27th Annual Molecular Parasitology & Vector Biology Symposium

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Program

8:30 AM  Registration and Poster Set-Up
9:00 AM  Opening Remarks: Dennis Kyle, Director of CTEGD
9:10 AM  Beatrice L. Colon, Dept. of Infectious Diseases, UGA
          Discovery and Evaluation of New Drugs for the Treatment of Primary Amoebic Meningoencephalitis
9:30 AM  Molly Savadelis, Dept. of Infectious Diseases, UGA
          Assessment of Parasitological and Clinical Findings in Heartworm-Infected Beagles Treated with Advantage Multi and Doxycycline
9:50 AM  Edward L. D’Antonio, Dept. of Natural Sciences, University of South Carolina Beaufort
          Discovery of Novel Trypanosoma cruzi Glucokinase Inhibitors with Biological Activity by High-Throughput Screening
10:10 AM Break — Poster Viewing
10:50 AM Emily M. Carpinone, Dept. of Microbiology, UGA
          Identification of Putative Secreted Proteins of the Endosymbiont Wolbachia pipiens Using the Surrogate Eukaryotic Host, Saccharomyces cerevisiae
11:10 AM Satyanarayana Lagisetty, EPIC and Genetics & Biochemistry Department, Clemson University
          Acetate as a Potential Alternative Carbon Source During Cryptococcus (Eukaryotic) Pathogenesis
11:30 AM Yi H. Yan, Institute of Bioinformatics, UGA
          Multi-omic Analysis of Severity of Infection in Macaca mulatta Infected with Plasmodium cynomolgi
11:50 AM Carly Dameron, Dept. of Biological Sciences, Clemson University
          Disruption of the de novo Heme Biosynthesis Pathway of Toxoplasma gondii Leads to Parasites with Growth Defects and Decreased Virulence
12:10 PM Lunch — Poster Viewing
1:30 PM  Mary J. MacLean, CTEGD and Dept. of Infectious Diseases, UGA
          Out of the Abdomens of Gerbils: the Effects of Anthelmintic Drug Treatment on B. malayi in Vivo
1:50 PM  Teresa Cruz-Bustos, Center for Tropical & Emerging Global Diseases, UGA
          Inducible Knockdown of Trypanosoma cruzi Gene Expression Using the glmS Ribozyme
2:10 PM  Karla M. Márquez Nogueras, Center for Tropical & Emerging Global Diseases, UGA
          The Role of Calmodulin-Like Proteins in Toxoplasma gondii Calcium-Signaling
2:30 PM  Break
3:00 PM  Adam Sateriale, Center for Tropical & Emerging Global Diseases, UGA
          A Natural Mouse Model for Cryptosporidiosis
3:20 PM  Ash Pathak, CTEGD and Dept. of Infectious Diseases, UGA
          Cryo-Preserved RBCs Support Plasmodium falciparum Development and Transmission to Mosquitoes: Present and Future Prospects
3:40 PM  Introduction of the Keynote Speaker
3:45 PM  Rick Fairhurst, M.D., Ph.D., NIAID Malaria Pathogenesis and Human Immunity Unit
          Pleraquine Resistance and Dihydroartemisinin-Pleraquine Treatment Failure in Cambodia
4:50 PM  Adjourn
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Oral Presentations

Discovery and Evaluation of New Drugs for the Treatment of Primary Amoebic Meningoencephalitis

Beatrice L. Colon¹, Christopher A. Rice², Abdelbasset A. Farahat³, David W. Boykin³, R. Kiplin Guy⁴ & Dennis E. Kyle²

¹Morsani College of Medicine, University of South Florida, Tampa, Florida ²College of Public Health, University of South Florida, Tampa, Florida ³Department of Chemistry, Georgia State University, Atlanta, Georgia ⁴St Jude Children’s Research Hospital, Memphis, Tennessee

