Dr. Mehnaaz Ali

Research: The focus of this proposed work is to develop aptamer-based sensors for the recognition of biomarkers. Aptamers are single stranded nucleic acid sequences, which can be selected for a specific target analyte, will be used as the target recognition molecule. The proposed sensors will link the recognition of a relevant analyte by an aptamer to the activation of apoglucose oxidase via its co-factor flavin adenine dinucleotide (FAD). Specifically, this strategy will be utilized within a displacement assay where a low affinity target analog *(modified with FAD)* bound to an aptamer can be displaced by a high affinity native target. By utilizing an enzyme-based detection module with high turnover rates, this sensor aims to provide excellent signal transduction and miniaturization capabilities. For our preliminary data an aptamer selected against model analytes (ATP and tryptophan) has been used to carry out the proof-of-concept work. An analyte binding event will trigger the co-factor FAD to activate a previously attenuated apoenzyme. The combination of aptamer based target recognition with an enzyme derived signaling output aims to provide an efficient tool for the sensitive and selective detection of relevant small molecules, proteins and nucleic acids which are important for clinical and environmental analysis.

Student Project: The *objective* of the student driven project is to show proof-of-concept for the proposed displacement assay using aptamers for target recognition with a co-factor-target trigger to signal the binding event. These studies will be tailored for the detection of small-molecules (that are relevant in clinical, environmental and forensics applications) where the recognition of this target molecule will initiate the signaling system. This strategy requires the conjugation of FAD to the model analyte, the results of which will serve as suboptimal target molecules for the aptamer based displacement assay. Thus, native analytes (free in solution) can be detected via the displacement of the prebound FAD-analyte via aptamer probes.

Dr. Gurdial Arora

Research: ***Pathophysiology of Urological Disorders in the Aging Male: The Regulatory Role of Hydrogen Sulfide and Nitric Oxide***

This study is designed to evaluate the regulatory effects of hydrogen sulfide (H2S) and nitric oxide (NO) during aging of human genitourinary tract and associated glands leading to the development of Urological disorders.

Student Project: We will select the student who has taken a basic statistics course and will teach the software needed to evaluate the data and statistical tools. Prof. Arora will help in promoting and coordinating the work of such selected student. Prof. Arora will teach the tools necessary tothe selected student to do the data analysis. Based on the courses taken by the student selected, we will prepare the timeline for the research planned.

Dr. Hector Biliran

Research: *Bit1 as a tumor suppressor and a therapeutic target in NSCLC*

The work in this laboratory has made significant contributions to the field of Bit1 functional biology particularly in the context of anoikis resistance and tumorigenicity of Non-Small Cell Lung Carcinoma (NSCLC), a deadly and the most prevalent form of lung cancer. Bit1 is a mitochondrial protein that is released to the cytoplasm following of loss of cell attachment, and associates with the transcriptional regulator protein Amino-terminal Enhancer of Split (AES) to trigger caspase-independent apoptosis. Our studies have shown that NSCLC cells are likely to bypass this pathway to become anoikis resistant and anchorage independent and provide the first evidence of the tumor suppressive function of Bit1 in NSCLC. Importantly, our recent findings indicate that Bit1 in addition to its anoikis function may negatively regulate Epithelial Mesenchymal Transition (EMT) in NSCLC cells. Hence, the observed tumor suppressive function of Bit1 may relate to its dual role in promoting anoikis and inhibiting EMT. This novel EMT regulatory function of Bit1 underscores its potential utility to circumvent the aggressive and metastatic phenotype of NSCLC.

Student Projects

Aim 1: To study the role of the Bit1/AES/TLE1 pathway in malignant transformation of human lung epithelial cells.
Rationale: Bit1 is a novel apoptotic pathway characterized by its unique regulation by integrins and its independence from caspases. As an anoikis effector and a guardian of anchorage dependence, the Bit1 pathway is likely to be circumvented by malignant cells to attain full anchorage independence and transformation potential. We previously showed that Bit1 pathway is selectively inhibited in NSCLC based on the selective downregulation of Bit1 expression and upregulation of the lung specific oncogene TLE1 in human NSCLC tumors. Targeted mitochondrial Bit1 expression in the anoikis resistant NSCLC A549 was sufficient to induce anoikis (despite defect in caspase activation) and attenuate anchorage-independent growth. Conversely, stable downregulation of endogenous Bit1 in these cells resulted in enhanced anoikis resistance and anchorage-independent growth in vitro and potentiated tumorigenic growth in vivo. Importantly, our recent data indicate Bit1 functions as an inhibitor of EMT in A549 cells. Taken together, these findings indicate Bit1 as a potential tumor suppressor gene in NSCLC, likely through its anoikis and EMT inhibitory function. To further examine the tumor suppressor hypothesis of Bit1, we will address the following questions: 1) whether Bit1 directly regulates transformation of lung epithelial cells and 2) what is the underlying specific mechanism of Bit1 effect on transformation. **Aim 1a** will determine if inhibition of the Bit1 pathway can induce transformation of immortalized HBEC and enhance the transformation of oncogene-induced HBEC. The latter is particularly important since a single or combination of genetic alterations (EGFR, hTERT, K-RAS, p16) can only confer partial malignant transformation of immortalized HBEC, and blocking the Bit1 pathway and/or activation of the TLE1 pathway may provide that additional needed oncogenic stimulus. Based on our findings that loss of Bit1 expression in A549 cells stimulates anoikis resistance and EMT, we will examine if Bit1 and/or TLE1 regulates apoptosis and the invasive/EMT phenotype of immortalized and oncogene-defined HBEC in three-dimensional (3D) tissue cultures (**Aim 1b**).
Experimental design:
Aim 1a: *Determine if inhibition of the Bit1 pathway can induce transformation of immortalized HBEC and potentiates the transformation potential of oncogene-induced lung epithelial cells*

Immortalized HBEC has been used extensively to study the contribution of genetic alterations in the pathogenesis of lung cancer and will be used as a model system in this study. Immortalized HBEC (carrying CDK4 and hTERT) will be purchased from ATCC and cultured in K-SFM (Life Technologies, Gaithersburg, MD) medium containing 50 ug/ml bovine pituitary extract (Life Technologies) and 5 ng/ml of EGF (Life Technologies). We will stably downregulate Bit1 expression in immortalized HBECs by using lentiviral shRNA. Ready to use lentiviral Bit1 shRNA expression constructs from Open Biosystems (Huntsville, AL) have been used in our lab to successfully knockdown Bit1 expression. We will use at least two lentiviral shRNAs that target different regions of the Bit1 gene. In parallel, stable control cell line will be created via the nontargeting shRNA construct. Following confirmation of Bit1 knockdown via western blotting/real-time PCR (to be performed by undergraduate students), the stable Bit1- and control shRNA HBEC lines will be subjected to a soft agar assay to assess their anchorage-independent ability. Briefly, 1,000 viable cells will be suspended and plated in 0.37% agar in maintenance medium with or without EGF and layered over a 0.50% agar base. The results from this study will be confirmed by examining the effect of stable downregulation of AES and exogenous expression of TLE1. We have both the lentiviral AESshRNA and GFP-TLE1 expression constructs (Open Biosystems). The effect of exogenous TLE1 on the transformation ability of immortalized HBEC is particularly important in light of the recently reported lung-specific oncogenic function of TLE1.

To investigate the effect of the Bit1 pathway on the transformation potential of oncogene-induced HBECs, we will test if disruption of the Bit1 pathway will enhance transformation of Ras-induced HBECs. First, the immortalized HBEC line will be transduced with the KRasG12V or vector lentiviral construct (Open Biosystems) followed by drug selection to establish population of cells stably expressing oncogenic activated Ras. The expression of oncogenic Ras will be assayed by immunoblotting using pan-Ras antibody (capable of detecting both endogenous and ectopic Ras) and its functionality will be validated by evaluating the activation of downstream effectors (Erk and Pi3K pathways) through immunoblotting. The resulting Ras-induced HBEC cell line will be transduced with control shRNA and Bit1 shRNA lentiviral vectors carrying a different drug selection marker to generate the control shRNA/Ras and Bit1 shRNA/Ras cell lines. The knockdown of Bit1 in Bit1shRNA/Ras cell lines will be confirmed by immunoblotting. Both the control shRNA/Ras and Bit1 shRNA/Ras lines will be subjected to 1) soft agar assays (which are routine procedures to be performed by undergraduate students) and 2) three-dimensional Matrigel (3D) cultures. The 3D Matrigel assay will allow quantification of the acinar size and the rate of lumen/hollowing formation. It will be interesting to see if blocking the Bit1 pathway in Ras-induced cells may lead to increase acinar size and decrease lumen formation. Considering that the latter is impacted by rate of cell death or apoptosis, we will focus on the impact of Bit1 on the rate of hollowing out. In the above assays, the specificity of the effect of Bit1 downregulation will be confirmed by AES downregulation and exogenous TLE1 expression.

