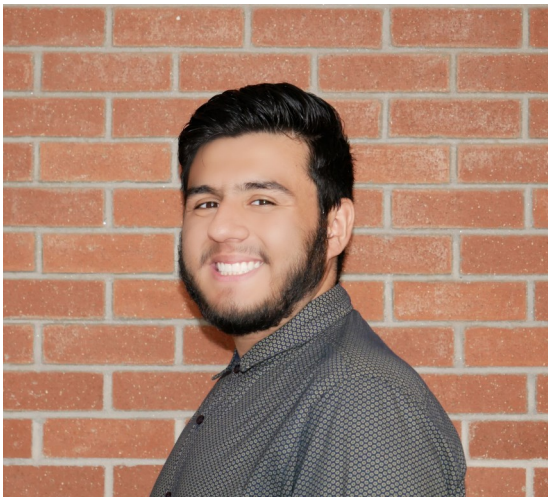
Maximizing Access to Research Careers (MARC)

Sundance Kemp

"Unraveling the Microvirus ssDNA Packaging Paradox: Genome Size Neutralization, and the Possible Role of a Non-Coding Structured DNA Sequence"

ABSTRACT: Viral DNA packaging requires the neutralization of the negatively charged genome. In single-stranded DNA microviruses, the DNA binding protein J performs this function. In addition to neutralizing DNA, the J protein also tethers the genome to the capsid's inner surface. Consequentially ~12% of the genome is icosahedrally ordered in the X-ray structure. The remainder appears as an electron dense core. Although the internal capsid volume of the three closely related microviruses φX174, G4 and α3 are nearly identical, genome size varies widely, from 5386-6067 nucleotides. Paradoxically, the virus with the smallest genome (φX174) encodes, and requires, the largest and most positively charged J protein. Thus, a charge neutralization model does not adequately explain ssDNA packaging. To more fully understand this phenomena a chimeric virus (φXG4J) was generated by replacing the indigenous J gene with one from G4. On the level of plaque formation, φXG4J was not viable unless complemented by the cloned φX174 J gene. However, chimeric virions can be produced with prolonged incubation in lysis-resistant cells. The resulting virions are significantly less stable and less infectious than wild-type. φXG4J utilizer mutations were isolated by selecting for plaques without complementation. The utilizer mutations contained duplications of the non-protein encoding J-F intercistronic region. The duplications restore particle stability and infectivity. These results demonstrated that a simple charge neutralization hypothesis does not adequately explain ssDNA packaging, and a non-protein encoding intercistronic region may regulate the fidelity of ssDNA packaging.

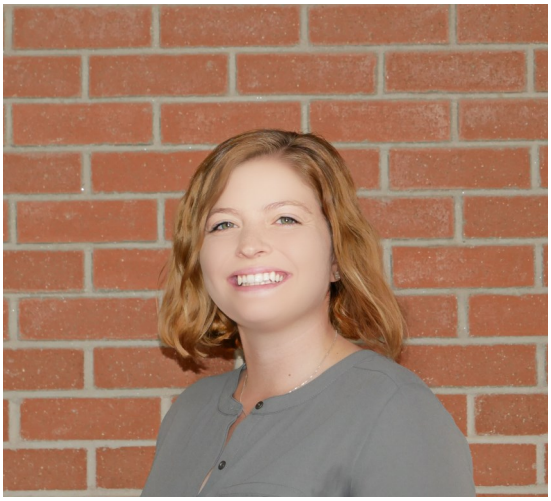
Frank Servin



"Assessment of Pulmonary Arterial Structure and its Association with Right Ventricular Function in Pulmonary Arterial Hypertension"

