ENYSCASM 2022 Trainee Symposium

Program and Abstracts

MARCH 24-25TH, 2022

EASTERN NY STUDENT CHAPTER OF ASM

2022 Eastern NY ASM Trainee Symposium

The Eastern New York Student Chapter of The American Society for Microbiology (ENYSCASM) proudly presents the free and fully virtual 2022 Trainee Symposium!

The annual ENYSCASM Trainee Symposium provides microbiology trainees (undergraduates, graduates, and post docs) with an opportunity to present their work to a network of peers.

We are eager to highlight trainee's work through two talk sessions and a poster exhibition. All talks and poster presentations will be evaluated by a panel of judges and eligible for awards and prizes. Prizes include monetary awards as well as one-year memberships with the American Society for Microbiology.

This year's symposium also features a career panel highlighting paths outside of academia, a science communication session (co-hosted by Scientista), and ASM distinguished lecturer Georgiana Purdy. See the full schedule below.

Registration: https://www.accelevents.com/e/enyasm
Sessions: https://www.accelevents.com/e/enyasm/portal

If you have any questions on Eastern NY ASM Student Chapter, contact us at: enyscasm@gmail.com

Event Sponsors

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The American Society for Microbiology (ASM) promotes and advances the microbial sciences. This event is co-hosted by Eastern NY ASM student chapter.

The Scientista Foundation is a national organization that empowers pre-professional women in science, technology, engineering, and math.

University Auxiliary Services (UAS) provides a variety of services to enhance campus life for University at Albany students, faculty, staff, alumni and guests.

Prize for Outstanding Student Talks
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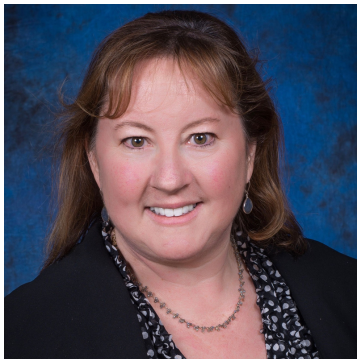
Krackeler Scientific is a leading distributor of products used in life science, pharmaceutical, biomedical, environmental and industrial laboratories.

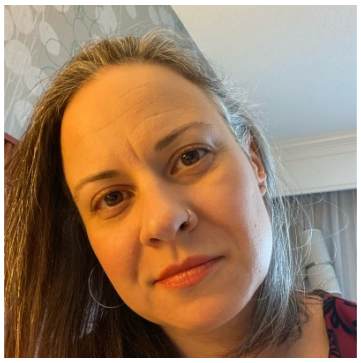
Agenda - March 24th

6:00 PM **CAREERS BEYOND ACADEMIA**

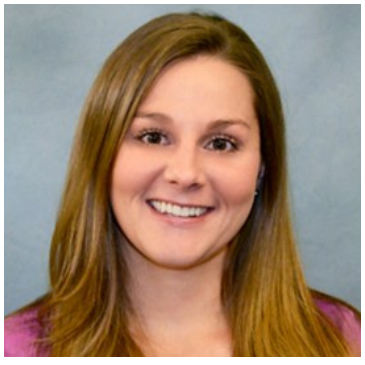
Join us for a panel on alternative careers paths in science as a part of the Annual trainee symposium hosted by The American Society of Microbiology eastern NY student chapter



Kimberlee A. Musser, Ph.D.
Clinical Director,
Wadsworth Center David Axelrod Institute
Chief, Bacterial Disease
Director,
Bacteriology Laboratory



Sarit Lilo, Ph.D.
Associate Project Manager, External Manufacturing, at Regeneron



Stacey Konkle, Ph. D.
Epidemic Intelligence Service (EIS) Officer at Centers for Disease Control and Prevention

Robin Moudy, Ph.D.
Senior Advisor and Team Lead, Emerging Infectious Diseases and Response Policy
Office of Global Affairs
U.S. Department of Health and Human Services

Agenda - March 25th

8:45 **WELCOME**

Dr. Shannon Murphy, ENYSCASM President

9:00 **SELECTED TALKS I:**

HOST DEFENSE & IMMUNOLOGY

Anthony Bui, Cornell University

Jacob Miller, Albany College of Pharmacy & Health Science (ACPHS)

Brittany Geiler, SUNY Upstate Medical

10:00 **POSTER SESSION**

11:00 **SCIENCE COMMUNICATION**

Dr. Maddie Sofia, Science Journalist

12:00 **LUNCH BREAK**

1:00 **SELECTED TALKS II:**

THERAPEUTIC TARGETS & TREATMENTS

Samantha Lindberg, University at Albany

Sydney Herring, University at Buffalo

Alexis Parry, ACPHS

Abdullah Al Hashimi, ACPHS

2:30 **KEYNOTE SPEAKER**

"THE BIG BALL OF WAX"

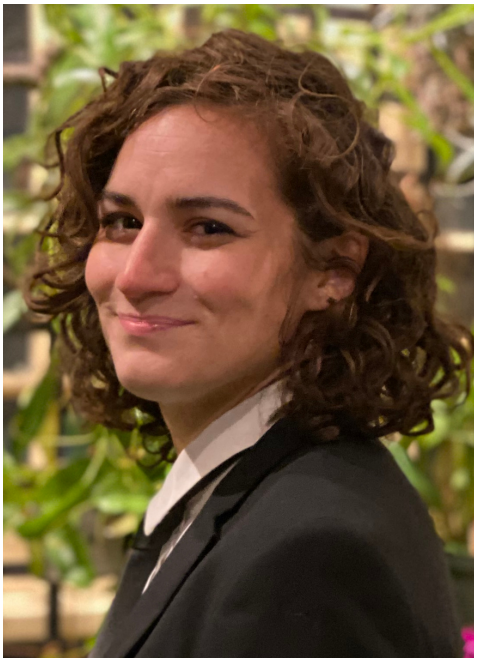
Dr. Georgiana Purdy, Oregon Health & Science University