Aim 1b: *Determine if Bit1 inhibition and/or TLE1 expression regulates apoptosis and controls the invasive/EMT phenotype of immortalized and oncogene-induced HBEC in three-dimensional (3D) tissue cultures*

The formation of hollow structures in 3D culture has been associated with induction of apoptosis.

Control and Bit1 shRNA cell lines derived from immortalized HBEC will be cultured in 3D matrigel in the presence or absence of EGF at days 5 and 8 and will be assayed for apoptosis by immunofluorescent based-TUNEL staining (Upstate Cell Signaling, Temecula, CA). The PI has extensive knowledge on TUNEL assay and will perform this work with assistance from undergraduate students. It will be interesting to see if basal apoptosis induced by 3D culture is enhanced by the lack of EGF and if this effect is attenuated by Bit1 suppression. To address if the observed apoptosis is caspase-dependent, we will prepare protein extracts from each of the cell populations by treating cultures with Dispase (Sigma) to digest the Matrigel and will be subjected to immunoblotting for inactive/cleaved caspase-3, caspase-8 and PARP (these are routine procedures to be performed by undergraduate students). To test the impact of Bit inhibition on oncogene-induced apoptosis under anchorage-independent growth condition, the control shRNA/Ras and Bit1 shRNA/Ras lines lines will be cultured in 3D Matrigel and assayed for apoptosis via TUNEL staining and detection of apoptotic markers (caspase-3, caspase-8, and PARP) by immunoblotting as described above. The specificity of Bit1 effect on basal apoptosis and oncogene-induced apoptosis in 3D cultures will be confirmed via downregulation AES and exogenous TLE1 expression in immortalized and Ras-induced HBECs.

To assess the effect of Bit1 inhibition on the invasive potential of immortalized HBEC, control shRNA and Bit1 shRNA HBEC lines will be subjected to a Matrigel/collagen 3D dimensional invasion assay as previously described. In this assay, acinar structures will be monitored for invasive behavior by measuring the development of multicellular protrusions and intercellular bridges. The use of Matrigel:collagen (1:1) mixture as opposed to pure Matrigel can enhance the formation of invasive branching morphogenesis of epithelial cells. In addition to monitoring for multicellular protrusions, we will use the 3D Spheroid Cell Invasion Assay Kit (Trevigen) to assess cell invasion. To determine the impact of Bit1 inhibition on the invasion potential of oncogene-induced HBEC, the control shRNA/Ras and Bit1 shRNA/Ras lines will be subjected to the above invasion assays in 3D culture conditions. In the aforementioned experiments, cell invasion will be visualized microscopically and quantitated through image analysis software. The second set of experiments will determine the impact of Bit inhibition on EMT in a) immortalized and b) oncogene-induced HBEC in both anchorage-dependent and independent conditions. Specifically, the i) control shRNA and Bit1 shRNA HBEC lines and ii) control shRNA/Ras and Bit1 shRNA/Ras HBEC lines will be cultured in attached or 3D Matrigel culture condition and examined for their changes in i) EMT-related morphology, ii) expression of EMT –related molecular markers via immunoblotting for the epithelial marker (E-cadherin) and mesenchymal markers (Vimentin and N-cadherin) and iii) migration/invasion potential. For Matrigel (3D) cultures, cell will be harvested after day 5 and 8 for immunoblotting to detect expression of EMT molecular markers and examined for invasive multicellular structures/protrusions to assess invasion as described above. The specificity of Bit1 effect on invasion/EMT of immortalized and Ras-induced HBECs will be confirmed by examining the impact of downregulating AES and/or expressing exogenous TLE1 in adherent and 3D cultures.

Aim 2: To determine the regulation of the TLE1 nuclear function by the Bit1/AES complex.

Rationale: Our cumulative data lend support to our hypothesis that the Bit1/AES axis negatively regulates the TLE1 survival and EMT promoting nuclear function to induce apoptosis and inhibit

EMT. Aim 2 will characterize the TLE1 nuclear pathway (via identification of its target genes and DNA binding interactants) and its ability to block Bit1 apoptosis and EMT inhibitory function. These studies will provide new insights on 1) how the Bit1/AES axis induces apoptosis and inhibits EMT and 2) the mechanism of TLE1 oncogenic function.

Aim 2: *Characterize the ability of the TLE1 nuclear pathway (via identification of its target genes and DNA binding interactants) to block Bit1 apoptosis and EMT inhibitory function.*

Initial studies will focus on the functionality of Bcl-2 to block Bit1 anoikis function and of E-cadherin to circumvent Bit1 inhibitory effect on EMT. Briefly, A549 or H460 cells will be cotransfected with mitochondrial Bit1 and/or Bcl-2 (Origene), and treated cells will be induced to undergo anoikis for 48h and analyzed for apoptosis assays as described above. Alternatively, Bcl-2 expression will be acutely ablated in mitochondrial Bit1 transfected cells, and cells will be subjected to anoikis assay as described above. If Bcl-2 expression cannot completely prevent Bit1-induced anoikis, such a finding suggests that TLE1 likely controls an entire apoptosis program, not just Bcl-2. Hence, we will identify novel TLE1 target gene(s) that regulate survival and apoptosis, and examine their individual and collaborative ability to block Bit1 anoikis (described below). To address the functionality of E-cadherin on Bit1 regulation of EMT, E-cadherin will be expressed in control or Bit1 knockdown cells and treated cells will be subjected to Boyden chamber migration and invasion assays. E-cadherin will also be acutely ablated in mitochondrial Bit1 transfected cells, and cells will be subjected to migration and invasion assays. Considering that loss of E-cadherin may also promote anoikis resistance, the effect of alteration of E-cadherin expression on Bit1 anoikis will be studied as described above.

To identify novel critical TLE1 target genes that may function downstream of the Bit1/AES axis, we will perform gene expression profiling via microarray analysis in control shRNA and TLE1 shRNA A549 cell lines. To eliminate false positives and focus on the core set of genes that exhibit functional role in anoikis, samples from controlshRNA and TLE1shRNA cells in both attached and detached conditions (24 h in suspension) will be used for RNA isolation, cDNA preparation, and downstream gene expression analysis. The resulting gene array data will be analyzed by the Affymetrix Gene Expression Analysis Software, and a set of genes will be identified that are positively or negatively regulated by TLE1 in detached condition as compared to TLE1shRNA attached or controlshRNA detached samples. The PI is aware that many genes will show differential expression and will focus on the category of genes that regulate apoptosis and/or EMT and exhibit transcription regulatory activity. The expression of promising core genes will be confirmed by real time-PCR/immunoblotting in controlshRNA/TLE1shRNA and control/GFP-TLE1 cell lines and subjected to the following functional analysis: i) their regulation by TLE1 will be confirmed by examining their expression following genetic alteration of TLE1 expression, ii) their ability (individually or collaboratively) to block Bit1 anoikis and EMT inhibitory function will be tested to identify downstream target gene(s) of the Bit1 pathway, and iii) the promising TLE1 target genes that exhibit inhibitory/regulatory effect on Bit1 pathway will be examined by ChIP assay for TLE1 to indicate their direct regulation by TLE1. We have already optimized the ChIP protocol, and specific TLE1 antibodies functional for ChIP assay are available from Active Motif. Because TLE1 lacks a DNA-binding domain and is recruited to target genes via interaction with DNA binding transcription

factors, we will identify these DNA binding transcription factor(s) via a large scale co-immunoprecipitation. Here, TLE1 will be immunoprecipitated from controlshRNA/TLE1shRNA and control/GFP-TLE1 cells cultured in attached or detached conditions. The PI has extensive experience and is knowledgeable of the constraints associated with this assay. We have specific anti-TLE1 (Abcam) as well as anti-GFP (Sigma) antibodies that are functional for immunoprecipitation. Because of close homology of TLE1 with other TLE homologues (such as TLE2 and TLE3), we will deplete the TLE homologues (using TLE2 and TLE3 specific antibody) in the sample prior to the actual specific TLE1 immunoprecipitation. The resulting specific co-precipitating proteins will be identified using mass spectroscopy (LCRC proteomics facility). Proteomic data will not only be validated by co-immunoprecipitation studies with specific antibodies but will be analyzed for DNA binding/transcriptional activity and their association with cell attachment or detachment. Importantly, the promoter of promising TLE1 target gene(s) derived from microarray studies will be examined for binding sites of candidate DNA binding transcriptional factors identified from proteomic studies.