Primary amoebic meningoencephalitis (PAM) is a disease with a 97% fatality rate that is caused by the free-living amoeba, Naegleria fowleri. These amoebae are ubiquitously found in the environment. Disease occurs when the amoebae enter the nasal cavity and make their way to the frontal lobes of the brain where they cause significant pathology. New drugs are urgently needed to treat this usually fatal infection, so we established high-throughput assays to identify compounds that inhibit N. fowleri within 72 hours. In this study, we screened diverse libraries of compounds to identify new leads or drugs that could be repurposed. These included a library of >3000 bioactive compounds and FDA-approved drugs in addition to the 400 diverse compounds in the MMV Pathogen Box. We screened in single point assays at 5 uM and then derived quantitative dose-response data to validate hits. From both collections we identified multiple compounds that produce IC50s < 1 uM. Interestingly, posaconazole was a potent hit in both compound collections. Next, we validated new methods to assess the rate of action of the hits and to determine if their activity was static or cidal. The rate of action assay demonstrated multiple amidino derivatives that significantly inhibit growth inhibition within 8-12 hours of drug exposure; remarkably, this was approximately 48 hours faster than the current treatments. In addition, we found posaconazole to be cidal within 24 hours and roxithromycin and ketoconazole to be cidal after 48 and 72 hours of drug exposure, respectively. These data demonstrate potential for new drugs optimized from amidino scaffolds and that drug repurposing may be possible for this fatal, neglected tropical disease.

Assessment of Parasitological and Clinical Findings in Heartworm-Infected Beagles Treated with Advantage Multi and Doxycycline

Molly Savadelis¹, Michael Dzimianski¹, Amanda Coleman¹, Gregg Rapoport¹, Ajay Sharma¹, Cameon Ohmes², Joe Hostetler², Andrew Moorhead¹

¹University of Georgia College of Veterinary Medicine ²Bayer HealthCare, Animal Health Division, Shawnee, KS

Anecdotal reports state that the adulticidal heartworm treatment utilizing doxycycline and Advantage Multi® has successfully converted antigen-positive dogs to antigen-negative. To date, no experimental studies have demonstrated the adulticidal efficacy of this treatment regimen. The aim of this study was to evaluate the parasitological and clinical effects of Advantage Multi® (10% imidacloprid, 2.5% moxidectin) and doxycycline on heartworm-infected beagles. This study utilized 16 dogs, 8 dogs in each of control and treated groups. A total of 16 adult Dirofilaria immitis (Missouri strain) were surgically transplanted into the jugular vein of all study dogs. The treatment regimen of monthly Advantage Multi® topically for 10 months and 10 mg/kg doxycycline BID orally for 30 days was initiated 30 days post-surgical transplant. Echocardiograms, radiographs, complete blood counts, clinical chemistry profiles, heartworm antigenemia, and microfilariaemia were evaluated every 4 weeks. All dogs tested positive for the presence of heartworm antigen post-surgical transplant and prior to treatment. Control dogs have remained antigen-positive and 7 of the 8 have detectable microfilariae to date. No microfilariae in treated dogs were detected after 21 days post-treatment. Heartworm antigen levels began declining in treated dogs 3 months post-treatment. Serum samples were tested for heartworm antigen using the DiroCHEK® heartworm antigen test kit. Radiographs of dogs depict right heart enlargement and main pulmonary artery enlargement. Echocardiograms were unable to detect adults as early as 3 months post-treatment in 3 treated dogs. This treatment regimen demonstrated a 95.9% efficacy in eliminating adult heartworms. Preliminary study data indicates that this treatment regimen successfully eliminates D. immitis microfilariae by 21 days post-treatment, reduces heartworm antigen concentration over time, and successfully eliminates adult heartworms. This treatment regimen is an appropriate treatment option for heartworm disease when the approved adulticidal therapy is not an option.
Discovery of Novel *Trypanosoma cruzi* Glucokinase Inhibitors with Biological Activity by High-Throughput Screening