3:30 **INFORMAL CHAT WITH DR. PURDY**

4:00 **AWARDS CEREMONY & CLOSING**

Dr. Shannon Murphy, ENYSCASM President

Science Communicator: Maddie Sofia, PhD



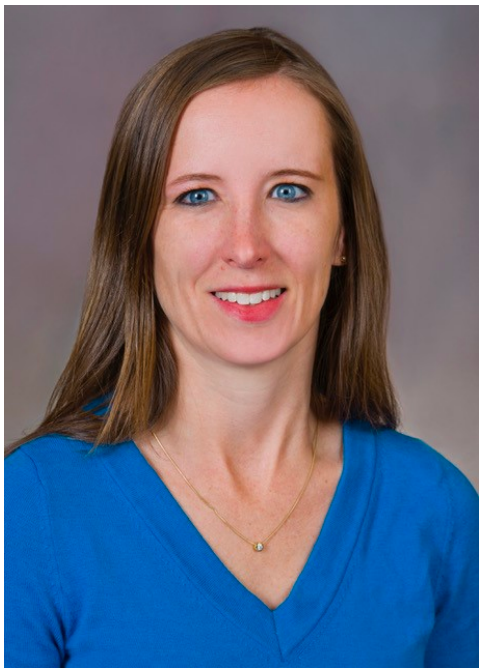
Maddie Sofia, Ph.D. is a freelance science journalist/ communicator and former host of the NPR podcast "Short Wave". Maddie earned her Ph.D. in Microbiology and Immunology from the University of Rochester in 2016. During graduate school, I realized I was more interested in helping people communicate their science than doing experiments in a lab.
Before hosting Short Wave, she hosted the NPR video show "Maddie About Science". She also co-developed the worldwide NPR Scicommers program which supported scientists interested in building their communication skills

The Eastern New York American Society of Microbiology Student Chapter and the Scientista Chapter at the University at Albany is excited to welcome Maddie Sofia to the Symposium. This guest lecture is made possible by the University Auxiliary Services (UAS), which provides a variety of services to enhance campus life for University at Albany students, faculty, staff, alumni and guests.

Maddie Sofia, Ph.D.

11:00 am: Science Communication Lecture

Keynote Speaker: Georgiana Purdy, PhD



Dr. Georgiana Purdy is a Professor of Molecular Microbiology and Immunology at the School of Medicine at Oregon Health & Science University. She is an NIH-funded investigator focused on tuberculosis. The goals of the Purdy Lab are to further define the intrinsic resistance of *Mycobacterium tuberculosis* to the host immune response and antibiotics, delineate pathways in mycobacterial cell wall biogenesis, and identify targets and new strategies for future drug therapy. The lab combines the approaches of bacterial genetics, biochemistry and cell biology to achieve these goals.

Dr. Georgiana Purdy is an
ASM Distinguished Lecturer

2:30 pm: Keynote Address

"The Big Ball of Wax: The Role of MmpL Transporters in Mtb Cell Wall Biosynthesis, Virulence and Non-replicating Persistence"

3:30 pm: Meet the speaker

Keynote Speaker: Georgiana Purdy, PhD

The Big Ball of Wax: The Role of MmpL Transporters in Mtb Cell Wall Biosynthesis, Virulence and Non-replicating Persistence

Mycobacterium tuberculosis (Mtb) is a successful pathogen because it survives within immune cells and effectively establishes and maintains a latent tuberculosis (TB) infection. Therefore, understanding the mechanisms underlying the establishment or maintenance of dormancy can inform new strategies for TB therapeutics. My lab has characterized the function of the Mycobacterial membrane protein large (MmpL) cell wall lipid transporters in pathogenic and non-pathogenic mycobacteria. MmpL proteins are integral to Mtb survival and pathogenesis. In particular, our current focus is on the MmpL3 and MmpL11 transporters. MmpL3 is essential and transports trehalose monomycolate (TMM) to the mycobacterial surface where it is utilized to generate trehalose 6,6'-dimycolate (TDM) and the mycolyl arabinogalactan-peptidoglycan (mAGP). As such, MmpL3 is a validated Mtb drug target. MmpL11 plays a central role in Mtb non-replicating persistence by transport of dormancy-associated "storage lipids", the very long chain triacylglycerol (LCTAG) and mycolate wax ester (MWE). The fate of LCTAG and MWE once it is extracellular is not known. This talk will describe the role of the essential MmpL3 transporter in cell wall biogenesis and show the contribution of MmpL11 to virulence and non-replicating persistence. I will also give some recent insights in to the structure and function of these proteins.