Dr. Robert Blake

Research: The overall goal of my research is to increase fundamental knowledge about the nature and behavior of biomolecules and living systems. Research projects in my laboratory generally contribute to one of two principal topics: either (i) the enzymology and physiology of bacteria; or (ii) the role of antibodies in the immune system.

Student Projects:

Student Project #1

Transmission Electron Microscopic Analyses of 'Nanowires' Produced by Bacteria Associated with Solid Particles of Pyrite

The hypothesis of this student project is that acidophilic bacteria that exchange electrons with redox-active insoluble substrates produce conductive pili, termed 'nanowires', that facilitate extracellular electron flow between the bacterium and a solid electron donor or receptor. We formulated this hypothesis based on the reports of conductive pili that are produced by selected neutrophilic bacteria that electrochemically reduce insoluble iron oxides under anoxic conditions. To our knowledge, no one has reported the results of a search for pili that are produced by acidophilic bacteria that either oxidize or reduce insoluble iron.

Student Project #2

Transmission Electron Microscopic Analyses of Electron Dense Bodies on Bacteria that are Adhered to Pyrite

The hypothesis of this student project is that all acidophilic bacteria that respire aerobically on insoluble pyrite contain numerous small electron dense bodies when the bacteria are adhered to the surface of the solid. We formulated this hypothesis based on transmission electron microscopic (TEM) observations that both *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* contained small electron dense bodies at or near their cell surfaces when the sessile bacteria were located in close association with the surface of the pyrite. These electron dense bodies were not

observed when planktonic bacteria derived from the same cultures were not physically associated with the solid pyrite. The premise is that these electron dense bodies repre- sent nanoparticles of insoluble substrate that have been cleaved from the exposed surface of the solid and distributed around the outer periphery of the bacterium.

Student Project #3

Transmission Electron Microscopic Analyses of Bacteria that Create Corrosion Tunnels in Solid Pyrite

The hypothesis of this student project is that acidophilic bacteria that respire aerobically on insoluble pyrite create corrosion tunnels within the solid mineral that are approximately the dimensions of individual bacteria. We formulated this hypothesis based on microscopic observations obtained when *Acidithiobacillus ferrooxidans* respired at the expense of particulate pyrite (1-3), as well as our own observations that aerobic respiration on pyrite by *At. ferro- oxidans* greatly increased the surface area of the solid (4). The latter observation was consistent with a propagating pore model of corrosion whereby corrosion tunnels are created that increase the total surface area of the particles with time and inconsistent with a shrinking core model of corrosion whereby the surface area decreases with time as the particle size shrinks uniformly.

Student Project #4

Absorbance Measurements of Electron Transfer Reactions in Live Bacteria that Respire Aerobically on Soluble Iron

The hypothesis of this student project is that phylogenetically distinct microorganisms express different types of electron transport biomolecules to achieve respiration on extracellular iron.This hypothesis is based on spectroscopic and structural studies conducted by both my own and many other laboratories using cell-free extracts derived from the different bacteria.

Student Project #5

Cooperative Behavior in Antibody-Antigen Binding Interactions

The hypothesis of this student project is that a bivalent antibody that exhibits positive cooperativity when it binds its antigen will have a higher ratio of biliganded to monoliganded antibody than that anticipated for an antibody that binds its two antigens in separate and independent binding events. This hypothesis is based on kinetic exclusion assays conducted in my laboratory that showed that five of the monoclonal antibodies analyzed from the National Cancer Institute's (NCI) Clinical Proteomic Technologies for Cancer initiative exhibited homotropic positive cooperativity when they bound their respective protein antigens. That is, each of these bivalent antibodies bound their second equivalent of antigen much more tightly than it did their first equivalent of antigen.

Dr. Jeremy Cohen

Research: In the Fall 2012 semester, Dr. Jeremy Cohen began a tenure track Assistant Professorship in the Department of Psychology at Xavier University of Louisiana. Dr. Cohen is a Cognitive Neuroscientist with an undergraduate degree in Cell and Molecular Biology from Tulane University and a PhD in Neuroscience from Tulane University. His graduate work focused on studying human brain anatomy related to persistent developmental stuttering. From there his research interests branched into emotional processing and the neural systems that underlie our emotional experiences. With a particular interest in understanding how changes in the brain relate to emotional dysregulation, he obtained a postdoctoral fellowship at Stanford University in the Center for Interdisciplinary Brain Sciences Research within the Psychiatry Department at the Stanford School of Medicine. There his research focused on brain structure and function that relates to anxiety (Williams Syndrome and Fragile X Syndrome) and depression (Psychotic Major Depression) in developmental populations. His publications in these populations indicated that insular cortex has a specific role in the formation of anxiety in developmental populations. Furthermore, more subtle changes to anterior insular cortex are related to an increase in psychotic symptoms while have no relationship with depressive symptoms. Dr. Cohen received an LBRN Summer Fellowship in 2013 to investigate anatomical abnormalities arising in insular cortex in Alzheimer's disease (AD) using the same manual morphometric method used in his previous publications. The insula is a known interface among memory, emotion and cognitive functions and shows significant progressive histological changes across AD course. The study revealed total and posterior insular volume reduction in AD compared to Mild Cognitive Impairment, which is a less severe albeit noticeable cognitive decline that typically precedes full AD development. Dr. Cohen is already collecting data for studies aimed at continuing investigations of insular morphometric changes in Autism through existing collaborations with researchers external to Xavier University of Louisiana. This will build upon his work in other developmental disorders with distinct social-behavioral profiles and symptoms on the Autistic spectrum. He also looks to expand his research into the exploration of the brain in a more system-wide approach to better understand how the brain interacts within itself to derive function. There is a growing consensus that the brain does not function as a series of distinct independent regions, but rather that it is the interaction of many specific brain areas simultaneously that predicts function. The growing use of graph theoretical techniques, along with established whole-brain automated processing techniques, will allow him to utilize graph theory to quantify changes in brain connectivity (both structurally and functionally) and its relationships to function. The hope is the approach can support single subject analysis to be compared to known normal brain organization given a subject's age and sex. Such a comparison could be a significant breakthrough in the use of imaging as a diagnostic tool sensitive enough to detect subtle brain abnormalities before clinical symptoms appear. This would allow for the earliest possible interventions via behavioral and pharmacological therapies.

Student Projects: There are several on-going research projects in my lab that could be suitable for student involvement. All projects currently revolve around studying human insular anatomy. Students would learn the anatomy of the insula, learn to identify this anatomy in standard MRI brain scans and utilize the established insular tracing method to become a reliable rater for obtaining regional volumetric measurements. Once the student shows statistical reliability with knowledge of insular anatomy, the student will create insular tracings to help inform a computer learning algorithm to automate the drawing procedure. Once the procedure becomes automated,
the student would be responsible for setting landmarks to guide the automated process and then to check the outputs when they are done. Once the automated pipeline is created for the insula, other brain regions that are a part of the salience network (including the anterior cingulate cortex, amygdala, and thalamus) will go through the same process of learning anatomy, protocol for tracing

each region and automating the drawing process. The drawings will be done in healthy children, children with autism, and children with Down's syndrome, as well as children and adults from the Stanford Reading study. The objective is to create a whole network brain mask paying close attention to obtaining inter-individual variability in anatomy.

