**D 1** **Abrol, Ravinder**  **California State University Northridge** **1R16GM153621-01**

**Structural dynamics and energy landscapes of G protein signaling**

Extracellular signals like hormones and neurotransmitters use G protein coupled receptors (GPCRs) to activate multiple signaling pathways inside the cells. GPCR‐mediated signaling is involved in many physiological processes, making them attractive therapeutic targets in a broad range of disease areas that affect metabolic, nervous, cardiovascular, and immune systems, to name a few. These receptors have emerged as dynamic signaling machines controlled by their ligands, where each receptor can potentially signal through a specific or multiple heterotrimeric G proteins and each ligand is capable of signaling through multiple G proteins by binding to G protein‐selective receptors. In addition, these receptors also utilize G protein independent signaling pathways mediated by other transducer proteins like arrestins. The receptors are no longer considered simple on‐off switches due to their complex signaling profiles. The heterotrimeric G proteins have emerged as maestro controllers of receptor‐mediated signaling, capable of coupling as heterotrimers to receptors that results in G protein dissociation into Gα and Gβ subunits, which can couple to downstream effectors (like adenylyl cyclases, phospholipases, ion channels, or receptor kinases) and to regulators (like RGS proteins ‐ regulators of G protein signaling). This signaling control by G proteins is orchestrated via conformational changes triggered by binding to receptors, GDP/GTP‐exchange, dissociation into Gα and Gβ subunits, GTP hydrolysis, and binding to RGS proteins. G proteins have also been shown to couple with some receptors unproductively, i.e., no GDP/GTP exchange or G protein activation takes place. This proposal leverages the experimental structures available for a handful of G protein conformations and uses computational approaches like molecular dynamics to provide a mechanistic basis for G protein activation. The main objectives in this proposal aim to determine the structural determinants and thermodynamic landscapes for G protein coupling with the receptors that causes GDP release, their dissociation from the receptors after GDP‐GTP exchange, and the effect of ligands on their constitutive activity. These computational studies will be complemented with experimental biophysical and biochemical methods and will provide unprecedented mechanistic insight into molecular determinants of G protein activation.

PUBLIC HEALTH RELEVANCE: G protein coupled receptors are therapeutic targets in a wide range of diseases and utilize G protein signaling for most of their effects. This project is aimed at using computational methods to understand the molecular mechanisms underlying G protein signaling. This provides fundamental knowledge about how these proteins work, which can help in the future in targeting their pathways for drug discovery.

**Key Personnel Institution Role**

Ravinder Abrol California State University, Northridge PI

**B 2** **Ashley, Amanda K** **New Mexico State University Las Cruces** **1R16GM153578-01**

**Investigating DNA-PKcs Mediated Regulation of CTCF**

DNA damage is a major hallmark of cancer, and mutation or misregulation of proteins that maintain genomic fidelity are observed in multiple cancers. Following DNA damage, cells initiate a coordinated set of pathways, collectively termed the DNA damage response (DDR), critical for DNA repair, genomic stability, and suppressing tumorigenesis. Clinically, many cancer drugs induce DNA damage, thus the DDR regulates cellular responses to chemotherapy and development of drug resistance. Accordingly, numerous novel therapeutics have been developed and are under development based upon a clear understanding DDR networks. The most deleterious type of DNA damage is a double strand break, which are repaired by two main pathways: homologous recombination (HR) and nonhomologous end-joining (NHEJ). We are interrogating the interactions of two proteins, one in the HR pathway, CTCF, and another core component of the NHEJ pathway, DNA-PK, which may interact with one another. CTCF mutations are commonly observed in endometrial and breast cancer, and mutations in CTCF consensus sequences are also frequently seen in multiple cancer subtypes. The goal of our project is to further interrogate and elucidate the interaction between these two DNA repair proteins. We will use genetically defined cells and breast cancer cell lines to accomplish our objectives. In Aim 1 we will delineate the role of DNA-PK activity on CTCF recruitment and DNA repair following DNA damage. We aim to uncover whether there is a functional interaction of these proteins regulated by DNA damage and assessing whether one targets the other. In Aim 2 we will determine whether DNA damage response proteins, including DNA-PK and related proteins, regulate cell death in breast cancer cells following CTCF depletion. We will determine if DNA-PK can modulate how CTCF associates with known genomic regions, especially following stress. CTCF aberrant expression, mutations, or haploinsufficiency each promote tumor formation; thus, understanding key regulators of CTCF function has significant clinical ramifications. Uncovering how DNA-PK modulates CTCF-mediated DNA repair and ultimately, cell survival will inform future studies aimed at targeting those tumors harboring alterations in these key regulatory proteins. Overall, completing this research will reveal novel protein interdependencies that may be exploited for personalized medicine and may be used to stratify patients based upon CTCF aberrations to improve patient success.

PUBLIC HEALTH RELEVANCE: Understanding how genetic differences confer specific vulnerabilities of cancer cells to new therapies is a promising area of research. Our goal is to elucidate the basic biology of how cancer cells survive genotoxic therapy, uncovering new ways to specifically target these cells while sparing the normal cells.

**Key Personnel Institution Role**

Amanda K Ashley New Mexico State University Las Cruces PI

**drug**

**B 3 Bishop, Thomas Connor Louisiana Tech University 1R16GM153672-01**

**SURE: Sequence-structure-function Unified and Recycled for Extrachromosomal circular chromatin studies.**

Techniques were recently developed that enable researchers to isolate extrachromosomal circular DNA (eccDNA) from linear genomic DNA. There is a rapidly growing interest in eccDNA as a biomarker and a therapeutic. DNA in eccDNA is a three dimensional closed space curve subject to the topological constraint Lk = Tw + Wr. Here Tw indicates Twist in the DNA, Writhe(Wr) measures the non-planar bending and Linking number(Lk) the number of times the DNA backbones crossover one another. Lk is fixed unless the backbone is broken and religated. Thus, any Writhe introduced into eccDNA by histones or others proteins must be compensated for by changes in Twist. This proposal focuses on the association of eccDNA with histones(eccChrom). Our hypothesis is that eccChrom’s function is effected by its sequence and structure. Because eccChrom is circular, its structure and topology are determined by properties of the linker DNA between nucleosomes, the spacing and variation of nucleosomes along the DNA and the relative orientation of the nucleosomes. The latter is in turn influenced by nucleosome-nucleosome interactions. EccChrom containing 1-3 kbp is sufficiently compact that it can be investigated with all atom molecular dynamics simulations making it an ideal system for exploring the biophysical properties of linear and circular chromatin, nucleosomes, and DNA. Our approach is to unify sequence-structure-function across our entire workflow. “Unify” has a very specific meaning here. Unification combines data from otherwise disparate sources into a single entity. It often relies on a transformation between data representations. We developed the “genome transform”, a generalization of the DNA helical parameters, to unify 1D chromosome coordinate data(sequence) and 3D Cartesian coordinate data(structure). Function is an observed public health outcome associated with sequence or structure. Our genome transform is therefore sufficient to unify sequence-structure-function. Significance: eccChrom is a functionally complete, topologically constrained, chromatin complex with the potential to radically change our understanding of gene amplification, adaptation, development and evolution in atomic detail. Innovation: Unifying sequence, structure and function across our entire workflow enables us to efficiently and collaboratively navigate disparate datasets from theory, experiment and simulation and cross-check hypotheses regarding eccChrom as a biomarker and therapeutic agent. Specific Aims: Our specific aims develop and apply methods, protocols, and metrics for the sampling and analysis of polynucleosomal eccChrom topology in atomic detail.

PUBLIC HEALTH RELEVANCE: Efficient protocols for isolating extrachromosomal circular DNA (eccDNA) from linear genomic DNA were recently developed and promise to rapidly change our understanding of gene amplification, adaptation, development and evolution. We will build research capacity at Louisiana Tech by recruiting undergraduate and graduate students, and training them in the art of molecular dynamics simulations and the mathematics of DNA topology. We will apply this knowledge to determine sequence-structure-function relationships for chromatinized eccDNA (eccChrom) that functions as a biomarker and therapeutic agent.

**Key Personnel Institution Role**

Thomas Connor Bishop Louisiana Tech University PI

**C 4 Chen, Joseph Chiung-Chu San Francisco State University 1R16GM153570-01**

**Liquid-liquid phase separation of Sinorhizobium meliloti RNA degradasomes during symbiosis with legume host**

The proposed project will elucidate how subcellular compartmentalization of the RNase E endonuclease into bacterial ribonucleoprotein granules (BR-bodies) in Sinorhizobium meliloti contributes to successful host colonization. S. meliloti serves as a model for investigating infection by alpha-proteobacteria, which include human pathogens such as Brucella, Bartonella, and Rickettsia. Understanding the molecular and cellular mechanisms that underpin effective host colonization can provide critical insights for preventing and treating infectious diseases, particularly as bacterial resistance to antibiotics continues to emerge. S. meliloti establishes mutualistic symbiosis with compatible legumes, including the genetically tractable reference species Medicago truncatula, by inducing development of root nodules, colonizing them, and fixing nitrogen in exchange for nutrients from host plants. We found that removing the C-terminal intrinsically disordered region of RNase E in S. meliloti led to ineffective symbiosis, eliciting nodule development but producing etiolated plants that grew poorly compared to plants inoculated with wild-type bacteria. RNase E is an essential ribonuclease involved in RNA processing and decay, and its C-terminal domain, while dispensable for viability, was shown to be necessary and sufficient for the formation of liquid-liquid phase-separated biomolecular condensates, named BR-bodies, in alpha-proteobacteria. Sequestration of RNase E into such BR-bodies facilitates mRNA decay and modulates the cellular RNA pool. The goal of this project is to determine how phase separation of RNase E influences RNA processing in S. meliloti and enables effective symbiosis by accomplishing the following three specific aims. (1) We will assess the symbiotic defects of the RNase E C-terminal deletion mutant in detail by genetic analysis. (2) We will decipher the role of BR-bodies during host colonization using high-resolution microscopy. (3) We will examine how transcriptomes differ between wild-type and mutant S. meliloti, both free-living and symbiotic, as well as compare gene expression profiles of host plants colonized by wild-type versus mutant bacteria. While RNase E has been linked to pathogenesis of various bacteria, including Brucella, relatively little is known about the specific microbe-host interactions involved. Results from the proposed investigation will help identify and provide better understanding of genetic and cellular factors that allow infecting bacteria to bypass host defenses. In addition to accomplishing the scientific objectives described above, this proposal will allow students, particularly those from underrepresented backgrounds, to gain research training and preparation for biomedical careers.

PUBLIC HEALTH RELEVANCE: The proposed research aims to unveil molecular mechanisms that enable bacterial cells to infect and colonize their host organisms. Results from the investigation will provide insights into microbe-host interactions, both beneficial and harmful, and ultimately improve the prevention and treatment of microbial diseases. In addition, the project will provide immersive research experiences for students, particularly those from traditionally underrepresented backgrounds, and encourage their pursuits of biomedical careers, thus strengthening the workforce pipeline in health-related fields.

**Key Personnel Institution Role**

Joseph Chen San Francisco State University PI

Sharon R. Long Stanford University Other Significant Contributor

Jared M. Schrader Wayne State University Other Significant Contributor

**C 5 Chen, Kuan-Hui Texas Tech University 1R16GM153648-01**

**Dissecting the role of mirtrons in human cancers**

Mirtrons are a new class of small RNAs. Previous findings show mirtrons are generated by splicing and are less stable than canonical miRNAs. Thus, the expression of mirtrons is often negligible. Additionally, most mirtrons exhibit distinct evolutionary properties relative to solo and mammals have way more mirtrons than lower organisms suggesting some unknown importance of mirtrons in mammals. Recently, elevation of mirtrons has been linked to human cancers and immune disorders. However, the biological significance of mirtrons in humans is not well understood due to very limited efforts of mirtron research in humans. It is therefore important to investigate how biogenesis of mirtrons is stabilized during disease development and how increased mirtron expression promotes tumor/disease progression. In ongoing experiments, we show most mirtrons elevated in human cancers contain guanine rich sequence that forms G-quadruplex, a secondary structure that has been implicated as a strategy adopted by some RNA viruses to evade the host exoribonucleases, XRN1/2 mediated digestion. We also show XRN/1/2 are crucial enzymes for mirtron stability that mirtrons in cancer cells significantly increase when XRN1/2 are knocked down. Additionally, all cancer mirtrons are featured with many (long) terminal nucleotide additions which result in abundance of mirtrons present in the cancer derived exosomes. Furthermore, we also show mirtrons from the cancer derived exosomes bind to the endosomal Toll-like receptor 7 (TLR7) of macrophages suggesting mirtrons are novel ligands of TLR7 and potentially modulate immune responses. Given these findings, we hypothesize that cancer cells selectively express mirtrons exhibiting high guanine contents that could form G-quadruplex structure to bypass XRN1/2 mediated degradation. The nucleotide addition to the 3’ tail provides preferential sorting of mirtrons into exosomes. Cancer exosomes can be taken by immune cells through endocytosis providing the opportunity for mirtrons to bind to TLR7. The binding of mirtron to TLR7 serves as an allosteric inhibitor to block TLR7 signaling and the associated downstream immunological responses. These hypotheses will be addressed in the experiments of the following Specific Aims: (1) to determine the impact of G-quadruplex structure of mirtrons on the resistance to exoribonucleases, XRN1/2; (2) to determine the consequence of altered mirtron precursor tailing on exosomal sorting; and (3) to evaluate the immune modulatory role of cancer mirtrons through binding to TLR7. Should this exploratory study reveal novel mechanisms of cancer mirtron stabilization and sorting into exosomes, and should these cancer mirtrons negatively regulate the TLR7 signaling and the related immune functions, novel therapeutics aimed to destabilize mirtron, to prevent their sorting into exosomes, and/or to compete mirtron binding to TLR7 may provide benefits to cancer treatment in addition to advance current knowledge of mirtrons in humans.