Edward L. D’Antonio1,a,* Gustavo F. Mercaldi2,a, Ana Rodriguez3 & Artur T. Cordeiro2
1Department of Natural Sciences, University of South Carolina Beaufort, 1University Boulevard, Bluffton, South Carolina 29909, United States of America 2Brazilian Biosciences National Laboratory, Brazilian Center for Research in Energy and Materials, Campinas, SP, Brazil 3Department of Microbiology, New York University, School of Medicine, 341 East 25th Street, New York, New York 10016, United States of America

*aAuthors made an equal contribution to the work. *To whom correspondence should be addressed. Telephone: (843) 208-8101; Email: edantonio@uscb.edu

Chagas’ disease is a neglected tropical disease caused by the protozoan parasite *Trypanosoma cruzi* and affects approximately 6 – 7 million people in Latin America. The available treatments for Chagas’ disease, such as benznidazole and nifurtimox, are problematic because they are intolerable from adverse side effects and present toxicity concerns. Furthermore, studies have found benznidazole resistance in *T. cruzi* and alternative options are therefore in high demand. Our laboratory has an interest in finding different targets in *T. cruzi* and we have made an effort with *T. cruzi* glucokinase (*TcGlcK*). *TcGlcK* is found at the nodal point for two essential metabolic pathways, including glycolysis and the pentose phosphate pathway. This enzyme allows for the phosphorylation of D-glucose with co-substrate ATP to yield glucose-6-phosphate. Inhibition of this drug-target leads to apoptosis. The goal of the study was to have *TcGlcK* proceed into a small target-based high-throughput screening (HTS) campaign of ~13,000 molecules from a diverse chemical library to identify new classes of potent inhibitors. Our successful HTS campaign resulted in the discovery of various novel and potent *TcGlcK* inhibitor leads that were also confirmed to be bioactive compounds through cell-based in vitro studies.

Identification of Putative Secreted Proteins of the Endosymbiont *Wolbachia pipiensis* Using the Surrogate Eukaryotic Host, *Saccharomyces cerevisiae*

Emily M. Carpinone1 & Vincent J. Starai1,2
Departments of Microbiology and Infectious Diseases University of Georgia, Athens, GA

*Brugia malayi* (*Bm*) is a pathogenic nematode that is the causative agent of lymphatic filariasis in humans. Its pathogenesis requires the presence of the Gram-negative bacterial endosymbiont, *Wolbachia pipiensis* (*Wb*), which survives inside of the cells constituting the nematode lateral chord. Treatment of lymphatic filariasis with antibiotics that kill *Wb* provides positive clinical outcomes nearly identical to those seen in cases where solely antihelminthics are used to clear the nematode. Our understanding of *Wb* invasion, replication, and persistence within *Bm* is therefore of great importance to the development of new targeted treatments for nematode infections, and yet, our current molecular knowledge of *Wb* is limited due to the fact that *Wb* is unable to be grown in pure culture. Through the use of *Saccharomyces cerevisiae*, a well defined surrogate eukaryotic “host,” we have developed a rapid screening system to identify putative secreted proteins which may alter eukaryotic cell biology, and can further characterize these proteins to understand the *Wolbachia*:host relationship. Here, we show – through toxicity studies and analysis of vacuole morphology – that of the forty-seven genes thought to encode for putative secreted effectors from *Wb*, fourteen induce abnormal cellular physiologies upon expression in yeast. Though the mechanism of activity remains unknown for these proteins, our data show that some proteins clearly alter eukaryotic membrane trafficking and actin dynamics pathways, which likely play a vital role in the *Wolbachia*:host relationship. We aim to characterize the activity of these proteins with further molecular and biochemical techniques in an effort to elucidate the primary proteinaceous players in the *Wolbachia*:host relationship, and define additional protein targets for the treatment of lymphatic filariasis.
Acetate as a Potential Alternative Carbon Source During *Cryptococcus* (Eukaryotic) Pathogenesis

Satyanarayana Lagishetty¹,² & Kerry Smith¹,²

¹Eukaryotic Pathogen Innovative Center ²Genetics and Biochemistry Department, Clemson University, Clemson, South Carolina 29631