Dr. Cecily Defreece

Research: The main interest of my laboratory is the study of the human mobile element LINE1 and its contribution to shaping the human genome. The mobile element LINE1 is a mutagen that is active in germ line and somatic tissues. LINE1 expression is observed in many types of cancers, including the cancers that are typically refractive to treatment, such as colon, lung, and ovarian cancers.

Student Project: **Fall 2015.** During this semester the student will work to identify small molecule inhibitors of the LINE1 endonuclease by screening a small molecule library that the lab has previously obtained. The student will also learn how to confirm any positive hits from the library by testing the ability of the inhibitors to prevent the activity of a related endonuclease, the APE1 endonuclease. The project has many positive attributes as the student will be (1) trained very well on proper pipetting technique, (2) taught how to perform biochemical assays used in high throughput screening, (3) shown how to use Excel and Prism programs to analyze and present data, (4) exposed to interpreting their data and decide on next steps of their project.

Spring 2016. During this semester the student will work to confirm any small molecule inhibitors that they discovered during the Fall semester. This part of the project will heavily involve tissue culture work by the student to determine if the molecules are toxic to cells and if the inhibitors can prevent LINE1 retrotransposition in cells. This part of the project is beneficial for student learning because they will learn (1) how to perform tissue culture, (2) assays that are commonly used in
the field of mobile elements and (3) how a scientific project utilizes many different techniques from bench top work to cellular work.

Summer 2016. During this time period, we would want to verify further that the molecule is directly binding to the LINE1 endonuclease and also test what affects the molecules may be having on cells if there does not appear to be a specific inhibition of the endonuclease activity. My current collaborators are at Tulane University in New Orleans, LA and include, Dr. Prescott Deininger, Dr. Astrid Engel, Dr. Victoria Belancio and Dr. Jeff Han. As of yet, I have not requested formal collaboration from other members in the field that work in different states. However, my goal would be to ask leaders of the field including Dr. John Moran, University of Michigan, Dr.
Haig Kazazian, The Johns Hopkins or Dr. Jef Boeke, NYU Langone Medical Center.

Dr. Stassi DiMaggio

Research:

I. The multibillion dollar chemical and polymer industry across Louisiana and Mississippi represents a sizable fraction of total U.S. chemical manufacturing and accounts for a significant fraction of the total regional economic output. Polymerization processes are fraught with problems of waste, inefficiency, and failed products with a large environmental footprint. These problems can be traced in large part to lags in polymerization process monitoring, which traditionally relies on laborious manual product sampling and slow lab turnaround. As next-generation stimuli responsive polymers, with applications in medicine, coatings, environmental remediation, *etc*., enter commercial production these issues will be exacerbated as a result of the more stringent processing constraints required to maintain desired polymer responses. Quantitative decision-

making enabled by polymerization reaction monitoring and control technologies could thereby provide significant returns across all sectors, and represents a strategic opportunity for research investment that will sustain and enhance regional competiveness into the future.

II. Synthesizing Dendrimer with Precisely Defined Numbers of Click Ligands for Target Delivery Application

Nanomaterials provide a potential platform for cancer treatment due to their ability to simultaneously detect early stage cancers, target cancer cells over non-cancerous cells, directly deliver an active anti-cancer therapeutic, and monitor treatment efficacy. Poly(amidoamine) (PAMAM) dendrimers are a class of nanomaterials ideally suited for use as a drug delivery platform due to their controllable size, uniformity, and ample number of versatile terminal amine groups that allow for effective conjugation reactions. Thus, functionalizing dendritic polymers with small-molecule conjugates yields nanomaterials having desirable characteristics for treating cancer with greater selectivity and efficacy. To this end, dendrimers conjugated to targeting agents, dyes and anti-cancer drugs have been used to track and selectively deliver chemotherapeutics to tumor cells. Conjugation of small hydrophobic ligands such as fluorescent dyes, click ligands to nanomaterials containing high numbers of functional site result in a Poisson distribution of ligand/particle ratios in the product obtained. The presence of these distributions complicates understanding of the chemical properties of the stochastic mixture of the nanomaterials thereby limiting their effectiveness for biomedical applications. To address this problem we are going to utilize Click chemistry to stochastically functionalize the dendrimer platform. The alkyne ligand used will results to distribution of components that can be resolved by high performance liquid chromatography (HPLC) to isolate precisely defined click ligand/particle ratios. The precisely defined dendrimer –ligand material will be used as a specific point of attaching the targeting agent such as peptides, folic acid and other small molecules with biological application. Once conjugated, the PAMAM-ligand products will be characterized by HPLC, Proton NMR, and Matrix Assisted Laser Desorption Ionization-TOF.

Student Projects:

Project I. Over two years, a student would synthesize Generation 2 and 3 bis-MPA polymers. The polymers would then be functionalized with biologically active conjugates (fluorescent dyes for cellular uptake tracking, cell penetrating peptides for gene delivery, tumor targeting agents, chemotherapy drugs, etc) and the quantity of the conjugates would be controlled and fully characterized. The material synthesis could then be monitored with the Stimuli Responsive Automatic Continuous Online Monitoring of Polymerization Reactions (SR-ACOMP) analysis platform at Tulane University. The student would be trained to monitor polymer onset and evolution in real time and record material properties and property changes as a result of external stimuli.

Project II. A student would be synthesizing a series of PAMAM dendrimers with precisely defined numbers of click ligand conjugates and fully isolate ad characterize each population. Then, small, biologically active molecules will be conjugated (dyes, peptides, drugs) for cellular uptake studies. Advanced characterization and fluorescence microscopy studies can be performed at the University of Michigan in the labs of our collaborators.

Dr. Maryam Foroozesh

Cytochrome P450 enzymes are the most important enzymes responsible for the biotransformation and metabolism of endogenous and xenobiotic compounds including procarcinogens and pharmaceuticals. The long-term goals of many of our projects are to design mechanism-based irreversible inactivators selective for human cytochrome P450s 1A1, 1A2, 2A6 and 2A13. Selective mechanism-based (suicide) inhibitors are valuable experimental tools for studying the active sites of P450 enzymes.
Over the past years, our efforts have focused towards the design, synthesis and bioassay of new families of P450 inhibitors with structural similarities to known P450 substrates and inactivators. Many of these inhibitors are metabolized by P450 enzymes into reactive intermediates that can covalently bond to the nucleophilic amino acid residues in the active sites leading to irreversible inhibition. These covalently bonded intermediates can provide new and important mechanistic information regarding the mechanisms of action and active site structures of the P450s studied. The information obtained from such studies can lead to the synthesis of more efficient drugs with lower side effects. The 2A6 and 2A13 inhibitors can also potentially be used in conjunction with various nicotine replacement therapies (NRT) for tobacco dependence and enhance their therapeutic outcomes.

Dr. Galina Goloverda

Research: *Inorganic Nanoparticles in Non-Polymeric Organic Coating for Biomedical Application*

Development of functional inorganic nanoparticles for biological and clinical applications remains one of the popular fields of investigation for more than 2 decades. It involves a truly multidisciplinary and global endeavor and is one of the most challenging research areas in chemistry and materials science. Application of these nanoparticles has been already explored in a wide variety of medical treatment and diagnostic procedures, such as cell labeling, cancer therapy, gene therapy, blood de-toxication, radiation treatment, drug and radioisotope delivery, hyperthermia, and magnetic resonance imaging (MRI). Despite a substantial amount of work done in this field we still do not have the reliable biocompatible materials which are ready for clinical use. On one hand, such a material has to be strong in its physical property, magnetic or optical; on the other hand it has to be dispersible and stable in physiological fluids. Physical property is governed by the quality of the nanoparticles in terms of their purity, crystal structure, shape and size distribution. Dispersibility in physiological fluids strongly relies on the nanoparticle's organic coating. Currently used polymeric coatings facilitate the aqueous colloidal stability; however often obscure physical properties of the inorganic core, the permeability and mobility of the resulting composite in blood vessels. Due to large diamagnetic component, polymers suppress the composite's response to an external magnetic field.