PUBLIC HEALTH RELEVANCE: In this study, we propose to investigate the biogenesis of mirtrons in cancers and to examine the potential roles of mirtrons in the modulation of tumor immune responses. Mirtron is an emerging new RNA subclass in the field but its biological importance in humans is completely not understood. Thus, successful completion of the project will advance current knowledge of mirtrons and their involvement in the immune regulation that will inform therapeutic strategies to treat cancers.

**Key Personnel Institution Role**

Ethan Chen Texas Tech University PI

Alex Sutton Flynt University of Southern Mississippi Co-Investigator

**D 6 Cheng, Hai-Ping Herbert H. Lehman College 1R16GM145526-01A1**

**The cellular targets and identification of bacterial compounds with antibiotic activities**

The global spread of antibiotic-resistant bacteria has led the WHO and CDC to declare a worldwide emergency and call for an effort to develop new antibiotics against multi-drug-resistant pathogens. While finding a novel antibiotic has been a global effort for decades, it remains a challenge due to the lack of new cellular targets and new compounds with antibiotic activity. In our search for new antibiotics, we have developed a reproducible method to produce a bacterial-derived culture supernatant with strong and broad-spectrum antibiotic activity (active supernatant) or without antibiotic activity (inactive supernatant). Unlike bacteriocins and microcins, our active supernatant inhibits the growth of the producer strain and all the bacteria that we have tested thus far, including Mycobacterium smegmatis and multi-drug resistant ESKAPE Gram+ and Gram-pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudo- monas aeruginosa, and Enterobacter spp.), which were obtained from the NIH funded Antibiotic Resistance Leadership Group (ARLG). In addition, exposure to the active supernatant caused S. aureus, P. aeruginosa, and Escherichia coli cells to burse into small vesicles. Our active supernatant also eliminates persister cells, which are often associated with the recalcitrance of chronic infections. Tests done in collaborators’ labs showed our active supernatant is not toxic to human red blood cells, rat hepatocytes, and yeast. All our preliminary results suggest our antibiotic compound is different from known bacteriocins and microcins. Our working hypothesis is the killing of S. aureus, E. coli¸, and P. aeruginosa, and all other pathogens we tested, is due to the presence of a novel antibiotic compound(s) that disrupts a fundamental bacterial cellular process that is absent in human and eukaryotic cells, making the active compound(s) a potential candidate for development into a novel antibiotic. We propose to 1). Determine the phenotypic and transcriptional responses of E. coli to the active supernatant treatment by determining the effects of our active supernatant on cell envelope structure, membrane integrity, chromosome integrity, and changes in gene expression; 2) Identify and determine the structure of the active compound by concentrating the active compound, obtaining an active fraction using function-guided HPLC fractionation, and then identifying the active compound in active supernatant using NMR. The completion of the proposed research will lead to the identification of the target of the antibiotic compound and make it possible to further investigate and identify the proteins involved in sensing and signaling the presence of our antibiotic compound. The completion of the research will also enable us to synthesize the compound in large quantities and subject it to different chemical modifications to maximize its antibiotic activity for various applications, including animal studies. Altogether, our preliminary data, the identification of cellular targets, and the elucidation of the structure of the antibiotic compound in the active supernatant, could form the basis for a new broad- spectrum antibiotic able to combat the spread of infectious bacteria around the world.

PUBLIC HEALTH RELEVANCE: We propose to further characterize a bacterial liquid culture supernatant that has broad- spectrum antibiotic activity that can induce the lysis of Gram+ and Gram- bacterial cell and eliminate persister cells. The completion of proposed research will allow us to identify the antibiotic compound in the supernatant and determine the bacterial cellular process that is the target of this antibiotic compound.

**Key Personnel Institution Role**

Hai-ping Cheng Lehman College PI

**C 7** **Chrestensen, Carol Ann** **Kennesaw State University** **1R16GM149476-01A1**

**Investigating gene and isoform specific differences of MAP-kinase interacting serine/threonine-protein kinases 1 and 2**

MAP kinase interacting serine/threonine-protein kinases (MKNK1/2 also known as Mnk1/2) are implicated in cancer, diabetes, and immunity. Despite these important physiological connections, relatively little is known about the gene and isoform specific differences of MKNK1 and 2 proteins. MKNKs bind all eukaryotic initiation factor (eIF) 4G gene products and this binding results in phosphorylation of eIF4E and changes in translation. Loss of the proteins (there are five known protein products from the two genes), or inhibition of the kinases can stop, or slow tumor growth and the mRNA of MKNK2-2 is upregulated in tumors while MKNK2-1 mRNA is downregulated. The MKNK1/2 proteins are understudied despite key functional differences and involvement in many important physiological and pathophysiological processes. This SuRE R16 proposal seeks to investigate functional aspects of the gene and isoform differences of MKNK1/2 proteins while mentoring and training undergraduate researchers at Kennesaw State University. We aim to: 1) Identify gene and isoform specific differences of MKNK1 and 2 proteins including protein-protein interactions with eIF4Gs and phosphorylation of peptide substrates. This will be accomplished using bacterial expression paradigms to produce the isoforms which will be characterized in vitro using kinase activity assays and determination of kinetic and affinity constants for binding to eIF4G peptides; 2) Explore the function of the unique 4-cysteine containing insert of the MKNKs, which bound zinc in the crystal structure of MKNK2, but not MKNK1. Bacterial expression paradigms of the protein variants will be made and then characterized with techniques used in aim 1; 3) Examine the where and when of intracellular MKNK1/2 and eIF4G1, 2, & 3 binding, using mammalian tissue culture, immunohistochemistry and proximity ligation assay; and, 4) Include students, ~39% URM, in several KSU chemistry courses in authentic research, testing the activity of MKNKs (using a simple fluorescence assay) and synthesizing isoform specific inhibitors. This SuRE proposal will support the research activities of the PI in training the next generation of biomedical research scientists including members of URM groups. The Chrestensen Lab trains students in many biochemically important techniques including, bacterial expression systems, protein purification, enzymatic analysis, biolayer interferometry, tissue culture and confocal microscopy. Students are involved in the execution of experiments, the interpretation of new results and the presentations of their finding through conference presentations and as authors of manuscripts.

PUBLIC HEALTH RELEVANCE: MAP kinase interacting serine/threonine-protein kinases 1 & 2 (MKNK1/2 also known as Mnk1 and Mnk2) are two genes with multiple isoforms (MKNK1 has three, and MKNK2 has two); they bind to eukaryotic initiation factor (eIF)4G and phosphorylate eIF4E both essential regulators of translation. Loss of the kinases has no discernable impact on development or procreation of mice, but they are implicated in cancers, diabetes, and regulation of immunity. We seek to investigate the meaningful differences in the isoforms especially in relation to binding with eIF4G to learn more about how they function in homeostatic and disease processes.

**Key Personnel Institution Role**

Dr. Carol Chrestensen Kennesaw State University PI

**B 8 Costantini, Lindsey M North Carolina Central University 1R16GM153198-01**

**Single Molecule analysis of KSHV/HHV8 DNA replication proteins**

Effective antiviral therapeutics are needed for the treatment of KSHV infection. We propose to pinpoint novel KSHV antiviral targets by identifying virus specific protein functions and conformations. This project aims to study the molecular interactions (protein-DNA and protein-protein) that govern KSHV DNA synthesis and genome replication using a highly specialized electron microscopy (EM) approach. Previous studies used sequence homology between KSHV and related herpesviruses to determine conserved protein functions of the seven essential core DNA replication genes/proteins encoded by KSHV: ORF6(SSB), ORF9(POL), ORF40/41(PAF), ORF44(HEL), ORF56(PRI), ORF59(PF) and ORF50(RTA). However, protein sequence homologies only range from 20-50% and thus poorly predict protein function; therefore, an ultrastructural characterization of purified KSHV viral DNA replication proteins, individually and in concert are needed to identify their full range of functions (Aim 1). To evaluate the in vitro activities, electron microscopy (EM) will be used to directly visualize viral proteins and viral DNAs. Our EM approach produces qualitative data (heterogeneous protein complexes, oligomeric state, DNA architecture) and quantitative data (DNA mapping of protein binding locations, molecular size comparisons). The first aim of this proposal will characterize the molecular interactions and activities of a subset of already purified KSHV DNA replication proteins. The findings will provide valuable insights into KSHV replication and inform future studies of proteins purified from a human cell culture system to directly compare the impact of viral protein post-translational modifications of proteins produced from insect cells with human cell native modifications. The second aim of this proposal is focused on generating viral proteins in physiologically relevant human cell lines. We have previously produced five of the seven KSHV replication proteins using an insect Sf9 cell system, but commercial and lab attempts to express and purify the remaining two proteins (PAF and PRI) from non-mammalian cells have been unsuccessful. We hypothesize that by utilizing relevant human cell types, we will overcome the challenges of producing viral proteins in non-human cell lines and enhance the functionality of the purified proteins (Aim 2). This in-depth molecular study of the core DNA replication proteins will advance the general understanding of KSHV biology and gamma-herpesvirus replication and the data generated from this proposal will provide the foundation for future proposals aimed at identifying virus specific inhibitors to prevent KSHV infection. Finally, trainees (undergraduate and graduate students) will gain exposure and expertise in a wide range of biochemical and molecular biology approaches while also learning unique, highly-specialized EM techniques while completing Aims 1 and 2.

PUBLIC HEALTH RELEVANCE: Two human herpesviruses, Epstein- Barr virus (EBV) and Kaposi’s sarcoma herpesvirus (KSHV/HHV8) are known to cause several human cancers. Effective antiviral therapeutics are needed for the treatment of KSHV infection. By using specialized electron microscopy (EM) to directly visualize protein-DNA structures and protein-protein conformations our goal is to identify novel anti-viral targets.

**Key Personnel Institution Role**

Lindsey Marie Costantini North Carolina Central University PI

**C 9 De La Parra, Columba Herbert H. Lehman College 1R16GM153947-01**

**The role of an alternate mechanism of translation initiation in TNBC Metabolism**

Metastasis constitutes the primary cause of death for patients with cancer. Specific traits in advanced cancer, including an altered metabolism and reprogramming of the translation machinery to establish selective translation of specific mRNAs including regulators of cell metabolism, anti-apoptotic and pro-invasion factors, leads to metastasis. Tumor cells under different physiological stresses such as hypoxia, nutritional deprivation, and oxidative stress, inhibit global protein synthesis by downregulating canonical mRNA translation, but in response, cancer cells utilize specialized mechanisms of protein synthesis to sustain their survival1-6. There is little research to determine which metabolic mRNAs are translationally maintained or even upregulated following stress, which needs to be elucidated. We propose to study the role of a non-canonical, cap-dependent mRNA translation mechanism that functions during cell physiological stress when canonical mRNA translation is inhibited, in the regulation of Triple Negative Breast Cancer (TNBC), focused on cell metabolism critical for metastasis. TNBC is one the most aggressive and highly metastatic subtypes and is associated with poor prognosis7. A feature of TNBC models and patient tumors is dysregulated glycolysis, which is linked to chemotherapeutic resistance8. In this application, we propose to continue our studies on two hallmarks of invasive cancers, translational control, and metabolic reprogramming. The goal of this proposal is to delineate the molecular mechanisms by which the selective mRNA translation by DAP5 regulated TNBC metabolism and identify the pathways and metabolites that could ultimately be targeted to improved clinical outcomes in advanced breast cancer. The central hypothesis is that DAP5 is critical in regulating the metabolism of TNBC through selective translation of mRNA encoding metabolic regulators, particularly glucose metabolism and calcium homeostasis, which affect metastasis, and this is favored under stress conditions, where canonical cap- dependent translation is attenuated. The role of selective translation initiation in cancer metabolism is almost completely unexplored and we propose an exciting and novel concept for understanding a non-canonical mechanism mediated by DAP5 in the regulation of metastatic cancer cell metabolism. To this end, we will (1) Identify the metabolic mRNAs translated by DAP5 that are maintained following oxidative stress and characterize the metabolic functions of DAP5 in TNBC cells. (2) Determine the role of DAP5 in the metabolite identity of tumors and metastasis in vivo. (3) Validate that increased expression of DAP5 is associated with metabolic molecular biomarkers in available human primary tumor biopsies by comparing non-metastasized primary tumor to primary tumors that gave rise to metastases in TNBC.