Abstracts

SELECTED TALKS I: HOST DEFENSE & IMMUNOLOGY

Anthony Bui, Cornell University

Structural characterization of non-canonical McrBC restriction complexes provides new insights into nucleotide-specificity

McrBC is a motor-driven endonuclease that functions in prokaryotic defense by cleaving foreign DNA. Canonical family members are comprised of McrB, a DNA-binding AAA+ protein that assembles into asymmetric hexamers, and a partner endonuclease McrC, which cannot bind DNA alone and thus associates with McrB oligomers to form functional restriction complexes. McrB differs from all other AAA+ enzymes in that it specifically binds and hydrolyzes GTP rather than ATP. Moreover, the interaction with McrC stimulates basal GTP hydrolysis, which in turn powers translocation on DNA substrates. Elucidating how McrB specifically recognizes GTP is therefore critical to understanding its mechanochemical activities. Recent cryo-EM structures of E. coli and Thermococcus gammatolerans McrBC complexes showed that guanine nucleotide specificity is achieved using structural elements that lie outside the core AAA+ fold and localize to either the flexible linker connecting the N-terminal DNA-binding domain or the very start of the AAA+ helix α1. Non-canonical McrBC family members like LlaI and BsuMI, however, lack a fused DNA-binding domain and instead are organized into three-component operons that contain a separate site-specific DNA targeting module (R1), a AAA+ motor protein (R2), and an McrC-like nuclease (R3). How these homologs discriminate between different nucleotides remains unknown. Here we present the 1.92-Å crystal structure of Lactococcus lactis LlaI.R2 in complex with GDP and the 3.1-Å cryo-EM structure of Bacillus subtilis BsuMI.R2 in complex with GTPgS. Although these R2 proteins retain all the key catalytic side chains required for nucleotide hydrolysis, they lack structural elements that explicitly provide selectivity for GTP over ATP, XTP, or ITP. Biochemical characterization of nucleotide-dependent R2 oligomerization alone and in the presence of the accompanying R1 proteins supports this inherent promiscuity. Our findings have important implications for the evolution and regulation of McrBC restriction complexes.

Jacob Miller, Albany College of Pharmacy and Health Sciences

The advancement of F. tularensis pathogenesis by oxyR, by means of host ROS scavenging

Francisella tularensis (Ft) is an intracellular gram-negative coccobacillus and the causative agent of tularemia. Ft has documented usage during World War II as a bioweapon and now is considered a potential bioterror agent by the Centers for Disease Control and Prevention (CDC) due to its low infectious dose, ease of dissemination via aerosolization, and high mortality rates. If left untreated, pneumonic tularemia has a fatality rate between 30-60% and for these reasons, the CDC has deemed Ft a Category A Select Bioterror Agent. Previous studies from our lab have shown that Ft evades the innate immune response of cells, such as macrophages and dendritic cells, via suppression of specific proinflammatory pathways that result in increased cytokine production, recruitment of innate immune cells, and bacterial eradication. A critical component of immunity to Ft is the host's cytosolic sensor, Absent in Melanoma 2 (AIM2) that senses dsDNA in the cytosol of infected cells and then assembles a multi-protein complex known as the inflammasome. The activation of inflammasome results in the secretion of bioactive IL-1β and IL-18, which are the key proinflammatory cytokines required to clear Ft infection. Studies have demonstrated that F. tularensis suppresses the activation of AIM2 inflammasome, however, its mechanism is currently unknown. We hypothesized that F. tularensis directly suppresses the AIM2-mediated responses by inhibiting the redox-dependent signaling that concomitantly leads to its priming and activation. To address this hypothesis, an Ft mutant deficient in the global transcriptional regulator of oxidative stress (ΔoxyR) was used and compared to Type B Francisella tularensis spp. holarctica, or the Live Vaccine Strain (LVS) wild-type. OxyR is a master regulator of key antioxidant enzymes of Ft and is also required for maintaining the redox homeostasis in infected macrophages. C57BL/6 BMDMs were infected at an MOI of 50 for 24 hours and then lysed. Their contents were separated through gel electrophoresis and probed through western blot analysis. Our results showed an elevated expression of of key signaling molecules in macrophages infected with the ΔoxyR mutant as compared to its wild-type counterparts. The elevated levels of IL-1β are associated with the activation of Caspase-1 in the ΔoxyR mutant infected macrophages. The expression of IRF1 and GBP2, important signaling components upstream of the AIM2 inflammasome, are also significantly higher in macrophages infected with the ΔoxyR mutant as compared to the wild-type. These results indicate that the redox environment modulated by oxyR of Ft may have a crucial role in the suppression of key signaling components of the AIM2 inflammasome. The ongoing studies are further investigating if the activation of the AIM2 inflammasome is due to the modulation of macrophage redox environment. Collectively, the findings from these studies will aid in extending the knowledge of how Ft-encoded factors subvert the host's innate immune responses.

Brittany Geiler, SUNY Upstate Medical

A Matter of Life and Death: Investigating How Survival is Mediated in HCMV-infected Monocytes