*Cryptococcus neoformans* is an invasive opportunistic pathogen of the central nervous system and the most frequent cause of fungal meningitis infecting one million people worldwide and killing approximately 625,000 per year (CDC report, 2009). Gluconeogenesis plays crucial role to generate ATP molecules and crucial metabolites from non-glucose source like two carbon acetate molecules. *Cryptococcus neoformans* undergoes drastic metabolic shifts in absence and/or low glucose environment, specially during *Cryptococcus* pathogenesis. Even though, various genes involved in acetate utilization/production has been reported, the complete pathway from extra-intra cellular and vise-versa of acetate utilization is not reported, yet. We are trying to identify, understand and analyze the various virulent metabolic pathways, mitochondrial ATP generation of Acetate utilization chaperones and overall kinase signaling mechanism connecting these pathways. Some of our important results: As XFP-ACK pathway is essential, *Cryptococcus* has two isomers of Cn-XFP1 and Cn-XFP2. Our structural-functional studies have conformed that Cn-XFP1 activity is regulated by phosphorylated through Cyclic AMP dependent Protein Kinase A (CAMP-PKA). Whereas Cn-XFP2 undergoes allosteric regulation of inhibition by ADP and, glycolytic and TCA cycle metabolites like PEP and OAA (Glenn, Ingram-Smith et al. 2014). Role of mitochondrial respiratory complex II/succinate dehydrogenase acetate utilization chaperones, ACN9 during ATP generation. Overall our goal is to understand the structural-functional relationship of *Cryptococcus* virulence proteins/enzymes and further identify and designing potential inhibitors, specially to cyclic AMP-PKA regulatory and catalytic subunits of this basidiomycete fungal pathogen (Choi, Vogl et al. 2012). Choi, J., et al. (2012). “Regulated expression of cyclic AMP-dependent protein kinase A reveals an influence on cell size and the secretion of virulence factors in *Cryptococcus neoformans*.” Mol Microbiol 85(4): 700-715.Glenn, K., et al. (2014). “Biochemical and kinetic characterization of xylulose 5-phosphate/fructose 6-phosphate phosphoketolase 2 (Xfp2) from *Cryptococcus neoformans*.” Eukaryot Cell 13(5): 657-663.

Multi-omic Analysis of Severity of Infection in *Macaca mulatta* Infected with *Plasmodium cynomolgi*

Yi H. Yan¹, Elizabeth D. Trippe¹, Diego M. Moncada², Mary R. Galinski³, Alberto Moreno³, Jessica C. Kissinger¹, Rabindra Tirouvanziam³, Juan B. Gutierrez¹, & the MaHPIC Consortium

¹University of Georgia, ²Universidad del Quindio (Colombia), ³Emory University

Severe anemia accounts for a large portion of the complications associated with malaria. This phenomenon is multifactorial, and the mechanisms that cause it remain elusive. A multi-omic approach comprised of immunology, transcriptomics, metabolomics, lipidomics, and proteomics was used to study five *M. mulatta* infected with *P. cynomolgi*. This experiment resulted in one death, two severe infections, and two mild infections. Particularly, we discovered that TLR3 and RIG-I pathways in macrophages and dendritic cells, conventionally linked to host antiviral responses, are upregulated in severe malaria. These pathways cross-talk, and their interaction had not been clearly implicated before in severe malaria. Associated with the activation of these pathways are changes in the effector response proteins IL1, AP1, MX1, MX2, OAS1-3, and PML. Our analysis provides novel insight into the molecular and cellular basis for the development of severe malaria. This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases; National Institutes of Health, Department of Health and Human Services [Contract No. HHSN2722012000031C] and the National Center for Research Resources [ORIP/OD P51OD011132].
Disruption of the de novo Heme Biosynthesis Pathway of *Toxoplasma gondii* Leads to Parasites with Growth Defects and Decreased Virulence

