is an adaptor protein that is widely expressed in organs and tissues. Originally called cupidin because of its binding affinity with a numerous proteins, it is known to interact with actin filaments and has been demonstrated to inhibit filopodia formation in HeLa cells expressing active Cdc42. The goals of my research are to investigate the nature of AFAP1/Homer2 interactions with regard to src activation and filopodia formation.

There are four projects that are currently available in my lab. The student or faculty member who chooses any of these projects will learn cell culture, cell transfection, western blot analysis and fluorescence microscopy techniques.

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**Finding New Medicines for Bacterial Infections and Cancer Through the Isolation of Novel Biologically Active Natural Products from Plants.**

We seek to discover new medicines through the discovery of novel structural templates. One such technique is to search for new biologically active compounds from plants. Plants have long been known to be a rich source of important medicines. Compounds such as Taxol from the bark of the yew tree, digitalis from foxglove, and Aspirin from the bark of the willow tree are just three prominent examples. We have begun the process of isolating anti-bacterial and cytotoxic compounds from the propagules of the red mangrove tree and also from the common flower *Centaurea cyanus* (bachelor button). We were led to the potential for activity in the red

mangrove through a partnership with the University of Belize and based on its use in folk medicine in that region. Other species from the genus *Centaurea*, that are endemic to Greece, have recently been reported to contain some anti-bacterial and cytotoxic compounds. Gratifyingly, we have antibacterial and cytotoxic activity in the crude ethanol extract from the red mangrove propagules. Anti-bacterial and cytotoxic activity has also been found in the ethanol extract of the flowering parts of *Centaurea nigra*, a locally growing invasive flower. Cytotoxic activity only has been found in the ethanol extract of *Centaurea cyanus*.

We have begun fractionation of these different sources by silica gel flash column chromatography. Our next step is to further separate each of the fractions using preparative high pressure column chromatography (HPLC). We have obtained a brand new, state of the art, preparative HPLC instrument for this purpose. The instrument is controlled by a computer. The operator need only inject a solution of the initial fractions and utilize the correct method on the computer. The operator will collect fractions by watching the increase or decrease in UV absorbance on the detector, which utilize UV spectrophotometry. The fractions will then need to be condensed using a rotary evaporator before being submitted for additional biological testing. Active fractions will be once again separated on the preparatory HPLC, although the method on the computer will be modified to accomplish further separation. This project can be accomplished by someone with no previous chemistry experience. Familiarity with computers is a plus.