Human cytomegalovirus (HCMV) is highly prevalent in the adult population with seroprevalence of 50-80% in the United States. HCMV infections are associated with severe morbidity and mortality in immunocompromised individuals, such as transplant and chemotherapy patients, as well as the leading infectious cause of permanent neurological sequelae in newborns. Hematogenous viral dissemination of HCMV relies upon the infection of monocytes, which function as vehicles to spread virions to end organ tissues. Biologically, monocytes have a short life span of 48hrs in the circulation that HCMV must circumvent. We have previously shown that HCMV infection promotes monocyte survival to bypass the 48-hour viability checkpoint by blocking the intrinsic biological programing of monocytes to undergo apoptosis, although the mechanisms governing this virally induced anti-apoptotic state remains unclear. We have found that HCMV entry blocks the activation of caspase 8, an initiator caspase of apoptosis. Although HCMV encodes a viral inhibitor of caspase 8 (vICA), this viral protein is not expressed in infected monocytes until differentiation into replication permissive macrophages has occurred. How HCMV blocks caspase 8 activation in infected monocytes is unknown. We found that HCMV stimulates the upregulation of the antiapoptotic cellular protein FLIP (cFLIP) rapidly following infection. Knockdown of cFLIP with siRNAs blocked the ability of HCMV to inhibit procaspase 8 cleavage resulting in the activation of the downstream effector caspase, caspase 3. We further demonstrate HCMV-induced cFLIP binds directly to procaspase 8 to prevent its cleavage and activation. Together, we believe understanding how HCMV interferes with the initiation of apoptosis will assist in identifying novel therapeutic targets designed at eliminating HCMV-infected monocytes to prevent viral dissemination and disease.

Abstracts

SELECTED TALKS II: THERAPEUTIC TARGETS AND TREATMENTS

Samantha Lindberg, University of Albany

Utilizing CRISPRi to Identify Genetic Drivers of Antibody-Mediated Agglutination in Salmonella Typhimurium

Salmonella Typhimurium (STm) is a human pathogen most commonly associated with self-limited infection of the intestinal tract. However, severely invasive and multi-drug resistant isolates of Salmonella have emerged in developing countries and pose a major threat to global public health. Secretory IgA (SIgA) antibodies act as the first line of defense against enteric pathogens like STm, and thus represent an attractive alternative to antibiotics. SIgA antibodies have several key effector functions, including immune exclusion, a process where invading bacteria are agglutinated by the antibodies, encased in mucus, and then cleared from the body via peristalsis. Sal4 is an anti-LPS monoclonal IgA antibody that has shown to confer protection against STm challenge in animal models. In addition, Sal4 rapidly triggers a number of phenotypic and behavioral changes in STm, including motility arrest, inhibition of the Type 3 Secretion System, increased exopolysaccharide production, and bacterial agglutination in vitro. While immune exclusion is an essential protective mechanism in vivo, this process cannot fully explain the phenotypes we have observed in vitro. We are applying a CRISPR interference (CRISPRi)-based library to screen the STm genome for potential genes of interest, with the ultimate goal of identifying the genetic drivers involved in Sal4-mediated agglutination of STm.

Sydney E. Herring, University at Buffalo

Mitochondrial ROS production in Neutrophils is required for host resistance against Streptococcus pneumoniae infection

Streptococcus pneumoniae (pneumococcus) cause serious infections including pneumonia, bacteremia and meningitis resulting in 1.6 million deaths worldwide per year. Polymorphonuclear cells (PMNs) also known as neutrophils, are required for control of S. pneumoniae infection. Neutrophils control bacterial numbers early in infection through their effector functions. These include NETosis, degranulation, phagocytosis, and ROS production. We previously found that PMNs produce both intracellular and extracellular ROS in response to infection, and that ROS was required for optimal antimicrobial function. However, NADPH oxidase activity was not needed for the ability of PMNs to kill pneumococci. In this study, we hypothesized that in PMNs, ROS produced by the mitochondria is what mediates the antimicrobial activity against S. pneumoniae. Using flow cytometry, we found that PMNs produced mitochondrial ROS (mitROS) in response to S. pneumoniae infection. Treatment of PMNs with a mitochondrial ROS scavenger MitoTEMPO resulted in a significant decrease in overall ROS production, but treatment with DPI, an NADPH oxidase inhibitor, had minimal effect on total ROS production. This indicates that the mitochondria is a major source of ROS in S. pneumoniae infected PMNs. We next investigated what host and bacterial factors may be triggering mitROS production. We found that mitROS is produced independent of bacterial capsule or pneumolysin but does require live bacteria for optimal mitROS production. To investigate if this is contact dependent, we used a series of approaches and found that while contact enhances the response, it was not required for mitROS production. As MyD88 is known to regulate mitROS production in macrophages we next investigated the role of MyD88 in PMNs. We found that in MyD88-/- PMNs failed to produce mitROS in response to S. pneumoniae. These findings suggest that released bacterial products acting as TLR ligands are sufficient for inducing mitROS production in PMNs. Finally, we investigated the role of mitROS in host resistance so S. pneumoniae infection. We found that mitROS is required for the ability of PMNs to kill S. pneumoniae in vitro. Importantly, treatment of mice with MitoTEMPO prior to systemic challenge with S. pneumoniae resulted in reduced survival and worsen disease score. In summary, here we have identified a novel role for mitROS production by PMNs in shaping host resistance against S. pneumoniae infection.

Alexis Parry, Albany College of Pharmacy and Health Sciences

Construction of the Bench-Scale Pipeline for Recombinant GFP Production and Purification at ACPHS's Stack Family Center for Biopharmaceutical Education & Training