PUBLIC HEALTH RELEVANCE: This proposal is relevant to public health because it will delineate the mechanism by which selective mRNA translation regulated metabolic reprogramming in Triple Negative Breast Cancer. Successful completion of the proposed aims will help to identify metabolites that could ultimately be targeted to improve clinical outcomes in advanced breast cancer; a clear emphasis of the NIH mission.

**Key Personnel Institution Role**

Columba Delaparra Lehman College PI

Julio Gallego-Delgado Lehman College Other Significant Contributor

Kelly Ruggles NYU School of Medicine Other Significant Contributor

Robert J Schneider NYU School of Medicine Other Significant Contributor

**C 10 Hollomon, Mario Texas Southern University 1R16GM153589-01**

**Role of FOXO transcription factors in redox homeostasis and response of osteosarcoma cells to anticancer drug treatment**

Osteosarcoma is the most common type of bone cancer found in children and teens. Although osteosarcoma survival rates have improved over the past two decades, there is still a need to better understand the biology of osteosarcoma so that novel therapeutic approaches can be developed. Reactive oxygen species (ROS) are generated during ordinary cellular processes or drug metabolism. Redox homeostasis involves the neutralization of ROS by endogenous antioxidants. An imbalance between ROS and endogenous antioxidants in favor of ROS results in oxidative stress (OS). High levels of OS can result in cell death. Therefore, endogenous antioxidants are essential for redox homeostasis and cell survival. Nuclear factor erythroid 2-related factor 2 (Nrf2) is considered the major redox-regulated transcription factor for the expression of endogenous antioxidants. Although Nrf2 is considered the major redox transcription factor for the expression of endogenous antioxidant genes, other redox transcription factors, such as forkhead-box class O (FOXO) and hypoxia-inducible factor-1 (HIF-1), also regulate the expression of endogenous antioxidant genes. Therefore, it is plausible that these and other redox transcription factors provide a significant contribution or compensatory function to redox homeostasis. There is a paucity of data on the contribution or compensatory function of redox-regulated transcription factors, other than Nrf2, to redox homeostasis. The central hypothesis presented here: FOXOs contribute to redox homeostasis and affect the response of osteosarcoma to anticancer drug treatment. Furthermore, the effect is cell line-, anticancer drug- or post-translational modification-dependent. The hypothesis is based on the observation made by the PI that chemical inhibition of FOXOs induced OS in osteosarcoma. The amount of increased OS was cell line- dependent. Additionally, FOXO1 inhibition reversed anticancer drug-induced cell death. Inhibition of FOXO1 induced G2/M cell cycle arrest and increased p21 protein expression. The amount of cell cycle arrest and p21 protein increase was cell-line dependent. Collectively, preliminary results suggest a context- dependent effect of FOXOs on redox homeostasis and response of osteosarcoma to anticancer drug treatment. The central hypothesis will be tested through the following three independent specific aims: 1) Determine the contribution of FOXOs to redox homeostasis in osteosarcoma. 2) Determine the effect of FOXOs on the response of osteosarcoma to anticancer drug treatment. 3) Determine the specific FOXO post-translational modification responsible for the FOXO-mediated response of osteosarcoma to anticancer drug treatment. Specifically, this study will determine the function of FOXOs in redox homeostasis and contribution to osteosarcoma resistance to anticancer drug treatment. PUBLIC HEALTH RELEVANCE: The proposed research aims to investigate the contribution of FOXO transcription factors to the maintenance of cellular redox homeostasis in osteosarcoma and their contribution to osteosarcoma resistance to anticancer drug treatment. The results of this study will lay the basis for the development of targeted therapy that involves FOXO transcription factors in osteosarcoma.

**Key Personnel Institution Role**

Mario Hollomon Texas Southern University PI

**C 11 Keniry, Megan E University of Texas Rio Grande Valley 1R16GM153687-01**

**Investigating fundamental signal transduction mechanisms impacted by FOXO transcription factors on NOTCH and JAK/STAT Pathways in stem cell contexts**

We propose a high impact collaborative project to overcome barriers to understanding the roles and regulation of Forkhead box subfamily O transcription factors FOXO -1, -3, and -4 (FOXO) in stem cell contexts. Stem cells repopulate the lining of the intestinal tract, muscle, and blood lineages, yet also drive devastating diseases such as cancer. Evolutionarily conserved, partially redundant FOXO transcription factors are needed for stem cell maintenance in a litany of contexts such as embryonic, cancer, mesenchymal, hematopoietic, and neural cell lineages, as well as direct specification of subsequent lineages in a context-dependent manner. Canonically, high PI3K output leads to cytoplasmic/inactive FOXO factors. Stem cells, however, are fundamentally rewired to have both high PI3K Pathway activity and nuclear/active FOXO factors. Prior work shows that FOXO factors directly bind to and activate stem genes in both embryonic stem cells and the poor prognosis cancer glioblastoma multiforme (GBM) to drive stem cell fate. However, the precise mechanisms utilized by FOXO transcription factors in driving stemness, hindering differentiation, and determining cell fate are incompletely understood. Our novel, preliminary insights indicate that nuclear-localized FOXOs engage both the NOTCH and JAK/STAT signaling pathways. Our preliminary evidence demonstrates a strong loss in the gene expression of NOTCH Pathway components NOTCH1 and NOTCH3, as well as their targets including HES1, in FOXO4 loss-of-function contexts. Our preliminary data indicates that FOXO factors share the ability to promote NOTCH output. Motivated by our preliminary findings and published work (by others), we propose to examine whether FOXO - 1, -3, and -4 redundantly drive NOTCH1 and NOTCH3 gene expression in GBM and myoblasts. Our preliminary work with FOXO transcription factor disruption mutants shows that FOXO4 acts uniquely to hinder JAK/STAT3 activation and expression of targets IGF1, PDGF and TH. Of note, FOXO3 disruption led to a loss in STAT3 activation, underscoring unique contributions of FOXO factors to the JAK/STAT Pathway. We propose to dissect the ability of FOXO factors to differentially regulate the JAK/STAT Pathway in GBM and myoblast settings. Our long-term goal is to delineate FOXO-driven fundamental biological processes that aid in stem cell maintenance and cell fate by promoting the NOTCH Pathway and differentially regulating the JAK/STAT Pathway. The proposed work will integrally involve student researchers (our group has successfully sent eleven students to doctoral studies) and will promote the research environment at UTRGV, the second-largest Hispanic serving institution in the United States.

PUBLIC HEALTH RELEVANCE: This study utilizes innovative genomics techniques to address critical barriers to understanding FOXO transcription factor-driven developmental and cellular processes that promote stem cell fate specification and function in the contexts of glioblastoma multiforme (GBM) and myoblasts. Single cell RNA Seq will be used to determine whether FOXO transcription factors drive the fundamental developmental NOTCH Pathway within the context of stem cells and/or in neighboring differentiated cells. This study will also investigate the differential impacts of FOXO factors on the JAK/STAT Pathway in stem cell settings.

**Key Personnel Institution Role**

Megan E Keniry The University of Texas Rio Grande Valley PI

**stress**

**D 12 Kondapalli, Kalyan C University of Michigan at Dearborn 1R16GM153701-01**

**Elucidating the role of luminal pH in regulating phagosome transport**

Macrophages capture harmful microbes by engulfing them and forming dynamic organelles called phagosomes. Following internalization, phagosomes undergo a graded process known as phagosome maturation. This involves the phagosome being transported by motor proteins along microtubules from the cell periphery to the lysosomes in the perinuclear region. During transport, the nascent phagosome sequentially fuses with early and late endosomes, leading to a gradual acidification of the phagosome lumen. Various pathogens, including Mycobacteria, Salmonella, Candida, and others, employ strategies to subvert or adapt to the acidic phagosomal pH, which can have serious consequences for human health. The roles of luminal pH in phagosome function and the factors that regulate phagosomal pH are not yet fully understood. Our recent study revealed a surprising new role for phagosomal pH in governing the transport of phagosomes and identified the sodium proton exchanger NHE9 as a regulator of phagosomal pH. Our long-term objective is to understand how the phagosomal environment impacts motor protein activity, to devise novel approaches for combating pathogens evading the acidic phagosome. NHE9 hinders the directed motion of phagosomes along microtubules and promotes early detachment from the microtubule tracks. However, the mechanism by which NHE9-mediated changes in pH within the phagosome lumen alter phagosome transport is not yet known. This application aims to achieve two objectives: first, to uncover the mechanism that links NHE9-mediated luminal pH regulation to phagosome motility, and second, to characterize the effect of this regulation on the mechanics of microtubule- associated motor transport. Dynein and kinesin motors both drive phagosome transport, but their opposing forces create a tug-of-war. Membrane lipids could play a crucial role in regulating this process by selectively determining which class of motors binds to phagosomes, tipping the balance in favor of one direction over the other. Specifically, phosphatidylinositol-3-phosphate (PI3P) on early phagosomes and cholesterol on late endosomes, are vital for motor protein recruitment and activity. Previous studies indicate luminal pH impacts both PI3P and cholesterol levels. Our central hypothesis is that luminal pH changes mediated by NHE9 are communicated to the motor proteins via the membrane lipids. We will test our hypothesis through the following aims: In aim 1, we will investigate the impact of NHE9 expression on PI3P and cholesterol levels on phagosomes, as well as the recruitment of motor proteins on these membranes. Luminal pH measurements will be conducted in conjunction with these experiments. In aim2, we will elucidate the impact of NHE9-mediated luminal pH changes on the mechanics of bidirectional motion. This research is significant as it will unveil the mechanism by which luminal acidification regulates phagosome transport, a crucial process for effective pathogen elimination.

PUBLIC HEALTH RELEVANCE: Macrophages, a type of immune cells, capture microbial pathogens in vesicles known as phagosomes, which are gradually filled with acid as they move towards the center of the immune cell where they fuse with another vesicle known as the lysosome for pathogen elimination. The goal of our proposal is to elucidate how the acid in the phagosome lumen governs the transport of the phagosome. Pathogens that cause severe diseases in humans employ various mechanisms to subvert phagosomal acidification, and therefore insights gained from this study will have significant implications for developing effective methods to combat these highly dangerous microbes.