Student Project: *Functional Capping Ligands Based on 2-hydroxyisophthalic Acid*

In the first year of this project a student will master synthetic strategies available in the lab and work on synthesis of 2,5-dihydroxyisophthalic acid. At this point we have good preliminary data for this synthesis, starting with 2,5-dihydroxybenzoic acid or 5-hydroxyisophthalic acid. The student will start with formylation of 2,5-dihydroxybenzoic acid (Duff reaction) followed by oxidation. The second route is currently under development and uses hydroxylation of 5-hydroxyisophthalic acid. It is expected that student will become relatively independent in calculations, scaling, planning and conducting synthetic, isolation and purification procedures during the first year.

We will explore possibilities for the student to receive an off-campus summer experience, however exact place for the student to go has yet to be determined.

In the second year the student will use the synthetic strategy developed in1 to synthesize a series of ligands of different length by reacting 2,5-dihydroxyisophthalic acid with allyl glycidyl ether under different stoichiometric ratios and reaction conditions. This part will be more exploratory as we cannot predict how 2,5-dihydroxyisophthalic acid will behave differently from 5-hydroxyisophthalic acid under reaction conditions. The student will master several protection-de-protection techniques. It is also expected that student will become good in spectra acquiring and interpretation. These will include but will not be limited to NMR, IR and MS. If time permits, the student will be engaged in Dynamic Light Scattering studies of the obtained ligands with iron oxide nanoparticles.

Dr. Elliot Hammer

Research: conducting experiments on factors that predict associations with skin bleaching and other body modification techniques. Although research on body modifications (e.g., tattoos, piercings, wigs) focuses primarily on psychological health and self-esteem, we are interested in body esteem and general affect as predictors, with a special focus on racial identity. We have identified a range of body modification practices and discussed which ones may have racial undertones to them (e.g., hair straightening, skin lightening, lip injections) and which do not (e.g., tattoos, false eyelashes, piercings). We expect that racial identity will predict attitudes toward race-related modifications but not the others. For the special focus on skin bleaching, which has grown in popularity recently, especially in developing countries, we are manipulating the message participants receive—through either a pro- or anti-bleaching video, and we are assessing attitudes toward the practice in three scenarios where an imaginary target engages in the practice.

Dr. Thomas Huckaba

Research: My laboratory studies a neurodegenerative disease known as Hereditary Spastic Paraplegia (HSP). While mutations in over 60 human genes have been shown to cause HSP, we are particularly interested in mutations in the kinesin, Kif5A, that give rise to the disease. Kinesins are molecular motors that are responsible for a variety of cellular functions, including organelle transport. We are using a variety of biochemical, biophysical, and cell biological approaches in order to understand the molecular basis for the neurodegeneration associated with each of the HSP-causing Kif5A mutations.

Student Project: To date, there are twenty different mutations in the human Kif5A gene that cause HSP. My laboratory has been working on a subset of these mutations, but there are plenty that remain uncharacterized. Following is a 2-year plan to characterize a single mutation from start to finish.

Year 1

☐ Mutagenesis of the Kif5A gene that has been inserted into a bacterial expression vector
☐ Expression and purification of the mutant protein
☐ *In vitro* biochemical/biophysical assays including solution ATPase activity, microtubule affinity assays in the individual nucleotide states, microtubule gliding assays, and single molecule motility assays
☐ Mutagenesis of full-length, mammalian expression vector for cellular studies

Year 2

☐ Neuronal cell culture training, including transfection of mutant Kif5A constructs
☐ Analysis of altered cargo dynamics in axons of transfected neurons using laser scanning confocal microscopy
☐ Preparation of protein and crystallography trays for structural studies

Summer in collaborator's lab at Dartmouth

☐ Solving the crystal structure of the mutant kinesin, including use of the beam line, and exposure to the computational tools necessary for solving the structure

Dr. Kelly Johanson

Research: Alveolar Rhabdomyosarcoma (ARMS) is a malignant, skeletal muscle tumor that occurs primarily in older children and adolescents and is primarily characterized by the presence of the oncogenic transcription factor PAX3-FOXO1. While many PAX3-FOXO1 transcriptional targets have been identified, little progress has been made in mapping the DNA binding sites of this transcription factor or the possible cooperation between transcription factors that may be essential for activation. PAX3-FOXO1 results from a chromosomal fusion of the N-terminal domain of PAX3 with the C-terminal domain of FOXO1. The fusion protein contains both PAX3 DNA-binding domains and the truncated but functional DNA-binding domain of FOXO1. Truncation of the FOXO1 DNA-binding domain results in a reduced specificity for known FOXO1 recognition sequences, and the fusion alters the specificity of both the PAX3 paired and homeodomains. Our lab is focused on understand the interactions between PAX1-FOXO1 and its target DNA sequences. We work on identifying the amino acids and nucleotides that are important for maintaining PAX3-FOXO1 DNA interactions as well as understanding the cellular environment that facilitates PAX3-FOXO1 directed gene activation.

Student Project: An examination of these putative binding sites has identified a number of common transcription factor binding sites that flank these regions, including those involved in muscle development and differentiation. While limited data exists about PAX3-FOXO1 interacting proteins, it is well known that interactions between FOXO1 and other transcription factors is necessary for both to bind to and activate promoter regions. Taken together, these findings indicate a need to screen PAX3-FOXO1 interacting proteins to determine if they correlate with the transcription factor binding sites near putative PAX3-FOXO1 elements. This project will focus on identifying proteins that interact with PAX3-FOXO1 both before and as part of DNA binding.

Summer 1

The goal of these first few months will be to identify potential PAX3-FOXO1 interacting proteins based on DNA sequence analysis. The student will be trained on the web-based tools used to screen DNA sequences for transcription factor binding sites near PAX3-FOXO1 recognition sequences. The student will select a small pool of sequences to begin analyzing through DNA pull down experiments. The student will begin by performing DNA pull downs with nuclear extracts from ARMS cell lines and a single DNA sequence in order to optimize the conditions for this experiment. The student will also learn how to perform Western blots to verify expression of PAX3-FOXO1 in the nuclear extracts.

Year 1

The student will begin to screen their target sequences using DNA pull down assays. Each sequence

will be tested using nuclear extracts from several different cell lines, including those that do not express PAX3-FOXO1. The student will use Western Blot to identify proteins that associate with PAX3-FOXO1 in the DNA complex. If multiple proteins are isolated, MALDI TOF MS will be used to determine the identities of the interacting proteins Immunoprecipitations using PAX3- FOXO1 as bait will also be performed under the same conditions to determine if the interaction between PAX3-FOXO1 and other proteins requires a DNA scaffold. Once interacting proteins have been identified in complex with a particular DNA sequence, the student will repeat the experiment with nuclear extracts expressing a DNA binding mutant of PAX3-FOXO1 to determine the effect on the interaction.

Summer 2

The student will travel to a collaborator's lab in order to perform one of several different experiments designed to map the interaction between PAX3-FOXO1 protein complexes and DNA. This can be done DNA Footprinting or through chromatin immunoprecipitation (ChIP). An alternative project would be knock down expression of a PAX3-FOXO1 interacting proteins using siRNA in and ARMS cell line. These cells would then be used for DNA pull downs to determine the effect on PAX3-FOXO1 DNA-binding. Luciferase assays can also be performed with these cells to determine the effect of reducing expression of the partner protein on PAX3- FOXO1 directed transcriptional activation. The siRNA project can also be performed at Xavier with the help of the RCMI Molecular Biology Core facility and will be rolled into the second year of the project if it does not fit within the summer research goals.

Year 2

The goal of this year is to use the information gathered during the summer to identify additional interacting partners and to determine the nucleotides that mediate the interaction with PAX3-FOXO1 and/or any partner proteins. Based on the results from the summer experiments, the student will design mutations in the DNA sequence designed to eliminate binding by PAX3-FOXO1 and/or the interacting protein. The mutant DNA sequences will then be tested in the pull down assay to determine the effect on PAX3-FOXO1 DNA binding and protein complex formation.