Green Fluorescent Protein (GFP) is a bioluminescence molecule natively produced by Aequorea victoria that is used extensively in scientific research. Currently, GFP is sustainably produced using recombinant DNA technology. Avirulent laboratory strains of Escherichia coli have been the preferred host organisms for the production of simple recombinant proteins such as GFP, owing to the microbe's rapid replication cycle, wide genome characterization and the ease associated with transforming its genome. The purpose of this work was to construct an upstream and downstream pipeline for recombinant GFP as a model recombinant biopharmaceutical to serve CBET's education and training scopes. Here, recombinant GFP was produced in E. coli K-12 using the T7-promoter-driven pET plasmid. The bacteria were transformed using heat-shock transformation and selected transformants were cultured in Terrific Broth. The transformants were fermented in a 3 L BioFlo 320 Fermenter with a 2.5 L working volume for 24 h, and GFP production was induced with 0.5 mM IPTG at t=6 h. Measurements of optical density at 600 nm and protein content were taken throughout the fermentation run to quantify biomass accumulation and GFP production, respectively. The maximum biomass concentration achieved was 1.6 g/L and the GFP concentration at the end of the fermentation run was 510 mg/L. The presence of GFP was confirmed throughout the production process via visualization under UV light. Downstream processing proceeded with centrifugation of the fermentation broth at 3900 rpm for 15 minutes, cell lysis via lysozyme addition and flash freezing at -80°C, vacuum aspiration with a 0.2 µm PES filter, and TFF with 50 kD and 100 kD filter cut-offs in 1X TE buffer. The crude GPF in 1X PBS was aseptically aspirated and then aseptically dispensed into 20 mL glass vials, which were subsequently finished, resulting in the completion of the pipeline.

Abdullah Al Hashimi, Albany College of Pharmacy and Health Sciences

Combating Antibiotic Resistance in MRSA

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the leading causes of nosocomial infections. Antibiotics such as Cotrimoxazole (SXT) and Ciprofloxacin (CFX) had been increasingly used to treat skin and soft tissue infections caused by (MRSA). However, their therapeutic efficacy is impaired by the formation of resistance due to their extensive use. Antibiotic resistance is linked to the initiation of the general stress response (SOS) caused by DNA damage and oxidative stress leading to mutagenesis. The SOS pathway promotes the activation of recombinant protein A (RecA) making it a good target for therapeutics. In this study, we evaluated Phthalocyanine Tetrasulfonic acid, a RecA inhibitor that blocks antibiotic-induced activation of the SOS response, and Edaravone (EDV), an antioxidant used to treat amyotrophic lateral sclerosis (ALS). Both compounds were tested alone and in combination with pre-existing antibiotics. We examined the survival of the two strains when treated with the antibiotic alone and in combination with the RecA inhibitor or Edaravone. In addition, we determined the emergence of mutants after treatment with the combination drugs. The results elucidated that combining the RecA inhibitor with either SXT or CFX resulted in a higher reduction in colonies forming units per ml after 24 hours of treatment when compared to antibiotics treatment alone. However, EDV appeared to synergize with CFX but not SXT. In summary, the data indicates RecA inhibitor compound is a promising agent in enhancing SXT efficacy and decreasing the emergence of drug resistance during treatment of antibiotics while Edaravone enhances Ciprofloxacin only.

POSTER PRESENTATIONS

Jasmine Uzzell, Albany College of Pharmacy and Health Sciences

Establishing seed train for growth of rest of CHO cells: towards scale-up bioreactor

The COVID-19 pandemic has disrupted the supply chain demand across biomanufacturing. For example, the lead time from ordering cell culture perishable (e.g., cell culture media) and non-perishables (e.g., cell culture shake flasks) are 6 months or more. The current study aimed to establish the characterization of suspension CHO cells in disposable and autoclavable spinner flasks. Specifically, growth characterization and metabolite profiling of a suspension CHO cell line was investigated from day 0 to day 10. Preliminary results showed that the highest viable cell density carried out in disposable spinner flasks was on day 8 at [8 ± 0.12] x10^6 cells/mL with % viability of 92.5 ± 0.12] %. The observation highlighted the peak growth conditions of suspended CHO cells for this knowledge can be implemented in future experiments. Afterwards, the preliminary experiments on characterizing the growth in disposable and autoclavable spinner flasks was compared for four days, showing that on Day 4 the autoclavable glass spinner flask yielded a viable cell density of 2.08 x10^6 cells/mL with 89.6 % viability while the disposable flask yielded 1.28 x10^6 cells/mL viable cell density and 84.6% viability. Cell growth characterization and metabolite profiling are critical for the scale-up of the cells to the bioreactor. Ideally, the CHO cells must be in their exponential phase, demonstrating how healthy and contamination-free they are and exhibit high % viability. Therefore, spinner flask conditions needed to be optimized such that the agitation does not negatively impact the % viability via shear stress to the cells. Our results show that we can successfully grow the suspension CHO cells using disposable and autoclavable spinner flasks. The next step will be scaling up to bioreactor while exploring the reproducibility from these preliminary results. In the future, experiments can be conducted using autoclavable glass spinner flasks along with characterizing scale up in the bioreactor while simultaneously analyzing metabolic profiling.

Julianna Jebaraj, Albany College of Pharmacy adn Health Sciences

Identification and Characterization of Novel Phage, OtterstedtS21

The bacteriophage population is estimated to consist of 10^31 phage particles, yet only 3,000 have been genetically characterized. In collaboration with the University of Pittsburgh's Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science Program, we utilized microbiology lab techniques to isolate phages that infect Gordonia rubripertincta (a gram-positive actinobacteria commonly found in soil) from the environment. We did three rounds of serial dilution to purify the phage and amplified the phage using the webbed plate method. A spot titer was then used to determine the titer of phage particles in the lysate. A full plate titer was also conducted to verify the uniform plaque morphology produced from a single purified phage. The genome was then characterized using a set of restriction enzymes, and the digest pattern was observed utilizing gel electrophoresis. The TEM imaging of the lysate depicted a phage with an icosahedral head and a long noncontractile flexible tail, meaning that the phage that was isolated would be placed in the siphoviridae class. The OtterstedtS21 phage DNA was sequenced, then using Glimmer and Genemark, the phage was auto annotated in DNAMaster. Using Pharmerator, Starterator, NCBI BLASTP, and coding potential data, we manually curated the start of the gene, and determined whether the gene should be included or not. This wet lab research coupled with bioinformatic analysis allowed for the discovery and characterization of the novel phage OtterstedtS21. The identification of these new phages will allow other researchers to target specific bacteria, and even treat patients experiencing health issues due to certain bacteria corresponding with these phages.