**Key Personnel Institution Role**

Kalyan Kondapalli Regents of the University of Michigan PI

**D 13 Liang, Fucheng Midwestern State University 1R16GM149504-01A1**

**Utilizing Membrane Protein Chaperone as Therapeutics to Prevent Protein Aggregation-Associated Disease**

Therapeutic efforts for preventing or reversing neurodegenerative disease have focused on clearing insoluble protein aggregates or inhibiting the aggregation of amyloidogenic proteins. However, no inhibitor/drug that can effectively cure protein aggregation-prone diseases, such as Alzheimer’s disease (AD) and Parkinson’s disease (PD). The novel protein-targeting machinery from the plant chloroplast, cpSRP43, is an effective ATP- independent membrane protein chaperone that prevents and reverses the aggregation of its membrane protein substrate. Membrane protein chaperone provides the first proof-of-principle model to prevent hydrophobic region of membrane proteins from aggregation. Most importantly, the membrane protein chaperone can actively disrupt protein aggregates without external energy, like ATP, emphasizing its unique function among protein chaperones. However, how membrane protein chaperone inhibits/reverses disease-related protein aggregation is unknown. My goal is to identify an effective chaperone to further prevent and/or reverse the protein aggregation and open a new avenue to improve the treatment for AD and PD. Importantly, the educational goal for this proposal is to create enhanced, relevant training opportunities for undergraduate students and M.Sc. students to participate in biomedical research. Project Objective and Hypothesis: The long-term objective of this R16 project is to apply the unique anti- folding and disaggregation properties of cpSRP43 as therapeutics for AD and PD. My educational objective is to encourage undergraduates and M.Sc. students to pursue cutting-edge research and as well as enhance diversity of students participated in biomedical research. I propose three specific hypotheses. H1: Chaperones will prevent Aß toxic oligomeric formation via inhibiting the surface catalyzed secondary nucleation step. H2: Chaperones can potentially prevent Tau aggregation formation. H3: Chaperones can potentially prevent ASyn oligomeric formation. Specific Aims: Aim #1: Apply membrane protein chaperone to prevent and disrupt Aβ aggregation. Aim#2: Inhibit and disrupt Tau aggregation with membrane protein chaperone. Aim #3: Inhibit and disrupt ASyn aggregation with membrane protein chaperone. Methodologies: I will utilize bacterial protein overexpression system; protein purification through affinity, ion-exchange and size-exclusion chromatography; Thioflavin T fluorescence assay; fluorescence resonance energy transfer assay; light scattering; sedimentation; dot-blotting assay and electron microscopy to probe protein aggregation. A high quality and innovative research opportunity for undergraduate and M.Sc. students will be provided to enhance problem-solving skills and independent thinking abilities. Enriching the research environment at MSU will provide quality hands-on experience on instrumental operation and data interpretation for students, thereby improving and motivating students’ passion for the biomedical research.

PUBLIC HEALTH RELEVANCE: Protein aggregation and the associated neuronal death pose a special challenge to protein homeostasis and lead to several neurodegenerative diseases (e.g., Alzheimer’s and Parkinson’s disease), but membrane protein chaperone provides the first proof-of-principle model for preventing and disrupting aggregation in hydrophobic regions of membrane proteins. This R16 proposal will build on these findings and further apply membrane protein chaperone as therapeutics to use its unique anti-folding and disaggregation properties to strategically develop and characterize an effective treatment for Alzheimer’s and Parkinson’s disease. My educational goal is to involve talented undergraduate and M.Sc. students in cutting-edge life science research and ultimately encourages students to gain experience via conducting biomedical research and make a major contribution towards building additional capacity at MSU.

**Key Personnel Institution Role**

Fu-Cheng Liang Midwestern State University PI

**C 14** **M Keppetipola, Niroshika A** **California State University Fullerton** **1R16GM153585-01**

**Determining the role of lysine acetylation in alternative splicing regulation**

The process of alternative splicing is regulated in part by RNA binding proteins (RBPs) that bind to corresponding sequence elements in the gene transcript and influence the assembly of the spliceosome components at adjacent splice sites. Recent studies highlight that many RBPs are modified at lysine side chains by the addition of acetyl groups. Acetylation neutralizes the positive charge of the lysine residue, which can disrupt its capacity to interact with the negatively charged RNA and its ability to interact with other proteins. Acetylated RBPs have been identified in many cancers and are linked to proteinopathy in neurodegenerative diseases. Similarly, spliced variants have been identified in many gene transcripts related to neurodegenerative diseases and cancers. Collectively, these data suggest a role for RBP acetylation in generating these disease- related spliced variants. A detailed mechanistic understanding of how lysine acetylation regulates RBP properties to elicit alternative splicing patterns will uncover enzymes and pathways that may serve as therapeutic targets in the treatment of splicing-related diseases. In this project, we propose to investigate the role of acetylation in alternative splicing regulation using the RBP polypyrimidine tract binding protein 1 (PTBP1). PTBP1 is a well-characterized RBP involved in alternative splicing regulation. We discovered that PTBP1 is acetylated at several lysine residues in each of the four RBDs and the nuclear localization sequence in the N-terminal region. PTBP1 most often functions to repress inclusion of regulated exons in spliced mRNA and the mechanism of PTBP1 mediated splicing regulation is well understood. We uncovered that PTBP1 is acetylated at highly conserved lysines that play a role in RNA binding, protein-protein interactions, and sub-cellular localization. Preliminary data demonstrate deacetylase inhibitors decrease PTBP1’s splicing repression activity. These findings suggest that acetylation might dictate PTBP1 alternative splicing activity. Our specific aims are to test this hypothesis. Aim1: Determine the role of RBD acetylation in PTBP1 splicing activity. Aim2: Determine the role of acetylation in PTBP1 cellular localization and splicing activity. Our studies will answer fundamentally important questions about how acetylation modulates RBD properties and in turn RBP splicing activity to dictate pre-mRNA splicing patterns in response to cellular metabolic state. The proposed studies will leverage experimental approaches established in the laboratory by previous undergraduate trainees, providing new students, including those from backgrounds underrepresented in biomedical sciences, an excellent opportunity to gain hands-on research experience in splicing biology. PUBLIC HEALTH RELEVANCE: Disease-related splice variants have been identified in many neurodegenerative disease and cancers underscoring the importance of alternative splicing regulation. We have discovered that RNA binding proteins including PTBP1, that regulate alternative splicing in part, are post-translationally acetylated in disease tissues including cancers. Learning how acetylation effects the activity of PTBP1 will enable a better understanding of how alternative splicing is regulated in response to cellular metabolic state and may facilitate the development of novel therapeutics for the treatment of splicing-related diseases.

**Key Personnel Institution Role**

Niroshika Keppetipola California State University, Fullerton PI

Alison Miyamoto California State University, Fullerton Co-Investigator

**D 15 Martinez-Ferrer, Magaly University of Puerto Rico Med Sciences 1R16GM149442-01A1**

**Andrographolide and its role modulating metabolism in prostate cancer.**

Prostate cancer (PCa) is the second most common diagnosed cancer and the fifth cause of cancer mortality in men worldwide. In the United States, PCa is the most common diagnosed cancer and the third cause of cancer mortality in men. PCa is often treated using radiation, chemotherapy or radical prostatectomy. However, the combination of natural products with the standard of care treatment is an emerging area of cancer therapeutics. Andrographolide, a labdane diterpenoid and the main bioactive component of the medicinal plant Andrographis paniculata, have shown a wide range of biological activities, among them, anticancer activity. Studies from our laboratory showed that andrographolide: (1) decreased PCa cell migration and invasion in vitro, (2) increased cell apoptosis in vitro, (3) decreased tumor volume and blood vessels formation in vivo, and (4) induced a DNA damage response in PCa cells. In addition, preliminary data identified alterations in genes associated with metabolism and mitochondria in tumors treated with andrographolide. These data suggest that andrographolide disrupts mitochondrial function and metabolic capability. However, there is a gap in knowledge about the mechanism by which Andrographolide alters mitochondrial function and bioenergetics in prostate cancer. The overall objective of our current study is to investigate the role of Andrographolide in metabolism and mitochondrial function. Based on our preliminary data our central hypothesis is that Andrographolide alters the bioenergetics profile of prostate cancer and decreases mitochondrial function. We plan to test our central hypothesis and, thereby, accomplish our overall objective for this project by pursuing the following two specific aims: (1) Determine the effects of Andrographolide in mitochondrial DNA integrity and mitochondrial dysfunction, (2) Determine the effects of Andrographolide in the bioenergetics of prostate cancer, and (3) To determine the effects of Andrographolide in autophagy-mediated clearance of dysfunctional mitochondria. Our approach will generate new information and lead to molecular insights into the molecular mechanisms of Andrographolide. These experiments are critical to move the use of Andrographolide for the treatment of prostate cancer from a basic science setting to a translational one. Understanding the Andrographolide mechanisms of action is crucial for identifying new prognostic and therapeutic targets. PUBLIC HEALTH RELEVANCE: Andrographolide, the main bioactive component of the medicinal plant Andrographis paniculata, have shown a wide range of biological activities, among them, anticancer activity. Previous studies from our laboratory showed that Andrographolide: (1) induces a DNA damage response in prostate cancer PC3 cells, (2) alters carbohydrate metabolism and, (3) alters mitochondrial associated genes in prostate cancer cells, and (4) leads to loss of the mitochondrial membrane potential (ΔΨm) and increased ROS production in 22Rv1 cells in vitro. These data suggest that andrographolide disrupts mitochondrial function and metabolic capability. However, the mechanism by which andrographolide alters mitochondrial function and bioenergetics in PCa represents a gap in knowledge. The overall objective of our current study is to investigate the role of Andrographolide in metabolism and mitochondrial function.

**Key Personnel Institution Role**

Magaly Martinez-Ferrer University of Puerto Rico Med Sciences PI

Carlos Torres University of Puerto Rico Medical Sciences Co-Investigator

**C 16 Molina, Carlos A Montclair State University 1R16GM149394-01A1**

**Establishing the role of the transcriptional repressor ICER in melanoma-genesis.**

The objective of this project is to investigate why some “forms'' of the transcriptional repressor ICER elicit aggressive tumors whereas others inhibit malignancies in a zebrafish model for melanomagenesis. The role of abnormal post-translational modifications (PTMs) on growth-related proteins during tumorigenesis is not well understood. My laboratory is focused on understanding how PTMs affect the activity of ICER at the molecular, cellular, and developmental levels. We and others have shown that ICER is regulated by phosphorylation and ubiquitination. ICER is phosphorylated on distinct Serine residues (Ser 35 and Ser 41) as a prerequisite for polyubiquitin-mediated proteasomal degradation, or altered cytoplasmic translocation. In order to study the role of ICER’s PTMs in melanoma, our laboratory has been working with a model transgenic zebrafish for skin cancer, Tg(mitfa:BRAFV600E); mitfa(lf); p53(lf), referred to as Triples. Our laboratory has generated fish lines for the expression of three different “forms” of ICER in these Triples; wild-type-(wt)-, No-lysines- and a phosphorylation mutant S35&41A-ICER. Tumor incidence curves showed that wt- and No-lysines-ICER significantly accelerates the onset of melanoma compared to the EGFP control. In contrast, the zebrafish expressing S35&41A-ICER had a significantly longer life than EGFP control. Preliminary pathological analysis of these tumors showed that wt-ICER melanomas are more aggressive than EGFP-expressing melanomas and that S35&41A-ICER shows benign lesions only. Presently, the main focus of my laboratory is to understand these observations. As a transcription factor regulating cAMP-dependent gene expression, ICER may be related to the observed resistance to inhibitors of the RAF/MAPK kinase pathway in human melanomas. The central hypothesis of this proposal is that more aggressive melanomas evolved and become refractory to the cell growth inhibitory activity of ICER. Our long-term goal is to understand the role of PTMs of ICER in malignancies using melanoma as a paradigm. The rationale is that the understanding of how ICER is post translationally regulated in tumor cells could lead to the development of inhibitors that will restore normal ICER protein levels contributing to their demise. Two specific aims will allow us to test our central hypothesis and meet our objective. 1) To characterize the malignancies of ICER-expressing transgenic zebrafish tumors, and 2) To determine the mechanisms of ICER-mediated malignancies using cell models. The expected outcome of this work is a comprehensive understanding of how ICER and ICER PTMs are associated with malignancies. The results will have an important positive impact as they will enhance our knowledge of the involvement of the cAMP pathway in melanomas, and hence the potential development of a new class of targeted treatments for cancer management. The research will involve students from diverse backgrounds, including those underrepresented in biomedical research.

PUBLIC HEALTH RELEVANCE: My laboratory seeks to understand the mechanisms involved in eliminating or misplacing a protein with cell growth suppression activity using animal models and cells in culture. The long term expectation is that this research will set the stage to test agents to specifically inhibit the degradation and abnormal localization of this protein in diseases such as cancer.