Dr. Vlajiko Kocic

Research: In the past twenty-five years, the area of difference equations has been one of the fastest growing fields in Mathematics. For a long time, this area was in the shadow of the well-developed theory of differential equations. While the differential equations are used in processes that are continuous in time, the difference equations are specifically tailored toward processes where the time is discrete. Many of the classical results in the area of difference equations, particularly in the case of linear equations, are analogues of corresponding results in the theory of differential equations. Approximately at that time the trend in the development of difference equations shifted toward the investigation of methods and techniques specifically tailored to address discrete problems. The wide mathematical community finally recognized this area as a completely separate discipline, independent of more developed differential equations. This fast development coincided with the appearance of the several specialized journals, an international society that organizes an annual international conference, and several research monographs. The American Mathematical Society (AMS) gave its contribution to the development of this field through the organization of special sessions on difference equation at practically every annual meeting in the past years, and at many regional meetings. Increased interest for discrete dynamical systems, advances in numerical analysis, discretization of continuous processes, and applications of difference equations in other disciplines such as Theoretical Physics, Mathematical Biology, Epidemiology, and Social Sciences also contributed to the rapid development of this field.

Student Project: Overall goal of the student project is to engage students in the research activities in the area of the dynamics and asymptotic behavior of discrete systems. Measurable outcomes are student's presentations (poster and oral) at students' meetings and at Joint Mathematics Meetings (annual joint meeting of all mathematical societies and associations), preparation of the joint research article to be published in professional peer reviewed journal, and acceptance to graduate school. This will be accomplished by carefully selecting research topic, providing student's training both on campus and off campus, guidance through the research process, preparation of presentations and finally writing the manuscript.

Dr. Anup Kundu

Research: **PROJECT TITLE:** ***Formulation of a targeted nanoparticle system for the treatment of breast cancer***

PROJECT SUMMARY

The goal of my research is to develop a targeted nanoparticle system for the treatment of breast cancer. Chemotherapy remains one of the major treatment options for metastatic breast cancer; however, acquired resistance to chemotherapeutic agents such as doxorubicin is a major reason for cancer treatment failure. Recently, it has been shown that knocking down the expression of MDR1 P-glycoprotein (P-gp) by P-gp specific siRNA could increase the delivery of doxorubicin to doxorubicin resistant breast cancer cells which could improve the metastatic breast cancer treatment. However, it will be more effective if the particles are selectively targeted to the cancer cells. The aptamer surface labeling will be used to selectively deliver both p-gp siRNA encapsulated nanoparticles and doxorubicin encapsulated nanoparticles to metastatic cancer cells. The efficacy of targeted versus non-targeted delivery of siRNA and doxorubicin nanoparticles to breast cancer cells will be examined. Our hypothesis is that conjugating nanoparticles with a cancer cell specific aptamer should allow selective delivery of therapeutic drugs to tumor cells leading to enhanced cellular toxicity and antitumor effect as compared to unconjugated nanoparticles. This hypothesis will be tested by the following two specific aims.

Specific Aim I: Formulation of nanoparticle-aptamer conjugates for targeted delivery of P-gp siRNA to breast cancer cells.
Specific Aim II: Formulation of doxorubicin encapsulated nanoparticles with surface aptamer for targeted delivery to breast cancer cells.

Dr. Candace Lawrence

Research: Currently in my lab, research is being conducted utilizing hydrogen-bonding to develop sturdy hydrogel systems for cancer drug delivery systems and to understand the characteristics of electronic transfer systems. These systems are being synthesized and developed by undergraduate students in my lab. Undergraduate students in my lab first learn how to research the literature and to find the important information pertaining to their project. Next, the students work directly with me in developing reactions and learning organic synthesis techniques. Finally, students will develop lengthy synthetic schemes on their own in respect to their project. I am devoted to working directly with the students in the lab to gain the needed experience and to learn basic and higher-level organic chemistry techniques. My students will also spend time analyzing the new organic compounds by Gas-Chromatography-Mass Spectrometry, by Infrared Spectroscopy, and by Nuclear Magnetic Resonance. Since I personally train all of my students, I make sure that each student develops at their own pace and are not rushed into completing experiments for which they are not ready. My undergraduate students must work directly with me on their projects and learn how to develop their own understanding of the experiments. During the summer, my students are expected to work alongside with me on the same projects and the effort is therefore a team situation. I am committed to teaching and training students on their level and work with them to develop higher-level experiments as they become more and more proficient.

Student Project: *Modified Nucleobases and Hydrogel Tissue Mimics and Drug Delivery Systems*

I. Brief Description:

The main goal of the proposed research is to investigate and study the formation of sturdy hydrogel systems that will incorporate natural and non-natural nucleic acid bases (nucleobases). These systems are used in the development of drug delivery systems to target cancer cells by appending cancer cell targeting polyamines. Most nucleobases that have been previously studied have shown a disruption to various pathways that contribute to cell proliferation. Therefore, the nucleobases have a twofold advantage in that they can become cancer cell drug targets and delivery systems. Specifically, we plan to 1) synthesize modified guanosine molecules to study the sturdiness of these hydrogels in biological media, 2) synthesize polyamine and amino acids as the cancer cell targeting unit, 3) combine both the guanosine and polyamine units, and 4) study the efficiency of the molecules as hydrogels. Once established, other nucleobases will be synthesized and studied as potential drug delivery systems. Research on this project includes both synthesis and purification of modified guanosine nucleobases and modified polyamine and amino acids. Subsequent research will include preparing and studying the synthesized nucleobases and the ability to form hydrogels. Analysis methods will include NMR, IR, HPLC-MS, and Fluorescence spectroscopy.

Dr. Kathleen Morgan

Current research in the Morgan lab focuses on simple carbohydrates and related compounds such as diols. Carbohydrates are ubiquitous in nature, yet there are still major gaps in our knowledge of sugar thermochemistry. Heats of formation ($\Delta_f H$) are being determined for simple monosaccharides having 2-4 carbons; remarkably, these fundamental values are unknown. A combination of reaction calorimetry, vaporization measurements, and high-level calculations will be used to obtain the $\Delta_f H$ for sugars and related compounds such as 1,2-diols. Although high-level calculations can often be used to obtain high quality thermochemical data, the compounds of interest have a large number of important conformations, making accurate calculations far from routine. The $\Delta_f H$ determined in this study will be used in conjunction with bond energies determined by collaborators, which afford the $\Delta_f H$ of free radicals that result from the degradation of carbohydrates.

II.

Thermochemical studies of diols: A student working on this project would start by determining reaction conditions for a reaction calorimetry study of diols. One reaction that seems promising is the hydrolysis of epoxides, and the student would need to further optimize the reaction conditions for our purposes. Once complete, this reaction would be used to measure the heats of reaction for a series of diols, via reaction calorimetry. At the same time, the student can work on calculations of the target diols to include various conformations. Other experiments are possible, such as determination of heats of vaporization and/or sublimation.

Thermochemical studies of simple sugars: A student working on this project would verify that the reaction conditions that have been developed will work on the carbohydrates planned for study, which will include glyceral and dihydroxyacetone. Then, the student will help with reaction calorimetry experiments that will, eventually, afford the heats of formation for the target compounds. The student will also work on calculations of the sugars and their reduced forms, in various conformations. Other experiments are possible, such as determination of heats of vaporization and/or sublimation.

Dr. Harris McFerrin

Although always focusing on the development and regulation of blood vessel formation, my research over the last ten years has touched on a number of topics: Human Immunodeficiency Virus (HIV), Human Herpes Virus-8 (HHV8), Herpes simplex virus-1 (HSV-1), Tumor Angiogenesis, Diabetes and Adult Human Mesenchymal Stem Cells (hMSC). These topics are all linked by their interaction with the vasculature through the up-regulation or down-regulation of the survival and growth of blood vessels. For example. HIV and HHV8 increase survival and growth of blood vessels to form lesions commonly known as Kaposi sarcoma. Diabetes negatively affects the vasculature, causing decreased blood flow and is linked to atherosclerosis and diabetic retinopathy. My lab has shown that the adult stem cells known as hMSC help to form blood vessels *in vivo* and are able to roll on and adhere to the vasculature through specific interactions, suggesting that they may be able to "home" to sites of damage.

Currently, my laboratory is investigating the role of cyclin-dependent kinases in HSV-1

infection of the eye. HSV-1 infects greater than 90% of humans worldwide and during ocular infection produces inflammation and neovascularization that can lead to blindness. In the United States, HSV infection is the leading cause of infection-induced blindness; nearly 40,000 new cases are reported, and 300,000 cases are treated yearly. We have demonstrated in a mouse eye model of HSV-1 infection that flavopiridol (a cyclin-depend kinase inhibitor) reduces neovascularization of the cornea as well as trifluorothymidine, the gold standard of treatment, and an international patent was submitted based on this research.