Phillip Truong, Albany College of Pharmacy and Health Sciences

Mitochondrial Trafficking During the Hyperglycemic Shift From TNF-α-Induced Apoptosis to Necroptosis

Necroptosis is a pro-inflammatory programmed cell death (PCD) pathway. Unlike apoptosis, necroptosis is caspase-independent and is mediated by the necrosome complex, consisting of receptor-interacting protein kinase 1 and 3 (RIPK1, RIPK3), and mixed lineage kinase domain-like pseudokinase (MLKL). We have previously discovered the hyperglycemic shift from TNF-α-induced apoptosis to necroptosis and wish to further delineate its mechanism. As we noted a central role for mitochondrial reactive oxygen species (mROS) in this shift to necroptosis, we aim to analyze the cellular trafficking of critical cell death factors as part of this mechanism. In this study, we show that RIP1, MLKL, and mitochondrial fission regulator, Drp1 traffic to the mitochondria during the hyperglycemic shift to necroptosis. We also show that this trafficking is primarily driven by ROS in experiments utilizing superoxide dismutase inhibitor, diethyldithiocarbamate (DDC), and antioxidant, N-acetylcysteine (NAC). DDC-induced ROS production led to the inactivation and loss of executioner caspases -3, -6, and -7 in the cytoplasm. Conversely, amounts of RIP1 and MLKL, and their phosphorylated forms increased and localized to the mitochondria following DDC treatment. DDC-induced ROS also led to increased translocation of pro-apoptotic proteins Bax and Bak, and dephosphorylated Drp1 to the mitochondria. Moreover, DDC-induced ROS promoted the oxidization/oligomerization of RIP1, MLKL, Bax, and Bak in the mitochondria. Contrastingly, inhibition of ROS by NAC treatment prevented the trafficking of RIP1, MLKL, and their phosphorylated forms to the mitochondria. NAC-inhibition of ROS also prevented the mitochondrial trafficking of Bax, Bak, and dephosphorylated Drp1. Finally, NAC-inhibition of ROS prevented the oxidization/oligomerization of RIP1, MLKL, Bax, and Bak in the mitochondria.

Ryan Hobson, Albany College of Pharmacy and Health Sciences

Preliminary Steps for Establishing CBETs Pipeline to Produce L-asparaginase using the Eukaryotic Yeast Pichia pastoris

The yeast, Pichia pastoris, seems to be forming the future of recombinant protein production in the biopharmaceutical industry. P. pastoris possess many capabilities that make it an attractive alternative for use in industry over E. coli; fast doubling time of 60-120min, low media complexity/cost, high expression, easily manipulable and last but not least high efficiency for post translational modifications. The ability of P. pastoris to perform PTM's allow proteins to be less immunogenic and more readily available for patients. The Stack Family Center for Biopharmaceutical Education & Training (CBET) see's this potential and envisions developing a pipeline to express L-asparaginase as a simple model biopharmaceutical for CBET's education and training scopes using P. pastoris. L-asparaginase is an anti-tumor therapeutic used to treat acute lymphoblastic leukemia. Using the yeast, it eliminates the immunogenicity encountered with proteins produced from prokaryotes. L-asparaginase is expressed in P. pastoris when induced with methanol when grown in simple media components under extremely regulated N-sources. With these parameters, CBET can expand its bioprocessing capabilities beyond recombinant GFP from E. coli. To establish this pipeline literature was compiled to evaluate media composition, induction agents and fermentation process information. P. pastoris was established in working and frozen cultures scaled up to shake-flask fermentation. Shake-flask fermentation pursuing benchtop bioreactor scale up in BioFlo 320 3L working volume Fermenters.

Alexa Boni, Albany College of Pharmacy and Health Sciences

Production and Analysis of a Monoclonal Antibody Utilizing a Hybridoma Cell Line in Small-Scale Experiments