**Key Personnel** **Institution** **Role**

Jinshan Gao Montclair State University Other Significant Contributor

Carlos Antonio Molina Montclair State University PI

Leonard Zon Harvard University (Boston Children's Hospital) Other Significant Contributor

**B 17 Mujtaba, Syed Shiraz Medgar Evers College 1R16GM153556-01**

**Deciphering Epigenetic Functions of MYST1 in Transcriptional Regulation of Androgen Receptor**

The long-term goal of our research is to elucidate the biological role of epigenetic mechanisms that regulate the transcription functions of the Androgen Receptor (AR). Despite blocking the physiological axis of androgen and AR, the mechanisms driving the development of castrate-resistant prostate cancer (CRPCa) are still not fully understood. Although the transcriptional coregulators are known to orchestrate AR functions, detailed mechanistic knowledge of the AR and coregulator interaction needs to be fully understood. Transcriptional coregulators mediated post-translational modifications and molecular interactions, which comprise epigenetic mechanisms, regulate genes that modulate cellular response. Published data underscore that the levels of acetylation at lysine 16 on histone H4 (H4K16ac) and trimethylation at lysine 20 on histone H4 (H4K20me3) on chromatin serve as markers for cancer progression. Coregulators MYST1 (MOZ, YBF2 and SAS2, and TIP60 1) and SUV4-20H2 are the primary enzymes that catalyze the H4K16ac and H4K20me3 marks, respectively. However, the molecular mechanisms intertwining the biological effects of H4K16ac and H4K20me3 for CRPCa growth are still unknown. We have demonstrated that MYST1 is an essential coactivator of AR transcriptional activation. Our data also showed that MYST1 is overexpressed in the human CRPCa tissues. The MYST1 protein is comprised of a chromodomain (ChD) and a histone acetyltransferase (HAT) module. Downregulation of MYST1 blocks the growth of CRPCa cells by triggering apoptosis. In CRPCa cells, the molecular interaction between MYST1 and AR is crucial for H4K16ac. Nevertheless, the mechanistic bases of MYST1 and AR interactions and H4K16ac remain elusive. Besides, preliminary data advocate defining the molecular basis and biological role of ChD-mediated MYST1 recruitment to H4K20me3 in CRPCa. Further, the biological significance of cooperativity between MYST1 and SUV4-20H2 must be investigated. Furthermore, the target genes regulated by the concerted functions of H4K16ac and H4K20me3 are unknown in CRPCa. We hypothesize that MYST1 synergizes the biological role of H4K16ac and H4K20me3 to promote CRPCa growth. Moreover, the MYST family of coregulators are targets for cancer drug discovery programs. The main goal of this proposal is to elucidate the molecular determinants of MYST1 functions that facilitate AR-driven CRPCa growth. Towards this objective, we will determine: i) Elucidate the Molecular Determinants of MYST1 and AR Interaction; ii) Elucidate the Biological Role of MYST1 ChD and H4K20me3 Interaction; and iii) Delineate the Target Genes and Pathways Controlled by H4K16ac and H4K20me3. One of the primary goals of our research program is to fulfill Medgar Evers College's mission to recognize minority students in the scientific community and advance its academic endeavors to mentor students to pursue careers to enrich STEM workforce diversity. Together, the emerging data will advance our knowledge of a new mechanism directing the AR pathway, which could be a new target for developing innovative CRPCa treatment modalities.

PUBLIC HEALTH RELEVANCE: We showed that MYST1 is a vital Androgen Receptor (AR) coactivator in Castrate Resistant Prostate Cancer (CRPCa). Besides, MYST1 downregulation triggers apoptosis in CRPCa cells. These data advocate unraveling the MYST1-mediated epigenetic mechanism for designing new strategies to block AR-directed CRPCa growth.

**Key Personnel Institution Role**

Syed Shiraz Mujtaba Medgar Evers College PI

**A 18 Murray, Sean California State University Northridge 1R16GM149447-01A1**

**Integrating DNA supercoiling with bacterial cell cycle progression**

Although required for bacterial proliferation, the regulation of the bacterial cell cycle remains poorly understood. Here, we aim to integrate DNA supercoiling into the transcriptional machinery responsible for the temporal and spatial regulation of genes during the bacterial cell cycle. We will use deep sequencing to uncover the genes that are differentially regulated by DNA supercoiling. Our preliminary results using a pharmacological approach have revealed that (1) two proteins change in abundance by Commassie brilliant blue staining of SDS-PAGE protein gels when DNA supercoiling is relaxed, and (2) that one of these proteins is the cell-cycle regulatory factor SciP, whose abundance varies as a result of altered transcription, and (3) 460 genes exhibited a 2-fold or greater change in occupancy of RNA polymerase when DNA supercoiling is relaxed. We plan to generate a temperature-sensitive allele of the gyrase subunit gyrB in Caulobacter crescentus, which will allow us to genetically inactivate gyrase activity, which is expected to validate and complement our pharmacological approach. We will use qRT-PCR to validate altered transcription in ~10% of the differentially-regulated genes identified via deep-sequencing, that are cell-cycle regulated, and then to determine if their temporal gene expression changes when DNA supercoiling is perturbed. This data will help us to understand how bacteria respond to the coumarin class of antibiotics that inhibit DNA supercoiling to prevent the growth of bacteria. Thus, our findings have the potential to improve human health as well as to demonstrate that DNA supercoiling may be a master regulator of the bacterial cell cycle.

PUBLIC HEALTH RELEVANCE: The proposed research will create a systems-level understanding for how DNA supercoiling integrates with the bacterial cell cycle. This can help us to better understand how bacteria transcriptionally respond to the coumarin class antibiotics.

**Key Personnel Institution Role**

Sean R Murray The University Corporation PI

**D 19 Noubissi, Felicite K Jackson State University 1R16GM153544-01**

**Colorectal Cancer Treatment and Drug Resistance; the Role of IGF2BP1**

Advances in chemotherapy treatment regimens have significantly improved colorectal cancer (CRC) patient survival, yet drug resistance remains an important clinical challenge that is associated with poor patient outcomes. Two major mechanisms employed by CRC cells are thought to be responsible for resistance to therapeutics: i) resistance to apoptosis usually achieved by activation of anti-apoptotic pathways; and ii) induction of multidrug resistance (MDR) membrane transporters that pump drugs out of the cells. We previously identified IGF2BP1 as a direct target of the Wnt/β-catenin signaling, a central mechanism that drives colorectal carcinogenesis. Further, we have shown that inhibition of IGF2BP1 sensitized CRC cells to drugs. Our long-term goal is to accelerate the development of clinically useful mechanism-based therapeutics for treating colorectal cancer resistance. Our objectives are to identify the mechanisms by which the inhibition of IGF2BP1 sensitizes colorectal cancer cells to chemotherapeutics in vitro and determine the in vivo efficacy of chemotherapeutics on tumor growth and progression when IGF2BP1 is inhibited. Our central hypothesis is that inhibition of IGF2BP1 promotes apoptosis and reduces migration, invasion, and drug efflux to sensitize CRC cells to chemotherapeutics in vitro and in vivo resulting in tumor regression. This hypothesis has been formulated largely on the basis of preliminary data described above as well as findings demonstrating that IGF2BP1 inhibition decreased resistance of chemotherapy-resistant CRC cells with activated Wnt/β-catenin signaling. Our rationale is that the determination of the preclinical therapeutic efficacy and associated mechanisms of IGF2BP1 inhibition is likely to provide strong basis for the development of a new class of drugs for an effective CRC treatment. The central hypothesis will be tested by pursuing two specific aims: 1) Identify the mechanisms of IGF2BP1 inhibition-dependent sensitization of CRC cells to chemotherapeutics. 2) Determine the in vivo effects of IGF2BP1 inhibition on tumor sensitivity to chemotherapy. We will use CRC cell lines to elucidate the mechanisms by which inhibition of IGF2BP1 sensitizes CRC cells to chemotherapeutics in aim 1 and use human xenograft tumor models and a mouse model of CRC to determine as proof of principle the anti-cancer therapeutic efficacy of chemotherapeutics when IGF2BP1 is inhibited in aim 2. The research is innovative in our opinion because of IGF2BP1’s unique characteristics. IGF2BP1 promotes cell proliferation and induction of MDR1 gene. In addition, it is absent or scarce in adult tissues, but over-expressed or reactivated in various tumors including primary CRCs. It is associated with aggressive cancers and its inhibition does not significantly affect normal cells. Our study is significant because in vivo, mechanism-based proof of principle evidence for a preclinical role of IGF2BP1 inhibition in sensitizing CRC to chemotherapy is likely to inform the subsequent development of novel strategies for reducing resistance in human patients. PUBLIC HEALTH RELEVANCE: The proposed research is relevant to public health because it focuses on developing a new approach to treat advanced and resistant colorectal cancer by inhibiting the function of an RNA binding protein that regulates numerous oncogenic proteins involved in growth and survival of colorectal cancer cells. This would provide a more effective treatment option for advanced chemo-resistant colorectal cancer and potentially reduce colorectal cancer mortality and survival disparity in our communities. Thus, the proposed research is relevant to the part of the NIH’s mission that pertains to reducing illness and disability.

**Key Personnel Institution Role**

Felicite K Noubissi Jackson State University PI

**D 21 Peric, Miroslav California State University Northridge 1R16GM153675-01**

**Effects of Statins on Alzheimer’s beta-Amyloid Peptide (25-35) in Phospholipid Bilayers**

Alzheimer’s disease (AD), a chronic neurodegenerative disease, is the most common cause of dementia in the elderly. Due to ever-increasing life expectancy, AD, whose symptoms usually first appear in adults after age 60, has become one of the scourges of modern times. AD is a complex disease caused by a wide range of biochemical and biophysical interactions between genetic, epigenetic, and environmental factors. One of the treatable medical conditions associated with an increased risk of AD is midlife hypercholesterolemia. Because of that, the role of statins, cholesterol-lowering medications, in preventing and treating AD has been investigated. Most human epidemiological studies, human neuropathology studies, and experiments using animals have found strong indications that high cholesterol adversely affects AD. However, human studies of statins have so far been inconclusive. According to a number of systematic reviews of existing studies, highly variable outcomes are very likely caused by too many confounding variables involved. One of the confounding variables is the existence of two types of statins: lipophilic and hydrophilic statins. The main goal of this project is to elucidate the interaction of lipophilic and hydrophilic statins with the β-amyloid peptide (25-35) in phospholipid model membranes with and without cholesterol by using the spin probe Electron Paramagnetic Resonance (sp-EPR) method. The working hypothesis is that the lipophilic statins penetrate into the phospholipid bilayer and thus change the dielectric properties, fluidity, and permeability of the bilayer to make the membrane more fluid. The new physical state of the bilayer prevents deeper penetration of water and cations into the bilayer, reducing its disruption, which in turn preserves the speed of signal transduction and neurotransmission, preventing βAP neurotoxicity. On the other hand, the hydrophilic statins (pravastatin sodium salt hydrate and rosuvastatin calcium) interact weakly with the surface of the phospholipid bilayer; therefore, they do not affect the physical state of βAP in the middle of the bilayer. To test the hypothesis, we propose conducting a range of electron paramagnetic resonance experiments on statin-phospholipid vesicles, statin-peptide-phospholipid vesicles, statin-cholesterol- phospholipid vesicles, and statin-peptide-cholesterol-phospholipid vesicles. These experiments are designed to give a complete knowledge of the changes in dielectric properties, membrane fluidity, and permeability across the phospholipid bilayer so that the location of the statin in the phospholipid bilayer and the structural changes of the aggregate as a whole can be determined. This information may provide new insight into how the two types of statins affect β-amyloid peptides in the phospholipid bilayer.

PUBLIC HEALTH RELEVANCE: Knowledge of the molecular interaction of statins and the β-amyloid peptides (βAP) in phospholipid- cholesterol membranes may provide new insights into the effect of statins on βAP. A better understanding of the interaction between statins and βAP in the phospholipid bilayer may improve existing treatments for AD patients. Since high cholesterol levels are detrimental to the health of the blood vessel wall, this understanding might be beneficial in treating diseases in which the health of the blood vessel is critical.