Dr. Krista Mincey

My research is looking at prostate cancer perception and risk in young Black males. Through focus groups with college-aged Black males 18-29, I seek to understand their prostate cancer knowledge and what behaviors or elements put them at greater risk for prostate cancer in the future. This information can then be used to inform prostate cancer programs geared toward prevention in young Black men.

Dr. Patience Obih

Title: ***Preparation and Screening of Medicinal Herbs for Antidiabetic Activity.***

Research

There are about 24 million Americans living with diabetes. Every year, 1.3 million are diagnosed with type 2 diabetes. Diabetes mellitus is a chronic disease associated with many debilitating complications which result in substantial morbidity and mortality. Though medicinal plants have been used historically throughout the world, few of them have been validated scientifically. Many studies have been able to isolate the compounds but have not gone beyond that to test them on animal or in human. The World Health Organization (WHO) is in support of the use of herbal remedies for the management of diabetes. Medicinal herbs are less likely to have drawbacks of the conventional drugs used for diabetes. Further research is required to validate the antidiabetic effects of medicinal plants. As a result of the limitations in many existing conventional antidiabetic drugs, it is crucial to search for alternative remedies for diabetes mellitus, and this has led many investigators to investigate traditional remedies for better alternatives. These traditional medicines are hoped to be cheaper, efficacious, more tolerable, and produce less side effects. The aim of our research therefore is to prepare and screen medicinal extracts for their antidiabetic activities. Our first goal is to examine the effects of medicinal herbs, Bitter leaf (*Vernonia amygdalina*), Dandelion (T*araxacum officinale*), Bitter melon (*Mormodica charantia),* Nchanwu (*Orignum vulgare,* Bitter Kola (*Garcinia kola*) on hyperglycemia using a glucose tolerance test on streptozotocin-induced diabetic mice. The second goal is to examine the mechanism of action of the medicinal herbs by evaluating their ability to inhibit alpha glucosidase and lipase enzymes.

Student Involvement:

In year 1, the first specific aim will be addressed. The students will learn how to prepare medicinal extracts from plants and follow up with glucose tolerance test with the extracts in

goal 1.

In year 2, the student will address specific aim 2 where he will conduct α-glucosidase and lipase inhibition studies *in vitro* to examine the mechanism of action of the medicinal extracts. He will also carry out chronic studies to evaluate the effect of the extracts on long term use. Phytochemical isolation of the active compounds will be carried out also during the second year.

Overall, the students will be trained in the following aspects of biomedical research:
A. research design
B. hypothesis testing
C. design & evaluation of diabetes research
D. data analysis and interpretation
E. presentation and publication of reports

Dr. KiTani Parker-Lemeiux

Title: Quiescence of noncancerous microenvironment promotes metastasis in triple negative breast cancer cells.

Abstract. The progression of breast cancer towards metastatic disease remains a poorly understood area of investigation. Among the most metastatic of breast cancers are those with the triple negative phenotype. Recently in my laboratory, we discovered that noncancerous breast epithelial MCF-10A cells secrete factors into their media that stimulate growth of triple negative breast cancer (TNBC) cell line MDA-MB-231 much more robustly than medium supplemented with fetal bovine serum. More interesting was the observation that these factors not only promoted growth and migration of TNBC a 2D cell culture system, they also substantially increased invasion of the MDA-MB231 cells. We propose to use noncancerous breast epithelial cell line MCF-10A in a 3D co-culture system and determine if the starved cells are undergoing quiescence, which may lead to apoptosis, or senescence, which may lead to metastasis.

Our *long-term goal* is to understand the molecular determinants that are critical in the progression, migration, and invasion of TNBC. The *objective* of this project, which is the next step toward the attainment of our long-term goal, is to determine whether nutritionally-deprived MCF-10A cells undergo quiescence verses senescence and to demonstrate cell if these noncancerous epithelial cells have undergone cell cycle arrest by measuring CCND1, which is cyclin D or if they are undergoing apoptosis by measuring the expression of TRAIL, which is part of the TNF superfamily. Caspases 3 and 8 will also be measured in nutritionally deprived MCF-10A cells. Our *central* hypothesis is that nutritionally-deprived MCF-10A cells undergo quiescence verses senescence, hence promotes proliferation, migration, and invasion of TNBC cell niches in vitro. The rationale for the proposed research is that better understanding the microenvironment of the TNBC cells will aid us in identifying molecular switches that may serve as pharmacological targets.

We plan to objectively test our central hypothesis and, thereby, attain the objective of this application by pursuing the following two *specific aims*:

1. ***To demonstrate that nutritionally deprived noncancerous MCF-10A cells undergo quiescence.***
Based on our preliminary data, our working hypothesis is that nutritionally-deprived MCF-10A cells undergo senescence, thereby promoting metastasis of TNBC cells. Our gene expression profile indicated that TNFSF10, or TRAIL is highly expressed in MCF-10A cells starved verses non-starved MCF-10A cells, suggesting these starved, noncancerous cells may undergo apoptosis and promote quiescence. Senescence may be evaluated by measuring CCND1, or cyclin D, while quiescence may be evaluated by measuring the expression of TNFSF10. This is important because there is a strong correlation between TNFSF10 expression, the up-regulation caspaces 3 and 8, and apoptosis, while high expression levels of cyclin D is indicative of cell cycle arrest leading to senescence. We propose 1) to evaluate markers associated with senescence and quiescence by: a) Western blot to measure the expression of cyclin D (CCND1) and TNFR10 (TRAIL) in each sample; b) use flow cytometry to evaluate the propidium-iodide-stained DNA in the control sample verses the test samples to determine the phase of the cell cycle of each sample; and c) perform IHC using dual-staining of cyclin D and TNFR10 in samples.

2. ***To determine the impact of nutritionally-deprived MCF-10A cells on metastasis of TNBC.***
Starved cells produce chemoattractants that promote the metastasis of TNBC cells. We propose to 1) to use cells grown in the alvetex 3D-co-culture system to conduct migration and invasion assays on TNBC cell line MDA-MB-231 treated with media from starved MCF-10A cells vs. non-starved MCF-10A cells 2) to evaluate the expressions of TNFR10 and CCND1 in the starved and non-starved MCF-10A cells and MDA-MB-231 TNBC cells grown in the alvetex 3D cell culture system. These data are expected to support the working hypothesis, which is that starved MCF-10A cells undergo senescence and promoting metastasis.

Florastina Payton-Stewart

Research: The long-term goal of this project is to develop novel therapeutic agents for treatment of ER+ metastatic breast cancer, in order to create therapy options for individuals who have failed to respond to, or relapsed on, current methods of treatment.

Student Project: ***Design and Synthesis of Anticancer Agents for Triple Negative Breast Cancer***

The undergraduate students will begin their research project during the summer, learning how to search the literature for current analogues of the known phytochemicals. The literature search will include gathering background information about their project. Students will explore background information about triple negative breast cancer, to increase their understanding of the critical need for therapeutic agents to treat the disease. Students will be expected to meet with PI to discuss background on phytochemicals and triple negative breast cancer on a weekly basis. Student will have the opportunity to explore alternative phytochemicals (phytochemical not currently explored in the Payton-Stewart research laboratory). Simultaneously, the student will learn the basic organic techniques through a series of synthetic steps. Currently, the analogues of the known phytochemical being research in our laboratory requires a large amount of intermediate product. The intermediate product requires reduction reactions. Through these reactions, students will apply basic chemical concepts learn in General and Organic chemistry. During the weekly meeting with PI, the student will learn about the chemical reactions and mechanism of all reactions. The students will review and/or learn how to setup basic reactions, become familiar with glassware in the laboratory, calculate molar equivalents and become familiar with the organic techniques such as recrystallization, extraction, and chromatography.