Carly Dameron1 & Zhicheng Dou1

1Clemson University

*Toxoplasma gondii* is an Apicomplexan parasite that may cause severe infectious disease in humans, with the ability to use foodborne, zoonotic, and congenital routes of transmission. As an intracellular parasite, *Toxoplasma* must acquire nutrients from host cells to survive and replicate. Heme is an essential nutrient in all organisms, serving as a prosthetic group found on many mitochondrial proteins. *Toxoplasma* encodes all the enzymes in the de novo heme biosynthesis pathway, suggesting that these parasites can synthesize this essential nutrient for themselves. In this study, the gene for the cytosolic coproporphyrinogen III oxidase enzyme was deleted in *Toxoplasma gondii*, generating a parasite strain with a disrupted heme synthesis pathway. TgCPOX-null mutants show replication defects, as well as decreased virulence in a murine model. However, the replication defect was not caused by an upregulation of phosphorylated eukaryotic initiation factor 2's alpha subunit, a classic indicator of heme deficiency. These findings suggest that *Toxoplasma* parasites may use an alternative mechanism to regulate protein translation to slow down parasite growth during heme deficiency, and the parasites may scavenge heme from host cells to supplement their own de novo pathway. This study plans to further assess the role of the de novo heme synthesis pathway in *Toxoplasma* infection.

Out of the Abdomens of Gerbils: the Effects of Anthelmintic Drug Treatment on *B. malayi* in vivo

Mary J. Maclean1,2, Walter W. Lorenz3, Christopher J. Anna4, Michael T. Dzimianski2, & Adrian J. Wolstenholme1,2

1CTEGD, University of Georgia, 2Department of Infectious Diseases, University of Georgia, 3Institute of Bioinformatics, University of Georgia, 4Perthera, McLean, VA

Lymphatic filariasis (LF) threatens nearly 20% of the world’s population and has handicapped one-third of the 120 million people currently infected. Current control and eventual elimination of LF rely on mass drug administration (MDA) programs with three drugs: ivermectin (IVM), albendazole (ALB), and diethylcarbamazine (DEC). Only the mechanism of action of albendazole is well-understood. In vitro, the amount of IVM required to impair parasite motility (IC50 = ~6µM) is over 100 times the peak plasma concentration following administration of a dose clearing microfilariae (Mf) from the bloodstream of patients, and DEC has no effect on Mf motility in vitro. These findings suggest that the rapid clearance of Mf observed after treatment with IVM or DEC is aided by the host immune system and does not simply result from the paralysis of the parasites. To gain a better insight into antifilarial drug action, we treated gerbils with patent *B. malayi* infections with DEC, IVM, or ALB mirroring the doses used in human MDA programs. Adults and Mf were collected 1 and 7 days post-treatment for RNA isolation and transcriptomic analysis. A total of 189 differentially expressed genes (DEG) were identified across drug treatment groups and parasite life stages. Nine DEG were chosen for validation with qPCR, and all agreed with the up or downregulation predicted by bioinformatic analysis. Among the identified DEG were those related to cell adhesion, proteolysis, and protein modification that are being explored to understand how these drugs work against filarial parasites.
Inducible Knockdown of *Trypanosoma cruzi* Gene Expression Using the glmS Ribozyme

Teresa Cruz-Bustos & Roberto Docampo
Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA

Methods for genetic manipulation of *Trypanosoma cruzi*, the etiologic agent of Chagas disease, remain relatively limited. Here we described a new strategy to manipulate *T. cruzi* using a ribozyme to create an inducible system to knockdown the expression of genes. This technique regulates the post-transcriptional level of mRNA using a ribozyme inserted in the C-terminal position of the targeted gene. The glmS ribozyme is the first known example of a natural ribozyme that has evolved to require binding of an exogenous small molecule for activity. We co-transfected a specific 3’ end-sgRNA/Cas9/pTREX construct with a specific DNA donor molecule for the C-terminal region of the gene of interest with an HA tag and the ribozyme glmS amplified from the pMOTag vector. The utility of the method was established by manipulating the gene encoding GP72, which is required for flagellar attachment. Parasites were grown in a modified SDM-79 medium without glucosamine, and after selection we confirmed the correct incorporation by PCR and sequencing, and the flagellar localization of GP72 using a polyclonal antibody against GP72 (Wic29.26). However, our results indicate that the system was already induced probably by an endogenous production of glucosamine-6-phosphate (GlcN6P), the ligand of the ribozyme. GP72 knockdown parasites showed the characteristic phenotype with detachment of the flagellum from the cell body and decreased motility. Western blot analysis showed lack of expression of GP72. The results suggest that further modification of the culture medium could improve the chances of getting an inducible system for gene expression in *T. cruzi*.

The Role of Calmodulin-Like Proteins in *Toxoplasma gondii* Calcium-Signaling

Karla M. Márquez Nogueras¹ & Silvia N. Moreno²
¹CTEGD, University of Georgia, Department of Microbiology ²CTEGD, University of Georgia, Department of Cellular Biology

*Toxoplasma gondii* is an Apicomplexan parasite that infects one third of the world’s population. The pathology of toxoplasmosis originates from its lytic cycle, which starts when the parasite invades a host cell, replicates inside a parasitophorous vacuole and finally egresses to find another host cell to invade. Calcium signaling in *Toxoplasma* regulates the activation/expression of effectors that decode into biological functions involved in the lytic cycle. An important source of calcium that triggers signaling is by influx from the extracellular environment. Our laboratory has shown Ca^{2+} influx, its regulation and its enhancing effect of each of the steps of the lytic cycle of *Toxoplasma*. Calcium-signaling domains have been described in mammalian cells as protein complexes that regulate calcium entry. Our hypothesis is that these Ca^{2+} channels at the plasma membrane are part of signaling domains, which are essential in activating the steps of the lytic cycle. Calcium-binding proteins, like calmodulins or calmodulin-like form part and play a role in activating other components of these signaling domains. These calcium-binding proteins would localize to these specific domains as well as other players like kinases, phosphatases and calcium channels. Essential Light Chain (ELC) 1 and ELC2 both Ca^{2+}-binding proteins, have been shown to localize to the glideosome, a protein complex involved in motility and we think that both proteins bind Ca^{2+}. Using CRISPR-Cas9 we generated single gene knockouts of these proteins and we were able to detect defects in calcium entry of the generated mutants. We propose that these calmodulin-like proteins have a secondary role in the regulation of calcium entry in addition to activation of the glideosome.
A Natural Mouse Model for Cryptosporidiosis

Adam Sateriale¹, Jan Slapeta², Rodrigo Baptista¹, Jessica Kissinger¹, Carrie Brooks¹, Gillian Herbert¹, Ravi Pulusu¹, & Boris Striepen¹

¹CTEGD, University of Georgia ²Veterinary Parasitology, University of Sydney

Cryptosporidiosis is a leading cause of diarrhea and an important contributor to global infant mortality. There are no efficacious drugs or vaccines available and our knowledge of Cryptosporidium biology to drive their development is scant. Cryptosporidium research is greatly hindered by the lack of a continuous tissue culture system and poor animal models. To develop a more facile mouse model of Cryptosporidium infection, we have isolated a strain of Cryptosporidium tyzzeri (C. parvum mouse genotype I) from naturally infected Mus domesticus; strains which we now maintain continuously in our animal facility. De novo assembly of the C. tyzzeri genome shows 96% overall nucleotide identity to C. parvum and hominis and a high degree of synteny. The highest burden of C. tyzzeri infection is found in the distal ileum of the mouse small intestine, yet there is also significant infection of the jejunum and duodenum, similar to what is seen in human cryptosporidiosis. Perhaps of greatest importance, C. tyzzeri produces significant infections in healthy C57/BL6 mice. These infections produce high parasite burden but are self-limiting, and mice that have cleared C. tyzzeri appear resistant to future infection. Using CRISPR directed homology repair we have genetically engineered C. tyzzeri strains to express reporter genes for in vivo imaging and localization. In summary, we now have access to a natural mouse model that closely resembles the human infection in which both host and parasite are genetically tractable. We envision this model will lead to better understanding of cryptosporidiosis susceptibility, resolution, and subsequent protection in the context of a functioning immune system.