**Key Personnel Institution Role**

Miroslav Peric California State University, Northridge PI

**D 22 Pluth, Janice M University of Nevada Las Vegas 1R16GM153540-01**

**Mechanistic understanding of role of ATF2 signaling in radiation-induced esophageal cancer**

The objectives of this proposal are to define fundamental mechanisms that drive radiation-induced esophageal squamous cell carcinoma (ESCC). There is compelling evidence linking an increased risk of developing ESCC with a history of therapeutic radiation exposure. Elucidating the machinery that drives tumor susceptibility, is of major import to NCI, as ESCC is the deadliest of all human squamous cell carcinomas. Abnormalities in stress signaling, in proteins such as ATF2 are strongly implicated in cancers. Additionally, a central role of epithelial to mesenchymal transition (EMT) in the promotion of cancer has gained prominence. Knowledge of pathways that translate radiation-induced aberrant signaling to changes in the microenvironment is lacking and essential to assess cancer risk post radiation exposure. This is a major gap in the field, which the current project proposes to bridge. Our preliminary studies suggest a strong association between persistent pATF2ser 490/98 signaling and EMT, both events regulated by transforming growth factor β (TGFβ). Our main objectives are to understand the role of TGFβ-mediated pATF2 signaling and its relationship to EMT, to provide insight into how changes in the microenvironment allow radiation-induced tumor initiation and progression and identify biomarkers for ESCC radiation-induced carcinogenesis. We hypothesize that TGFβ driven persistent pATF2 signaling triggers EMT post radiation exposure, and this signaling network creates a tumor permissive microenvironment. To test this hypothesis, we will use well-characterized normal and transformed cells containing genetic alterations commonly occurring in esophageal cancer, and 3D cell culture systems, closely mimicking the in vivo physiological environment of the esophagus. High and low dose fractionated radiation will be used to simulate the exposure to the esophagus from radiotherapeutic cancer treatments. CRISPR technology will be used to mutate the ATF2 phospho-sites to define dependency of this signaling. Immunofluorescence, immunohistochemistry, and RNA seq will be used to address the specific aims and gain a mechanistic understanding of these events. We will (1) test our hypothesis that EMT induction is a consequence of fractionated radiation-induced aberrant pATF2 signaling, and (2) investigate the effect of pATF2 signaling on tissue architecture, promotion of a tumor phenotype, and transcriptomics post radiation exposure. Our contribution is expected to provide an innovative approach to understand how radiation, through its influence on intercellular communication and interactions with the microenvironment affects levels of biological organization and promotes cancer. This proposal addresses relevant scientific areas of emerging importance such as the contribution of persistent stress signaling, and microenvironment changes in promoting carcinogenesis. These studies are especially significant, as elucidating the underlying mechanisms that promote radiation-induced carcinogenesis could aid in predicting patients at an increased risk of developing ESCC and give mechanistic insights into the radiation-related carcinogenesis of other tumor types including esophageal adenocarcinoma (EAC).

PUBLIC HEALTH RELEVANCE: Esophageal squamous cell carcinoma (ESCC) is the deadliest of all human squamous cell carcinomas, and has a relatively low five-year survival rate. Abnormalities in stress response signaling and epithelial to mesenchymal transition (EMT) have gained prominence as cancer promoting mechanisms. These studies will define whether radiation-induced persistent pATF2 stress response signaling, is a key node in promoting EMT and changes in the microenvironment that facilitate radiation-induced ESCC initiation and/or progression.

**Key Personnel** **Institution** **Role**

Lung-Chang Chien University of Nevada, Las Vegas Biostatistician

Hiroshi Nakagawa Columbia University Irving Medical Center Other Significant Contributor

Janice M Pluth University of Nevada, Las Vegas PI

**D 23 Raychoudhury, Samir Benedict College 1R16GM153611-01**

**Effects of PAHs on NOX2-induced redox signaling in breast cancer cells**

The proposed research aims to understand how the molecular signals inside the breast cancer cells are associated with the change in cell shape, size, growth, and cell cycle progression under varying exposure to BaP and FLA-PAHs. Since some of the PAHs are known as endocrine disruptors (ED), we plan to use MCF-7 (estrogen receptor-positive) cells, MDA-MB 231 (estrogen receptor-negative) cells, or unrelated (macrophage) cells. This research asks the basic biology questions: Do the PAHs-induced events in MCF-7 (estrogen receptor-positive) cells and MDA-MB 231 (estrogen receptor-negative) cells exacerbate via NOX2 dependent pathway? Does the functional NADPH oxidase pathway share regulation by lipid rafts? In specific aim 1, we plan to study the role of NOX activation of PAH-induced DNA damage, mitochondrial dysfunction, and apoptotic response to oxidative stress in estrogen receptor (ER) - positive and ER-negative cells. We will examine ROS production and NOX expression levels. The level of p47phox knockdown will be assessed by RT-PCR and Western blotting. In specific aim 2, we plan to study the functional alterations of lipid rafts associated NOX pathway. Here, we will characterize the expression of gp91phox and p22phox subunits and their co-localization with lipid rafts using several independent approaches, including enzymatic assays, immunological assays, and RT-PCR analysis. In specific aim 3, we prepare an ER knock-out breast cancer cell line by mutated ER in MCF-7 cells and compare NOX-activation, oxidative stress, and mitochondrial dysfunction due to PAH exposure. In addition to MCF-7 cells, we plan to use hTERT-HME1 [ME16C]-immortalized breast cells exhibiting epithelial morphology. The ER-α gene knockout in these cells will be carried out by using the most innovative and available CRISPR (clustered, regularly interspaced, short palindromic repeats)/Cas9 (CRISPR- associated protein 9) tool. The project will select and train two undergraduate researchers (sophomore or junior) and two high school students (junior or senior), creating a pipeline of well- trained graduating seniors and college students.

PUBLIC HEALTH RELEVANCE: The goal of this SuRE application is to examine the role of NOX2 in PAH (fluoranthene and benzo[a]pyrene)-induced DNA damage, mitochondrial dysfunction, and apoptotic response to oxidative stress in estrogen receptor (ER)-positive and ER-negative breast cancer cells. We will study the functional alterations of lipid rafts associated NOX pathway and test the association of estrogen receptors in the NOX activation process. Minority student training is an integral part of this proposed research.

**Key Personnel Institution Role**

Samir Raychoudhury Benedict College PI

**D 24 Rotenberg, Susan A Queens College 1R16GM153696-01**

**Phosphorylation of α/β Tubulin is a Toggle Switch in Human Breast Cancer**

The α/β-tubulin heterodimer is the building block of microtubules (MTs) which comprise an essential component of the cell cytoskeleton. MTs undergo growth/shrinkage episodes as dictated by whether GTP is bound to β-tubulin (β:GTP) (assembly) or is hydrolyzed to GDP (disassembly). Protein kinase C (PKC) is a key signaling enzyme that controls these MT events in human breast cells. In this regard, PKC phosphorylates α-tubulin at Ser165 (α:Ser165) resulting in persistent MT elongation and breast cell motility while proliferation is suppressed. Molecular dynamics simulations and validating experiments showed that phosphorylation of α:Ser165 misaligns the catalytic residue Glu254 in α-tubulin (α:Glu254) with its substrate β:GTP, leading to diminished hydrolysis of β:GTP, thereby increasing GTP caps and causing persistent MT elongation. β-tubulin undergoes phosphorylation at Ser172 by cyclin-dependent kinase-1 (cdk-1) which promotes proliferation, a process that requires α/β-tubulin to form spindle fibers during cell division. Thus, PKC and cdk1 produce opposing effects on microtubule structure, GTP binding/hydrolysis, and cell proliferation. By alternating the phosphorylation states of α− and β- tubulin in microtubules using phospho-mimetic mutants, a “toggle switch” model will be explored as a novel decision point that governs cancer-related phenotypes. This model will be rigorously tested by co-expression of site-directed mutants of α-tubulin (S165D or control S165N) and β-tubulin (S172D or control S172N) in human breast cells. By use of these constructs, proliferation and motility as well as EMT markers (E- vs. N-cadherin) will be evaluated. Molecular dynamics simulations will be used to screen the alignment of α:Glu254 with β:GTP both in the α/β-tubulin mutant constructs and in those mutants to be identified in tumor genomic databases; their significance will be verified experimentally by the presence of GTP caps and cell phenotypes (proliferation, motility). In vivo support for the α/β-tubulin toggle switch will be sought in an orthotopic animal model with highly aggressive breast cells to evaluate: 1) the impact of phospho-mimetic mutant constructs of α/β-tubulin, and 2) the efficacy of small molecule inhibitors of PKC and cdk1. The purpose of these studies is to establish a novel framework for assessing metastatic potential and to explore improved strategies for breast cancer chemotherapy.

PUBLIC HEALTH RELEVANCE: Alternating phosphorylation states of α− and β-tubulin in microtubules define a “toggle switch” model to be explored as a novel decision-point in cell behavior. Phosphorylation of α-tubulin or β-tubulin could determine whether a breast cancer cell metastasizes to a remote site or proliferates into a primary tumor, respectively. The α/β-tubulin toggle switch could have intrinsic biological significance as well as clinical value as a therapeutic target and prognostic indicator of metastatic potential.

**Key Personnel Institution Role**

Adriana Haimovitz-Friedman Memorial Sloan-Kettering Cancer Center Co-Investigator

Yoko Nomura Queens College/CUNY Collaborator

Baofu Qiao Baruch College - City University of New York Co-Investigator

Susan A Rotenberg Queens College – CUNY PI

**B 25 Schiller, Martin R University of Nevada Las Vegas 1R16GM153593-01**

**Synonymous Variants of Uncertain Silence**

Synonymous variants are generally considered to be silent for any effect, because they do not change the amino acid sequence of a protein. Over the past decade, there is a growing body of work challenging this dogma with conclusive examples of synonymous variants associated with a disease, exerting selective pressure in fitness assays, or effecting protein expression and function. At this point, it is crucial to understand if these synonymous variants with effects are rare occurrences, or if they are more commonplace than previously appreciated. We recently reported that 50% of synonymous variants (n =89) were not silent when the transcriptional activity of the HIV Tat transcription factor was measured with a LTR-GFP reporter. This evaluation of synonymous variants has the advantages over previous work in that it measures many variants at once and is a direct assay of the molecular function of the gene, rather than a far downstream measure like fitness, which has many unknown sources of potential error. While our assay system has an accuracy of 95% for all 1,615 designed single missense variants in a saturating mutagenesis experiment, the synonymous variants assessed were much fewer because they arose from errors in oligonucleotide synthesis. These variants could be isolated because each molecule in our assay has a random barcode unique molecular identifier (UMI) that can be tracked. In this proposal, we will further test the hypothesis: “Many synonymous variant alleles are not silent” with experiments on a saturating set of synonymous variants in Tat, and expanding the analysis of variants in Erbb2, an endogenous human gene measured with a separate direct molecular assay. In Aim 1 we will Create, detect, and measure the transcriptional activity of all Tat synonymous single site variants. In Aim 2, we will determine the percentage of Her2 synonymous single site variants that have reduced autophosphorylation to assess a second gene. In Aim 3 we will create stable cell lines for 6 variants for each of the two genes and measure the gene- specific activity in an orthogonal assay. This will validate the GigaAssay results. If the results of these experiments support our hypothesis, this would be a more rigorous test and stimulate investigation of inferred variant silence routinely used in medical genetic clinics.

PUBLIC HEALTH RELEVANCE: Synonymous mutations are a type of genetic variant that are thought to have very little effect on genes. This project will extend and validate recent studies suggesting that many of these variants may affect cells and disease. If our preliminary work is verified, this would justify new efforts to improve the interpretation and accuracy of molecular genetic diagnostics for clinical care.

**Key Personnel Institution Role**

Martin R Schiller University of Nevada Las Vegas PI

**D 26 Stedman, Kenneth Mark Portland State University 1R16GM154184-01**

**Determining the molecular basis for extreme virus stability, a structure-guided approach**

In the long-term we aim to understand the genetic, biochemical and structural basis for extreme virus function and stability. Sulfolobus Spindle-shaped Virus 1 (SSV1) is astonishingly stable to both high temperatures (>80°C) and low pH (3 and below). This stability is despite being composed of proteins and nucleic acids with similar compositions to viruses and other macromolecular complexes found in less extreme conditions. However, it is not clear how SSV1’s extreme capsid stability is determined. We have developed robust genetic tools for SSV1 and standardized techniques for working with these extreme viruses, and demonstrated their use in undergraduate research. Together, these tools and the recently-determined structure allow probing of the determinants of stability as follows. Determine the amino acids in the SSV1 major capsid protein VP1 that are important for thermal stability using site directed mutagenesis (Aim 1). We have determined that the spindle-shaped SSV1 virion is composed of seven intertwined helical strands of the major capsid protein, VP1. Using the predicted structure of VP1 and the virion structure, we will make site-directed mutations in 3 sets of amino acid side chains: those predicted to be in loops and glycosylated, those predicted to make interactions between strands and those predicted to form intramolecular interactions in VP1. These mutant viruses will be screened for activity at multiple temperatures. Use in vitro evolution to select SSV1 VP1 and VP3 mutants for increased thermal stability (Aim 2). In order to perform an unbiased screen of amino acid substitutions that could confer stability to SSV1 we will make a library of mutants that contain random substitutions in the major and minor capsid proteins VP1 and VP3. These mutants will be selected in vitro for their ability to maintain infectivity at 80°C and 90°C. Comparison of mutant libraries before and after selection with high throughput sequencing will be used to determine which substitutions are permitted and which, if any, stabilize the virus. Impact: Characterizing SSV1 mutants in vitro (Aim 1) and in vivo (Aim 2) will provide insight into fundamental aspects of protein and virus stability including glycosylation. The identification of amino acid substitutions that stabilize or destabilize SSV will help refine the SSV1 structural model. The results obtained in this project could facilitate the creation of highly stable SSV-based nanoparticles, methods to destabilize pathogenic viruses and produce stabilized vaccines. Finally, this project will allow Portland State University (PSU) students to engage in cutting edge biomedical research and strengthen the PSU research environment.