ABSTRACT: Pulmonary arterial hypertension (PAH) is a progressive disease that results in right ventricular (RV) failure. Mean pulmonary arterial pressure (mPAP) and pulmonary vascular resistance (PVR) increase due to a decrease in vascular radius. Current clinical assessments of vascular structure have not been linked to RV function. The aim of this preliminary study is to 1) develop a method to quantify pulmonary vascular structure from cardiac magnetic resonance angiograms (MRA) and 2) quantify associations between vascular structure and RV function in patients to distinguish unique PAH phenotypes. This study included patients who underwent right heart catheterization (RHC) and a clinical MRA. The patient's pulmonary vasculature was volume rendered in SimVascular (v5.23.2018 Stanford University), then the centerline and perimeters were measured for the principal pathway, anterior artery, medial basal artery, and anterior basal artery. The cross-sectional area (CSA), pathlength, and number of segments of each artery were compared in patients with PAH (mPAP ≥ 25 mmHg) and without pulmonary hypertension (PH) (mPAP < 25 mmHg). Hemodynamically, PAH patients had increased mean PA pressures and PVR, decreased PA compliance, and decreased RV function. Patients with PAH have increased CSA and diameter in the principal vascular pathway compared to patients without PH. In three arteries, PAH patients had fewer segments than patients without PH. Preliminary results show it is feasible to detect altered pulmonary vascular structure in patients with PAH from cardiac MRAs. Combining structural vascular assessment with hemodynamic assessment of RV function could be used to identify unique PAH phenotypes.

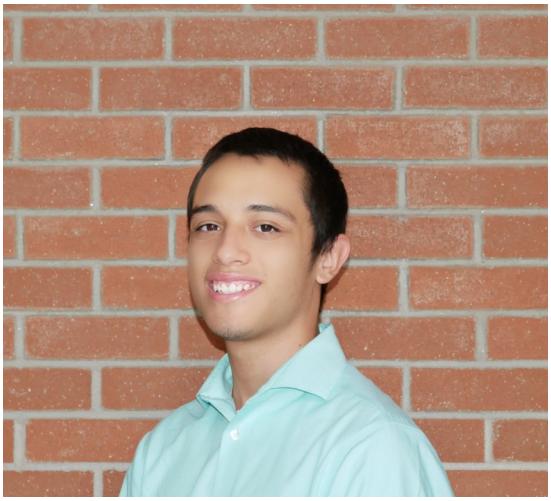
Alana Gonzales



“Paper-based vertical assay for detection of metastatic breast circulating tumor cells”

ABSTRACT: Metastasis, the primary cause of death in cancer patients, occurs when secondary tumors arise from circulating tumor cells (CTCs), which shed from the primary tumor and circulate through the bloodstream to other parts of the body. As an alternative to tissue biopsy, current technologies use liquid biopsy of the blood to detect and count CTCs as a method for measuring patient prognosis and determining treatment options. These technologies are often costly and require highly trained personnel. This project seeks to overcome these issues by using a low-cost, paper-based vertical flow immunoassay and a smartphone-based microscope as a novel biosensor for quantifying CTCs. Antibodies specific for protein expression markers of epithelial and mesenchymal cell morphology, respectively, were conjugated with fluorescent polystyrene particles, and these particles were mixed with both epithelial and mesenchymal breast cancer cell lines to model the diagnostic assay in suspension and on paper. The paper-based vertical assay device is designed to filter red blood cells and platelets, leaving white blood cells and CTCs. Fluorescent microscope images were taken, and a MATLAB program was developed to detect the particles bound to CTCs, enabling the accurate counting of CTCs towards the single cell level. Imaging was repeated using a smartphone microscope device. Results demonstrated specific binding of antibody-conjugated particles to their respective cell lines, and future experiments aim to increase the accuracy of image processing. Successful detection of CTCs as epithelial or mesenchymal using this method will provide a cost-effective, less invasive way to assess metastasis and prognosis of cancer patients.

Andres Sanchez



“Drug Resistance Mechanism Mediated by Fungal Sterol Transport Protein Tir3”