The Stack family Center for Biopharmaceutical Education and Training (CBET) at ACPHS strives to train a workforce in development of biopharmaceuticals. Establishing a characterized monoclonal antibody-producing cell line was a critical need for the downstream course to begin a pipeline. The development and characterization of a monoclonal antibody-producing cell line are time-consuming and a labor-intensive process. A well-established and characterized monoclonal antibody-producing hybridoma cell line was obtained from ATCC. Hybridoma technology was developed in 1975 by Köhler and Milstein who were awarded the Nobel Prize in Medicine in 1984. Currently, hybridoma technology is employed to produce monoclonal antibodies for drug discovery and used as reagents in drug manufacturing (FDA.org). The current study aims to establish and characterize a monoclonal antibody-producing hybridoma cell line at CBET, utilizing the state-of-the-art equipment made available in the Borisenok Family Foundation Upstream Laboratory. Specifically, growth characteristics, metabolic profiling, and production of monoclonal antibodies from the hybridoma cell line were investigated. The hybridoma cell line (HB-57 cells from ATCC) was first sub-cultured in T-75 flasks using the vendor-recommended media (37° C with 5% CO2). Then Hb-57 were adapted to 250 mL shake flasks at (37° C with 5% CO2, shaken at 125 rpm). However, the vendor recommended media formulation contains fetal bovine serum (FBS). FBS is an additive serum that is highly variable and can contain some harmful proteins to the cells, which would likely hinder growth and give inconsistencies in results (Waters, 2020). The first step of the project was to adapt the cells in serum-free media. Cell growth analysis showed that the cells were growing efficiently in serum-free media in shake flasks with a viable cell density of [1.02± 0.28]x106 cells/mL on day 3. In addition, metabolic profiling demonstrated that as the viable cell density was increasing, the cells glucose depleted from 3.89 g/L to 1.47 g/L, while lactate was produced from 0.14 g/L to 1.97 g/L. The monoclonal antibody titer was analyzed using immunoturbidimetric methods (Cedex Metabolite Analyzer, Roche) and results showed that in serum-free media, the cells were able to volumetrically produce 64.07 mg/L of antibody with a cell-specific productivity of 21.7 pg/cell/day. The FDA guidance documents for “Monoclonal Antibodies Used as Reagents in Drug Manufacturing” recommend various tests to characterize the monoclonal antibodies, including, reducing and non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern. Next, we analyzed the recovered the monoclonal antibody using SDS-PAGE under reducing and non-reducing conditions. As expected, results showed that when the monoclonal antibody was reduced, there were two bands representing the heavy and light chains of the antibody at 50 kDa and 25 kDa, respectively. While the nonreduced sample showed a band at 150 kDa, indicating the whole antibody was intact and no disulfide bonds broken. In summary, our results showed that we can successfully culture and grow the hybridoma cells at CBET, recover the monoclonal antibodies, and analyze using Cedex (quantitative) and SDS-PAGE analysis (qualitative). However, the SDS-PAGE also demonstrated that the product contains uncharacterized proteinaceous impurities. Future experiments will focus on developing, optimizing the purification of mABs from hybridoma cells, further characterization of residual process-related impurities, and establishing the short-term stability conditions of the monoclonal antibodies.

Vibert Putra, Albany College of Pharmacy and Health Sciences

Alternative Treatment Options against Carbapenem-Resistant Acinetobacter baumannii Infections

Within the last few decades, Acinetobacter baumannii has been declared a global threat due to its exceptional ability to attain or upregulate its antimicrobial resistance. This Gram-negative nosocomial pathogen has the propensity to persist within the healthcare environment and has been attributed to various forms of hospital-acquired infections: ventilator-associated pneumonia, skin and soft tissue infections, catheter-associated urinary tract infection and bacteraemia. Successful treatments have become exceedingly difficult with increasing reports of Ambler Class D (OXA-type) carbapenemases along with hyperproduction of the Ambler Class C Acinetobacter derived cephalosporinases (ADCs) in multidrug-resistant strains. Hence, new treatment strategies are needed to effectively treat CRAB infections. This project attempts to utilise a clinically available non-antibiotic combined with antibiotics as a novel strategy. We propose that novel combination treatment strategies will reduce CRAB bacterial burden and improve patients' outcome. Thioredoxin A (TrxA) is an electron-recycling enzyme in the thioredoxin antioxidant system that restores protein structures damaged by oxidative stress and regulates downstream effector proteins. TrxA is activated by reduced thioredoxin reductase (TrxR). We hypothesise that the thioredoxin system in A. baumannii modulates bacterial resistance responses to carbapenems. Using broth microdilution chequerboard assay and 24-hour static concentration time kill assays, inhibition of thioredoxin system using auranofin (FDA-approved rheumatoid arthritis drug that inhibits TrxR) in clinical isolates has shown to increase their susceptibility to meropenem. Additionally, disc diffusion assay showed that auranofin has additive effect against clinical isolates with various classes of antibiotics. Deletion mutant appears to have a significant increase in antioxidant activity than the wild type when treated with sub-inhibitory concentrations of meropenem. Thus, other antioxidant mechanisms are independent of thioredoxin A, and their activities appear to be upregulated in the presence of stressor. Therefore, increased sensitivity to antibiotics is not attributed to their loss of antioxidant activities. This suggests thioredoxin-related regulation of antibiotic resistance mechanisms. Using modified disc diffusion assay with non-specific pump inhibitor (CCCP)-infused plates, it was apparent that tetracycline and colistin efflux pumps were regulated by the thioredoxin system but not meropenem resistance-related pumps. Lastly, nitrocefin assay showed increased total β-lactamase activity when treated with sub-minimal inhibitory concentration of auranofin. This was unexpected as increased in total β-lactamase activity would result in greater resistance against meropenem. Interestingly, meropenem MIC of the wild type did not change after 24-hour incubation with auranofin despite this apparent increase in total β-lactamase activity. In closing, targeting the thioredoxin system showed great potential in re-sensitising CRABs towards various classes of antibiotics. More studies need to be done to elucidate the mechanisms in which the thioredoxin system is related to CRAB's resistance mechanisms.