PUBLIC HEALTH RELEVANCE: Sulfolobus Spindle-shaped Virus 1 is an astonishingly stable virus, but we do not know how it or many other viruses are made to be stable. This research will use mutagenesis and artificial evolution to determine how virus proteins fit together to make stable virus particles. These results should allow the creation of highly stable nanoparticles and stabilized vaccines and may provide insights into how to destabilize pathogenic viruses, while strengthening the Portland State University research environment.

**Key Personnel Institution Role**

Ignacio de la Higuera Hernandez Portland State University Research Associate

Kenneth Mark Stedman Portland State University PI

**D 27 Taheri Araghi, Sattar California State University Northridge 1R16GM153656-01**

**Unveiling the Impact of Physical Parameters on Antimicrobial Peptide Efficacy: A Single-Cell to Population-Level Investigation**

Antimicrobial peptides (AMPs) are naturally occurring molecules that have a broad spectrum of activity against bacteria and other pathogens. Although the molecular-level interactions of AMPs with bacterial membranes have been studied extensively, our understanding of the population dynamics of AMPs and their efﬁcacy in large bacterial populations remains limited. This is partly due to the complex electrostatic interactions between AMPs and bacterial cells, which modify the density of AMPs around the target cells and near their membranes. To bridge this knowledge gap, we propose a systematic approach to map out the impact of cell-speciﬁc and culture-speciﬁc physical parameters on the activity of AMPs in bacterial cultures. Our approach will incorporate a range of experimental techniques to collect precise data on the action of AMPs at both single-cell and population levels. By integrating this data with theoretical modeling, our goal is to map out the quantitative physical cues inﬂuencing the action of AMPs within bacterial populations. This project is organized into three speciﬁc aims. In Speciﬁc Aim 1, we will manipulate the physical dimensions of individual Escherichia coli cells to evaluate the impact of single-cell physical parameters on the outcome of AMP action in cell cultures. We will conduct microscopy and population-level assays to determine how cell length, width, and proliferation rate inﬂuence the susceptibility of E. coli cells to AMPs. In Speciﬁc Aim 2, we will evaluate the action of AMPs in E. coli cultures at various cell densities and ionic concentrations. By measuring the minimum-inhibitory-concentration (MIC) of AMPs as a function of cell density and salt concentration, we will demonstrate how population-level and environmental parameters impact the susceptibility of a population to AMPs. In Speciﬁc Aim 3, we will develop a theoretical model based on the principles of statistical physics and electrostatics theory. This model aims to predict the outcome of AMP action within a cell population, based on cells' and cell cultures' physical parameters. The anticipated outcomes of this project will offer a fundamental, quantitative understanding of how AMP behaviors in a population depend on physical parameters of target cells. The resulting theoretical model will further reconcile the stochastic dynamics at the single-cell level to the deterministic dynamics at the population level. Our proposed project is expected to attract a diverse group of students and promote interdisciplinary research opportunities at CSUN that will expand our institutional capacity and offer highly specialized and unique training for our students. The impact of our efforts will be signiﬁcantly attractive to the broader community who are designing and developing effective novel antibiotics. PUBLIC HEALTH RELEVANCE: Bacterial resistance to antibiotics is a growing concern, highlighting the need for new treatments. Antimicrobial peptides (AMPs) are a promising alternative, but their efﬁcacy in large bacterial populations is not fully under- stood. This project aims to elucidate the population dynamics of AMPs by integrating experimental techniques and mathematical modeling to identify the physical parameters that inﬂuence AMP activity in bacterial cultures.

**Key Personnel Institution Role**

Sattar Taheri Araghi California State University, Northridge PI

**C 28 Van Dyke, Michael W Kennesaw State University 1R16GM154191-01**

**Transcription Factor Discovery in the Opportunistic Pathogen Pseudomonas aeruginosa**

Pseudomonas aeruginosa is a gram-negative, aerobic/facultatively anaerobic, rod-shaped bacterium that is commonly found in freshwater environments. However, P. aeruginosa can become an opportunistic pathogen, causing increased morbidity and mortality in immunocompromised hosts, burn victims, and cystic fibrosis patients. For example, more than two million people are infected by P. aeruginosa annually, resulting in almost 90,000 deaths. Notably, the environmental versatility of P. aeruginosa, its multiple mechanisms for providing antibiotic resistance, and its wide range of dynamic defenses (e.g., biofilm formation), render P. aeruginosa a most challenging organism to effectively treat, even in today’s hospital settings. The genes responsible for P. aeruginosa virulence are regulated by a host of sequence-specific transcription factors. Unfortunately, our knowledge regarding their transcriptional regulatory networks and how they can be modulated is woefully incomplete. Our laboratory has developed an iterative selection method, Restriction Endonuclease Protection Selection and Amplification (REPSA), that has proven highly successful in identifying consensus DNA binding sequences and target gene promoters for a variety of transcription factors in bacteria such as Escherichia coli and Thermus thermophilus. We now propose to use REPSA to identify the binding sites and genes regulated by the MerR-family of transcription factors in P. aeruginosa, which are thought to be involved in mediating responses to environmental stimuli, e.g., oxidative stress, heavy metals, and antibiotics, involved in pathogenesis. Ultimately, such studies will illuminate global regulatory networks in this important pathogen, thereby facilitating the development of novel anti-P. aeruginosa therapeutics. Notably, per the goals of the SuRE award mechanism, this project will support the research activities of the PI, a former NIH grant recipient, and will enhance the training opportunities of undergraduate and master’s graduate students at a primarily undergraduate/majority-minority institution, a major focus for the PI. An award will allow the PI to continue a record of training students who have gone on to further biomedical education in graduate or professional schools and/or pursuing careers in STEM industries. It will also allow the PI to apply the skills honed through continuous NSF-funded basic research to questions of direct relevance for human health.

PUBLIC HEALTH RELEVANCE: Pseudomonas aeruginosa is the cause of most hospital-acquired infections, infecting more than two million people annually and resulting in almost 90,000 deaths. Notably, the environmental versatility of P. aeruginosa, its multiple mechanisms for antibiotic resistance, and its wide range of dynamic defenses (e.g., biofilm formation), render it a most challenging organism to effectively treat. Understanding the proteins that regulate these processes (e.g., transcription factors) will illuminate new pathways and targets for the development of anti-P. aeruginosa therapeutics.

**Key Personnel Institution Role**

John Barrows Kennesaw State University Co-Investigator

Michael Van Dyke Kennesaw State University PI

**D 29 Vazquez, Francisco X St. John's University 1R16GM153642-01**

**Multiscale Modeling of Dynamin Allostery and Conformational Changes Related to Membrane Scission**

The final stage of endocytosis involves the recruitment of the protein dynamin to the neck of the vesicle to cut the membrane and release the vesicle to the interior of the cell. Dynamin forms a helical protein coat around the vesicle neck that ultimately cuts lipid membrane. During scission, dynamin undergoes a large conformational change after catalyzing the hydrolysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP). Two mechanisms for dynamin induced membrane fission have been proposed. In the constriction model, the explanation is that hydrolysis leads to constriction of the helical coat, which destabilizes the membrane. In the disassembly model, hydrolysis leads to disassembly of the helical coat, which causes destabilization of the membrane neck. To be able to distinguish between these two models, we believe two key points need to be understood: 1) how does the free energy change from GTP hydrolysis lead to allosteric motion and 2) how is the PH- membrane interaction affected by allosteric motion? If hydrolysis causes allosteric motions that disrupt the PH-membrane interaction, it suggests that membrane fission occurs through disassembly. Otherwise, if the PH domain stays bound to the membrane when the protein undergoes large conformation changes, it suggests that fission likely occurs through the constrictase mechanism. The goal of this work is to develop a multiscale picture of dynamin induced membrane fission in endocytosis using molecular simulation and free energy arguments to understand how the allosteric conformational changes in the protein affect membrane binding. We plan to study allosteric conformational changes in dynamin proteins that involve coupled motions between the different domains; especially between the GTPase domain, which catalyzes guanosine triphosphate (GTP) hydrolysis, and the plekstrin-homology (PH) domain, which controls protein-membrane binding. By comparing all-atom (AA) dynamin dimers, coarse-grained (CG) oligomer models, and mixed quantum/classical (QM/MM) simulations of dynamin-catalyzed GTP hydrolysis, we will be able to create a multiscale picture of how the hydrolysis reaction energy leads to allosteric changes in the protein, and how that energy affects the interaction with the membrane. By comparing the free energy changes of allosteric motions with the PH domain binding strength, we can bring new insight and clarification into the mechanism of dynamin membrane fission.

PUBLIC HEALTH RELEVANCE: After neurotransmission, the cellular machinery required to send the neurotransmitters across the synapse is recycled back into the neuron using endocytosis, where the final stage of endocytosis involves the scission of the membrane vesicle by a helical dynamin protein coat. Two competing models of dynamin induced membrane scission have been proposed, but there is not a clear consensus on the actual mechanism. The goal of this work is to develop a multiscale picture of dynamin induced membrane fission in endocytosis using molecular simulation and free energy arguments to understand how the allosteric conformational changes in the protein affect membrane binding.

**Key Personnel Institution Role**

Francisco X. Vazquez St. John's University, New York PI

**D 30 Wang, Bi-Dar University of Maryland Eastern Shore 1R16GM153688-01**

**Molecular mechanisms of PI3Kδ splice isoform mediated drug resistance in aggressive prostate cancer and solid tumors**

Prostate cancer (PCa) is the most commonly diagnosed and the second leading cause of cancer deaths among American men. Notably, 2.3 higher incidence rate and 1.7 higher mortality rate of PCa were observed in African American (AA) compared to European American (EA) men. Besides the socioeconomic factors, accumulating studies have suggested that intrinsic genetic risk factors may account for, at least, part of the PCa disparities. Alternative splicing is a post-transcriptional process for acquiring proteomic diversity in eukaryotic cells. Emerging evidence has highlighted the critical role of mRNA splicing in cancer development and progression. Previously, our genomic study revealed >2,500 differential splicing (DS) events occurring between AA and EA PCa. RT-PCR validations further confirmed a dozen of oncogene and tumor suppressor genes were undergone DS in AA vs. EA PCa. Among these DS events, we particularly focus on understanding the functional impacts of DS in PIK3CD. PI3Kδ (encoded by PIK3CD) is frequently upregulated in hematologic and solid tumors. Recently, PI3Kδ has emerged as a promising therapeutic target in cancers with hematologic and non-hematologic origins. Idelalisib, an FDA-approved PI3Kδ-specific small molecule inhibitor, exhibits potent efficacies in hematologic cancers. However, resistance to Idelalisib observed in a subset of patients remains a clinical challenge, and the mechanism underlying this treatment resistance remains elusive. In this proposal, we hypothesize that aberrant splicing of PIK3CD may represent one of the genetic mechanisms conferring Idelalisib resistance. PIK3CD-S splice variant, where exon 20 is skipped from the PIK3CD pre-mRNA, appears to be overexpressed in AA PCa compared to EA PCa. Initial cell-free kinase activity assay showed that PI3Kδ-S (encoded by PIK3CD-S) exerts higher kinase activity than the full-length PI3Kδ-L. Also, the PIK3CD-S overexpressing AA PCa cells demonstrated an enhanced resistance to Idelalisib and Wortmannin (a pan-PI3K inhibitor), compared to the PIK3CD-L overexpressing EA PCa. These preliminary data suggested that the PIK3CD-S overexpressing AA PCa may be a useful in-vitro cell model to study the molecular mechanism underlying the splicing-mediated Idelalisib resistance in advanced PCa and solid tumors. Towards this hypothesis, we have performed a series of multi-layer analyses including RT-PCR, immunohistochemistry, western blot assays to examine PIK3CD-L and PIK3CD-S expression profiles at RNA and protein levels, in tissue specimens and cell lines derived from AA and EA PCa. Our preliminary data have, again, confirmed a general higher PIK3CD-S/PIK3CD-L ratio in AA PCa vs. EA PCa. In-silico molecular docking suggested that PI3Kδ/PI3K inhibitors fail to target the ATP pocket of PI3Kδ-S catalytic domain, where the drug binding residues are missing due to skipping of exon 20. Furthermore, ATP/drug competitive binding assays confirmed a lower binding affinity of drug with PI3Kδ-S vs. PI3Kδ-L. Take together, these results strongly suggest that upregulation of PI3Kδ-S contributes to the Idelalisib resistance in AA PCa. Guided by strong preliminary data, we propose to pursue three Specific Aims to elucidate the molecular mechanisms underlying the PI3Kδ-S mediated drug resistance in advanced PCa and solid tumors: 1) Validate the PIK3CD-L and PIK3CD- S expression profiles in a penal of cell lines and patient specimens derived from PCa and other solid tumors, including breast, colon, lung and pancreatic cancers; 2) Elucidate the molecular mechanisms underlying PI3Kδ-S mediated Idelalisib (and other PI3K inhibitors) resistance; and 3) Develop novel therapeutic strategies for reversing aberrant splicing and overcoming drug resistance conferred by PI3Kδ-S in aggressive PCa and solid tumors. Collectively, our proposed research will contribute to the field of AA PCa disparities and Idelalisib resistance by characterization of PIK3CD-L/S expression signatures and deciphering the functional roles of PI3Kδ-S in advanced tumors. It will also allow us to design proteomics and biochemical experiments to explore the molecular mechanisms underlying the PI3Kδ-S mediated drug resistance. The results are expected to facilitate the development of precision prognostic biomarkers and novel molecular therapeutics for advanced PCa, and ideally, all PIK3CD-S expressing cancers. Ultimately, the success of this SuRE proposal will strengthen the research/STEM program at UMES and enhanced PI’s competitivity to pursue a NIH competitive R01 grant.