Kevin Riley

Research: ***Computational Description of Halogen Bonds and Applications to Protein-Ligand Complexes***

Research in my lab focuses on the computational treatment of non-covalent interactions, which are the forces that hold molecules together. The goals of the research carried out in this laboratory are to understand the energetic and geometric factors governing the strength and directionality of non-covalent interactions and to gain an understanding of the role that these interactions play in biomolecular and material structures. One type of non-covalent interaction in which we are particularly interested is the halogen bond. A halogen bond occurs between a bound halogen and an electronegative atom, such as oxygen, nitrogen, or sulfur. This type of interaction is somewhat counterintuitive, as it depends on two electronegative atoms, namely a halogen and a Lewis base {ie oxygen or nitrogen) approaching each other very closely. Halogen bonds are an example of an electrostatic interaction that can only be understood through close inspection of molecular electrostatic potential maps. We conduct research to understand that strengths of halogen bonds using sophisticated quantum chemical methods, including the generation of electrostatic potential maps, coupled cluster calculations, and symmetry adapted perturbation theory (SAPT) calculations. SAPT allows for the decomposition of an interaction energy (the strength of a non-covalent interaction) into physically meaningful terms, namely: electrostatics, dispersion, induction, and exchange-repulsion.

Jayalakshmi Sridhar

Research: The objective of the proposed research is to find inhibitors of HER2 oncogenic isoform HER2_16 that can inhibit the growth drug-resistant breast cancer. The long term goals of this project are to provide therapeutic agents for the treatment of aggressive and drug-resistant breast cancer. Development of small molecule protein inhibitors had been my major research focus during my post-doctoral training period at Georgetown University under the mentorship of Dr. Alan P Kozikowski and Dr. Nagarajan Pattabiraman. I was trained in the molecular modeling techniques as well as synthesis of designed inhibitors during my work on the kinase inhibitors resulting in several peer reviewed publications and two patents. I have used molecular modeling tools in conjunction with organic synthesis to develop specific inhibitors for GSK3b and VEGFR.

My earlier training in molecular modeling and synthesis helped me to perform several computational studies on the development of cytochrome P450 inhibitors at Xavier University of Louisiana under the tutelage of Dr. Cheryl Stevens. These studies resulted in the design and synthesis of new inhibitors that have shown good promise as P450 mechanism based inhibitors. As an assistant professor at Xavier University of Louisiana, I have designed and developed inhibitors of protein kinases (HER2, CK1d, and Pim kinases) and cytochrome P450 enzymes (1A1,1A2, 2B1 and 1B2). I was awarded grants from DoD, LBRN-NIH, LCRC and RCMI-NIH for these research projects.

Student Project:

Year 1:

1. The student will be trained in the methods of organic synthesis such as reaction set-up, isolation purification and characterization of the product.
2. The student will also be trained in molecular modeling methods used in the field of drug design.
3. The student will initially be involved in the synthesis of core structures whose synthetic protocols are already established

Year 2:

1. The student will design new derivatives of the core structure using computational modeling tools.
2. The student will learn to device new synthetic schemes for the designed derivatives.
3. The most viable synthetic scheme will then be followed by the student to achieve the goal of synthesizing new derivatives as kinase inhibitors.

Summer Research:

1. The bioassays for our synthesized compounds are carried out in our collaborators laboratories. If the student is interested in gaining experience in such work, opportunities will be explored with our collaborators.

Zhe Wang

Research: Controlling the film forming parameters is the key challenge in this regard, having implications on sensitivity, stability and reproducibility, interference and coupling, and electrochemical connectivity factors of the sensor performance. In the course of this project, inverted mesa structures will be designed, fabricated, and validated to address these challenges of E-QCM integration on monolithic QCM wafers. Finalized structures will be evaluated for their sensor capabilities in detecting real-life high value targets in comparison to routinely used high tech instruments so as to provide a proof-of-concept. We will establish biofunctionlized array system for complex biodetection.

Student Project: In this project, the student will be exposed to analytical chemistry and be able to work hands-on with electrode fabrication and fine quantitative measurements. Learning how to handle platinum (Pt) and Gold (Au) electrodes will be one of the first skills the student will develop. The student will also learn how to use analytical instruments which will be used to observe the chemistry process. Besides the significant educational impact for training a student in this cross disciplinary project of developing a reliable biosensing interface and to overcome the associated challenges, this project is also expected to make various broad impacts to biochemistry and clinical medicine.

Beside hand-on experimental kills, the student will learn the electrochemistry methodology, environmental monitoring method, micro-fabrication process and instrumental integration during this interdisciplinary project.

First year: ***Biosensing interface fabrication and primary detection***

1. First peptide/nanomaterial-based bioactive interface will be designed and build on the electrode surface by May.
2. In the summer time, we will go to University of Alabama and University of Tennessee, Knoxville for surface characterization and preliminary testing for individual biodetection. We will use in-situ electrochemistry-XPS system, in-situ electrochemistry-FTIR system for this work.
3. More bio-specific interfaces will be finalized to target the multiple specific analytes in the complex system by end of first year.

Second year: ***Bioarray system integration and characterization***

1. The optimization of biodetection for a neat target system (buffer and animal serum) and start to do the data analysis.
2. In the summer time, we will conduct system integration at Oakland University and preliminary testing for biosensor array. We will integrate multi-mode EC instrumentation chip (MEIC) with our sensor system.
3. We will characterize bioarray system for complex bedside sample testing and collaborated computer science department with data analysis by end of second year.

Terry Watt

Research: Our research focuses on proteins called lysine deacetylases (KDACs). A common post-translational modification to proteins is addition of an acetyl group to a lysine residue.[1–5] This modification has the effect of changing the protein's overall size and charge, and hence influences its activity within the cell. KDACs are enzymes that are responsible for reversing this modification, thereby creating a system that allows for regulation of protein behavior through controlled addition and removal of acetyl groups. KDACs are directly associated with biological processes as diverse as the formation of memories, metabolic regulation, and the normal development and repair of organisms.[6–9] In addition, the mis-regulation of KDAC activity is associated with many diseases and physiological stresses, including cancers, asthma, infectious diseases, and neurological, vascular, and muscular disorders.[7,10–12] However, because it is largely unknown which proteins are being modified by KDACs, the reasons for the links to diseases are unclear. Our goal is to understand the physical interactions between KDACs and other proteins, using a model system that allows us to screen for biologically relevant interactions. Greater understanding of these interactions, and identification of particular target proteins, will lead to novel therapies and diagnostics for cancer via new cellular pathways and drug targets.

Student Project:

There is flexibility in the specific goals of the project, subject to student interest. Here we describe all possible goals, with the understanding that the student can select a subset (approximately half) of the goals as a realistic set of objectives for this project, with the other goals being performed in parallel by the RCMI core, the project mentors, and external collaborators.

Year 1

(i) Complete modeling (docking) of known substrates in the active sites of KDAC8, KDAC7, and KDAC4 to identify residues to target for mutations. (ii) Introduce 5-10 selected mutations (by site-directed PCR mutagenesis) into KDAC8, KDAC7, and/or KDAC4, then express and purify the resulting variant proteins. (iii) Characterize each of the purified variants with several known substrates to determine how effectively each variant catalyzes the reaction. (iv) Correlate observed changes in enzyme catalytic ability with mutations to determine the effect of specific residues on substrate interactions.

Year 2

(i) Generate *in silico* models of the generated variants, and repeat the initial modeling analysis to calibrate the calculated model to experimental results. (ii) Select and generate up to 25 new *in silico* mutations in the KDACs to identify new mutations for possible experimental verification. (iii) Select 5-6 of the potential new mutations, introduce the mutations into the KDACs, and then express and purify the variants (as done for the variants described in year 1). (iv) Characterize the substrate interactions of the resulting variants and correlate the results to the predicted effects from the computational analysis (as done for the variants described in year 1).

Off-campus experience.

The student will have completed the first round of testing variants before the summer spent off-campus. Therefore, several possible avenues exist for off-campus projects, depending in part on the interest of the student. The modeling of additional variants could be performed with a collaborator rather than by the RCMI core at Xavier, allowing the student to perform most or all of the relevant steps. Alternatively, at least one other research group that researches KDACs has developed a biologically-relevant assay, and the student could work with that research group to learn an alternative assay and compare the different methods to validate the data being obtained using our approach. If interested, there would also be value in considering crystallography of some of the KDAC variants to more directly determine structural impact of the mutations being made.