Audrey DeGraw, Albany College of Pharmacy and Health Sciences

The Discovery of the Bacteriophage Patos

Research conducted at Albany College of Pharmacy and Health Sciences resulted in the discovery of a new actinobacteriophage, Patos. The SEA-PHAGES program was administered in collaboration with the University of Pittsburgh to discover the new actinobacteriophage. Soil samples were collected from Lincoln Park in Albany, New York, which is where the phage Patos was found. Using microbiology lab techniques, we isolated the phage, then Patos was sent off to get the genome analyzed at the University of Pittsburgh. These lab techniques involved three rounds of serial dilutions and plaque assays to purify the phage. Webbed plates to increase phage titer and a TEM were used to recover images of the phage to determine its morphology. Based on the TEM image, Patos was found to be Siphoviridae, this means that it has a long non-contracting tail. The phage titer was calculated to be 1.53 E12. Patos DNA genome was sequenced, and then, using DNA Master, Glimmer, and GeneMark, the genome was auto annotated. A total of 97 genes were manually annotated. This included finding the purpose of each gene in the genome from function, gaps, and coding potential. Through annotating the genome, Patos genes were added, extended, and deleted. This allowed us to compare Patos with other phages of the same cluster and morphology.

Vincent Fazzari, Albany College of Pharmacy and Health Sciences

Understanding the Mechanism by which Sulforaphane Promotes SAMHD1 Activation to Protect Macrophages Against HIV Infection

Sulforaphane (SFN), a natural compound found in cruciferous vegetables, has been shown to block HIV infection of macrophages, a critical immune cell, through the upregulation of the transcription factor Nrf2. Unpublished work has shown that SFN causes the absence of an inhibitory phosphate from the antiviral protein SAMHD1, thus activating it. In this work we show that SAMHD1 is the major driver of SFN mediated protection of macrophages from HIV. We further show that Nrf2 is important for SAMHD1 dephosphorylation (activation) and exclude two potential mechanisms by which SFN could enable SAMHD1 dephosphorylation. Specifically we exclude a possible role for the cell cycle control protein p21 in promoting dephosphorylation. Additionally, we show that intracellular distribution of SAMHD1, which harbors a nuclear localization sequence, is not affected by SFN. Combined, these findings establish the importance of SAMHD1 in SFN/Nrf2 mediated protection from HIV and the narrow down the list of possible mechanisms by which SFN can activate SAMHD1.

Gaston Jofre Rodriguez, University of North Carolina at Chapel Hill

How to define species in Histoplasma

The most dominant concept to delimit species boundaries is the Biological Species Concept (BSC). Groups of individuals can be perceived as discrete groups of genetically differentiated gene pools if they maintain reproductive isolation (RI). However, the BSC does not apply to multiple organisms, including pathogenic fungi. The globally widespread pathogenic fungus Histoplasma capsulatum (commonly treated as a singular species) maintains most of the life cycle in an asexual state with infrequent sexual reproduction. Laboratory cross experiments to detect and measure genetic differentiation in the genus Histoplasma cannot be implemented. However, the advent of genome data allows to measure and detect genetic differentiation to delimit species boundaries. In this poster I will demonstrate four approaches that leverage whole genome sequences to delimit species boundaries in multiple populations of Histoplasma collected worldwide. Specifically, I will show how a population collected from India shows strong signatures of genetic differentiation from other Histoplasma populations.

Anisha Paudel, Albany College of Pharmacy and Health Sciences

Sulforaphane inhibits the reactivation of HIV-1 in latently infected cells

Background: Despite the massive research efforts and development of cART (combined Anti Retro-viral Therapy) strategies, complete eradication of HIV is still not achieved and remains a challenging task. cART has shown promising effects in reducing the new cycles of infection and lowering the viral load below the detection threshold along with the partial restoration of the immune system, but it is inefficient in providing the lifelong cure. The cessation of cART leads to the quick rebound of viremia within a few weeks. HIV remains incurable due to its ability to exist as an undetectable latent or silent reservoir in different tissues that are insensitive to antiretroviral therapies moreover the latent cells remain invisible to the immune clearance. So far, efforts to completely eradicate the latent reservoir have been a difficult and unsuccessful task. New approach “Block and Lock” is the more realistic approach to control the latently infected reservoir and to achieve a functional cure. This aims to permanently silence the latent reservoir using the latency-promoting agents (LPAs) to block the virus transcription and lock the virus promoter to keep it in the latent phase via epigenetic modifications. Recent reports have suggested Sulforaphane, an inducer of Nrf-2 (nuclear erythroid 2-related factor) mediated antioxidative signaling, to have an additional anti-HIV property by restricting HIV replication at early stages. However, the effect of sulforaphane on the expression of integrated provirus is still elusive. We propose SFN may act as Latency Promoting Agent (LPA) due to its anti-HIV activity.

Method: The potential of inducing Nrf2 signaling in promoting latency using latently infected monocytic (THP89GFP and U1) as well as T cell lines (J89GFP) was tested. We reactivated the cells using TNFα in the presence and absence of Sulforaphane. RT-qPCR assays were used to measure viral copy numbers in the supernatant as well as cell-associated HIV transcripts. ELISAs were performed to measure p24 levels. Finally, cell death analysis was performed by staining the cells with Annexin V and 7-AAD.

Result: We observed that Sulforaphane treatments significantly reduced the TNFα induced reactivation in all the tested cell lines. Viral RNA copy numbers released in the supernatant as well as HIV transcription initiation and elongation specific transcripts were significantly diminished. Cell-associated HIV p24 levels were also decreased by Sulforaphane treatments. Thus, our study present exciting opportunities to develop a novel latency-promoting agent that can be used in achieving functional cure by the ‘block and lock’ approach.