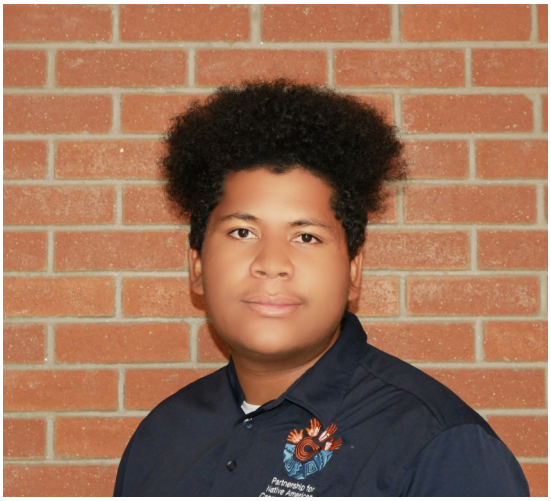
ABSTRACT: Fungal diseases present a rising clinical and agricultural threat worldwide. The treatment generally prescribed for such diseases are doses of antifungal agents known as azoles. Azoles work by targeting various enzymes in the biosynthetic pathway of ergosterol, a key component in the structure and maintenance of the fungal cell membrane. However, recently antifungal agents show less lethality to pathogenic fungi due to the rise of resistance to antifungals in pathogenic fungi. Although antifungals generally target the biosynthesis of sterols, fungal cells exhibit the ability to bypass this problem by importing exogenous sterols from the sterol-rich host, a process known as sterol uptake. Membrane protein AUS1 and cell wall protein Tir3 in the pathogenic fungus Candida glabrata are required for sterol uptake and survival in lethal concentrations of antifungals; however, the structure and mechanism of this sterol uptake system is unknown. We have developed an expression system that provides purified samples of truncated Tir3 constructs to examine its sterol uptake mechanism and determine where sterol uptake occurs. The results of our future experiments will provide a structure and mechanism of the Tir3-AUS1 system as well as targets for new drugs to inhibit the system.

Corinne Meinhausen

"Memory Retrieval Strategy Utilization at Various Time Points Following a Mild Traumatic Brain Injury"

ABSTRACT: Mounting evidence suggests that learning, memory and attentional deficits occur in the acute stage post-mTBI. This study aims to identify memory retrieval strategies post-mTBI to better understand how those deficits manifest. The California Verbal Learning Test, 2nd Edition (CVLT-II), an orally presented word list that provides quantitative and qualitative scores, was administered to participants at different stages of recovery and healthy controls (HC) to test for the prevalence of clustering techniques indicating how one retrieves, represents and organizes words. Based on the typical cognitive recovery pattern for mTBI, it was hypothesized that changes to attention, memory and learning would result in more semantic and less serial clustering during the acute stage (2-12 weeks post-mTBI) compared to those in the chronic phase (6-12 months post-mTBI) and those who have never experienced a concussion. Raw semantic and serial clustering scores were compared between the acute (n=26), chronic (n=20) and HC (n=23) groups using one-way ANOVAs. There was a main effect of group on serial clustering ($F(2,66)=3.142$, $p=0.050$, partial eta-squared=0.87). Post-Hoc comparisons showed the acute group had significantly fewer serial clusters than HCs ($p=0.031$) and the chronic group ($p=0.043$), clearly demonstrating the use of different memory retrieval strategies during the acute stage. There was also an observed tendency in the acute group to perform semantic clustering over the HCs and the chronic group. These findings, in addition to similar overall performance between groups on the CVLT -II, may indicate a compensatory effect during early mTBI recovery in response to deficits in the memory process.

Meucci Watchman Ilunga



"An Exploration of the Secretoglobin Gene Superfamily: A bioinformatic analysis of structural and genetic motifs across several mouse species and subspecies"

ABSTRACT: The Secretoglobins (SCGBs) are a family of small, dimeric proteins best studied in mammals . No member protein has had its primary function conclusively determined, leaving the family to be grouped together based on structural similarity. While prior studies have focused on individual member proteins and their possible functional roles, clear insight remains elusive.

In an effort to further address this question of function, our investigation has focused on genetic studies of the SCGB superfamily. We hope that understanding the relationships of family members will provide insight into functions for individual member proteins. The primary goal of this investigation was to compare SCGB genes and proteins across a variety of mouse species and subspecies. In particular, we sought to identify shared structural features and functional domains among SCGB member proteins. A secondary goal of the investigation was to gain insight about the evolutionary history of the family and build phylogenetic trees based on collected genetic data.

The investigation centered on predicting six SCGB genes in six mouse species and subspecies. We used BLAST to interrogate draft and completed genome data from Mus musculus domesticus, M. m. musculus, M. m. casteneus, M. spretus, M. caroli, and M. pahari. We then predicted introns and exons based on similarity to related genes identified the genome mouse and rat and translated protein sequence from the predicted mRNA. We made alignments of gene, mRNA and protein sequences and used these to identify shared and unique features and also to build phylogenetic trees.