PUBLIC HEALTH RELEVANCE: Emerging evidence has suggested that aberrant mRNA splicing paly critical roles in cancer aggressiveness and treatment resistance. Previously, we identified that PI3Kδ-S, an aberrant splice isoform of PI3Kδ, exhibits an enhanced oncogenic activity and confers resistance to PI3Kδ/PI3K inhibitors. Our proposed studies aim to understand the molecular mechanisms underlying the PI3Kδ-S-mediated drug resistance in advanced prostate cancer and solid tumors. This research will potentially facilitate the development of a novel prognostic biomarker and therapeutic strategies for treating aggressive prostate cancer and solid tumors.

**Key Personnel Institution Role**

Dr. Bi-Dar Wang Univ of Maryland Eastern Shore PI

**D 31 Wang, Yixuan Albany State University 1R16GM153664-01**

**Comprehensive understanding about the binding of estrogen-related receptor alpha (ERR-α) to inverse agonists with the first-principles based theoretical methods**

ERRα is one of three estrogen-related receptors (ERRs: ERRα,-β and -γ) that act as ligand-dependent transcription factors and share common target genes. Substantial expression of ERRα was found in a few types of breast cancers, and ERRα has thus been identified as a new target for breast cancer therapy. Breast cancers have become the most common cancers diagnosed in the United States and one of the leading cause of cancer deaths in women. Therefore, it is urgent to develop targeted therapy approaches for breast cancer treatment. A few classes of ERRα inverse agonists were reported to inhibit tumor development and progression by disrupting the interaction of ERRα with their coactivators (PGC-1α and PGC-1β). The crystal structure for the complex ERRα and an inverse agonist compound 1 (1-p-tolyl-1H-indol-3-ylmethyl- amine, the most studied inverse agonist) reveals the conformation change as compared with active apo-ERRα in the absence of compound 1, and illustrates the major residues in the ligand binding pocket (LBP). However, the quantitative binding affinities for the key residues with the inverse agonist are unknown, and the binding free energy for ERRα/compound 1 predicted by previous theoretical methods is far from the experimental one. Although the inverse agonist (thiadiazoleacrylamide, XCT790) and a few others also show strong inhibitory effects on the transcriptional activity of ERRα, the crystal structures for the complexes of these inverse agonists with ERRα are still unavailable, which considerably limits an understanding about the mechanism by which these inverse agonists bind to ERRα, and inhibit the coactivation of ERRα and PGC-1α. Overall, the binding mechanism of ERRα to the potential inverse agonists has not been well understood in terms of binding modes and thermodynamics, and the hot spot residues that play an important role in the binding have not been well defined. On the basis of our preliminary study we hypothesized that a) a potential inverse agonist needs to strongly interact with the residues of H3, H5, H6/H7 loop, and H11 helix in the ligand binding domain (LBD) through hydrophobic and hydrogen bonding. b) it is essential for an effective inverse agonist to strongly bind with the aromatic ring cluster consisting of Phe328(H3), Phe495(H11), and Phe382(H5/H6 loop) as well as Leu500. The first-principles-based theoretical methods such as molecular dynamics (MD) simulation and binding affinity calculations are able to provide meaningful insights into the binding mechanism of ERRα to the inverse agonists. Using large scale MD simulation with the updated force fields and a robust binding free energy strategy, the major goal of the project therefore is to gain a comprehensive understanding about the binding between ERRα and a few inverse agonists in terms of binding modes and binding thermodynamics. To test our hypothesis and gain a comprehensive understanding the specific aims are 1) Aim 1. To explore the binding mode and binding free energy of ERRα to inverse agonists with the new force fields and more robust method, alchemical binding free energy in AMBER22. Large scale MD trajectory will be used to analyze the binding modes with 3- D PyMol and 2-D LigPlot visualization software, and to calculate binding affinity with MM/PB(GB)SA approaches. Besides the standard MM/PB(GB)SA calculation for binding affinity, conformation change of unbound ligand and protein will be taken into account through separate MD simulations. Before applying to other inverse agonists, the employed theoretical methods and force fields will be carefully validated by comparing the theoretical results (binding modes, and binding free affinity) with the available experimental data for the binding of ERRα and compound 1. 2) Aim 2. To provide more accurate binding thermodynamics by developing a quantum mechanics based hierarchical approach and using Alchemical binding free energy strategy Predicting protein-ligand binding affinities is a major objective of structure-based drug design. In order to provide more accurate thermodynamics for the binding of ERRα to the inverse agonists, a hierarchical approach will be developed on the basis of the first-principles based theoretical methods such as dispersion-corrected density functional theory (DFT-D) and quantum mechanics/molecular mechanics (QM/MM) like 3-layer ONIOM scheme. DFT-D and QM/MM will provide accurate binding affinity for ligand-residue pairs and ligand-protein, respectively. Accurate binding thermodynamics are essential to identify the hot spot residues for the complexes of ERRα/inverse agonists. 3) Aim 3. To identify hot spot residues of ERRα and the essential (operational) groups of the inverse agonists The validated theoretical methods will be extended to a few more systems such as ERRα/XCT790 and ERR α/compounds 3-4 including large scale MD simulation and binding free energy calculation with new force fields. Accurate binding affinity per residue basis from DFT-D and binding pocket analysis with AlphaSpace will help identify the hot spot residues. The relative binding free affinities for different inverse agonists from Alchemical binding free energy (BFE) will be used to analyze the essential (operational) groups of the inverse agonists, which play the predominant role in the binding of ERRα with the inverse agonists.

PUBLIC HEALTH RELEVANCE: Estrogen-related receptor (ERRα) is a new target to combat a few types of breast cancers. Inverse agonists (anti- cancer drug) can inhibit the activity of ERRα, and reduce the proliferation and migration of cancer cells. The proposal will aim to provide new understanding for eventually developing more efficient inverse agonists.

**Key Personnel Institution Role**

Yixuan Wang Albany State University PI

**D 32 Williamson, Heather Rebecca Xavier University of Louisiana 1R16GM153658-01**

**Examining the thermodynamics of Hemoglobin Compound II auto-reduction**

The goal of this study is to identify the foundational mechanism of the auto-reduction of the high-valent iron species Compound II (Cpd II) in Hemoglobin which results in an oxidatively damaged Hemoglobin molecule. The hypothesis we are testing is that the two subunit types of hemoglobin (α and β) had two different rates for Cpd II auto-reduction based on different mechanistic thermodynamic biases but display similar rate dependencies by pH (proton transfer) and in ratio oxidative products (source of electron transfer). To test this assumption, the mechanism will be evaluated using time-dependent UV-Visible spectroscopy to establish an initial rate dependent temperature profile at all relevant pHs and explore proton and redox dependent affects. The first aim of the project is to isolate each independent rate for the α-subunits and the β-subunits and determine the thermodynamic parameters that affect the mechanism. The second aim of the project is to determine how the proton transfer is affecting the rate for each subunit and the tetramer via isotopic analysis (solvent kinetic isotope effects, temperature dependent isotope effects, and proton inventories). The third aim of the project is to examine how the electron acceptor and donor effect the kinetics and thermodynamics of the mechanism by removing or modifying potential donors, modify the initial acceptor, and exploring thermodynamic features of each site. The final aim of the project is to expand research capacity in the Williamson lab in order to facilitate student research opportunities and extend research training opportunities for postbaccalaureate students interested in biomedical postgraduate research programs. The proposal plans to support 1-2 undergraduate research students per year and 1 postbaccalaureate research technician per year for all 4 years of the grant.

PUBLIC HEALTH RELEVANCE: The relevance of this study is to answer the basic scientific question concerning the thermodynamic behavior of the auto-reduction of high-valent hemoglobin specifically in terms of the electron and proton transfers. This fundamental information is critical to understand why whole blood cell transfusion when stored at cold temperatures has a limited storage life that differs from its behavior in the body which has a vastly different reaction temperature and how these thermodynamic parameters can provide structural and chemical information needed to adapt current hemoglobin-based oxygen carriers (HBOCs) into safe biologically useful artificial blood substitutes. Additionally, this study should help support and develop several underrepresented undergraduate and postgraduate students as biomedical researchers, prepared for a successful future postgraduate research career.

**Key Personnel Institution Role**

Heather R Williamson Xavier University of Louisiana PI

**D 33 Xie, Chloe Yixin Kennesaw State University 1R16GM153635-01**

**Salt bridge database and protein mutation prediction**

Targeting lipid raft membrane domains is a new way for drug design and development for diseases including cancers, infectious diseases (such as HIV, COVID-19), neurodegenerative diseases (such as Alzheimer’s disease), and cardiovascular diseases. The traditional drug usually targets cellular components, including receptors, enzymes, and ion channels, which face two major problems: 1) It leads to off-target effects and unintended toxicity; 2) Cells develop resistance to traditional drugs making them less effective, due to mutations that alter the target of the drug and the signaling pathways. Lipid raft drugs have greater selectivity, reduced toxicity, and higher potential for combination therapy. However, lipid raft drugs are still in the early stages of development, it represents a promising new approach to drug development that offer advantages over traditional drugs. Computational methods have helped drug discovery and disease treatments in recent years, which have been proven to be effective and efficient, but the research for computational prediction tools for binding affinity and key residue analysis remains relatively underexplored. Particularly, salt bridge prediction and key residue analysis can be used to explore unknown drug targets or binding sites in protein-protein interactions (PPIs) in new complex structures of proteins. The objective of this study is to develop a protein-protein interaction analysis package, with the functions of performing potential mutations and evaluating the mutations. This work will reveal the mechanisms of lipid rafts in cancer prevention and treatments. Aim 1: Develop a protein-protein interaction analysis package, which is capable of understanding and revealing biological mechanisms. Aim 2: Perform potential mutations and investigate the binding affinity changes after potential mutations on lipid rafts. Aim 3: Reveal the lipid raft binding mechanisms in cancer cells based on the tools from Aims 1 and 2. This study is innovative in its use of 1) building up a key reissues database; 2) developing a prediction model for key residues; 3) analyzing protein-protein interactions automatically with the development of a protein-protein interaction analysis package; 4) performing and evaluating the potential mutations; 5) revealing the lipid raft mechanisms in cancer cells.

PUBLIC HEALTH RELEVANCE: This study describes the investigation of lipid rafts and its mutations by studying the binding affinities between membrane and target proteins. These microdomains play important roles in cell signaling, membrane trafficking, and cellular processes. Studying lipid rafts mechanisms benefits infectious diseases, neurodegenerative diseases, cardiovascular diseases, and cancer prevention and treatments.

**Key Personnel Institution Role**

Chloe Yixin Xie Kennesaw State University PI