Cry-Preserved RBCs Support Plasmodium falciparum Development and Transmission to Mosquitoes: Present and Future Prospects

Ash Pathak & Courtney Murdock

CTEGD and the Dept of Infectious Diseases, University of Georgia, Athens, GA

Human RBCs are the singular point-of-contact between Plasmodium falciparum, the etiological agent of the most lethal form of human malaria, and mosquitoes of the Anopheles species. In addition to serving as a substrate for the entire life cycle of P. falciparum, human RBCs are the sole source of nutrients for mosquitoes to generate progeny and maintain its gene pool. Starting with invasion of the RBC by a young merozoite, P. falciparum is destined to either proliferate asexually to generate more merozoites, or differentiate into sexually dimorphic pre-gametes tasked with establishing infection in mosquito midguts. While the asexual cycle is a relatively short 48 hours, differentiation into mature male and female gametocytes takes 10–12 days. These dynamics are largely mirrored in ex vivo cultures of the parasite in primary human RBCs. However, the long duration of gametocytogenesis necessitates the use of relatively fresh RBCs as prolonged storage of RBCs at 4°C is associated with often irreversible metabolic and/or bio-mechanical “lesions” which may not be conducive to parasite proliferation or differentiation. In the current study, we demonstrate that cryogenically preserved RBCs support asexual as well as sexually dimorphic stages of P. falciparum NF54. Moreover, the resulting gametocytes are fertile and are able to establish infections in the midguts and salivary glands of Anopheles stephensi mosquitoes when introduced via standard membrane-feeding assays. Finally, we show that cryo-preserved RBCs support highly fecund mosquitoes, at rates similar to, or better than RBCs stored at 4°C. In addition to the material benefits in terms of cost-savings, we envision broad applications for the approach. For instance, it is especially promising for long-term storage of rare blood-types and/or blood from individuals that may not be consistently available. This blood could then be tested for its relative ability to support parasite growth, in conjunction with mosquito life-history traits of fecundity and longevity and the cumulative effects on vectorial capacity.
**P1. Dual Role of the Vacuolar H\(^+\)-ATPase as a Guardian Against Ion Stress and Maturation of Secretory Proteins**

Andrew J. Stasic\(^1\,^2\), Stephen A. Vella\(^1\,^2\), Eric J. Dykes\(^2\), Vincent J. Starai\(^1\) & Silvia NJ Moreno\(^2\)

\(^1\)Department of Microbiology, University of Georgia, Athens, GA 30602-7400, USA
\(^2\)Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA 30602-7400, USA

Toxoplasma gondii is an obligate intracellular parasite of great medical significance. While searching for a host cell, the parasite is exposed to an environment with an ionic composition quite different from the host intracellular composition. T. gondii possesses a lysosomal-like compartment termed plant-like vacuole (or PLV) that appears to be involved in ionic homeostasis. Proteomic analysis of an enriched PLV fraction showed the presence of homologous subunits of the vacuolar-H\(^+\)-ATPase (V-H\(^+\)-ATPase), an evolutionarily conserved proton pumping complex. We inserted a hemagglutinin (HA) tag at the endogenous locus of the T. gondii a1, E, and G subunit genes. IFA analysis showed that the V-H\(^+\)-ATPase localizes to the plasma membrane and the PLV. We have created a VH\(\text{ha1}\) conditional knockout strain, which shows significant defects in motility, egress, growth/replication, and invasion/attachment. Biochemical function of VH\(\text{ha1}\) was validated by complementing VH\(\text{ha1}\) yeast mutants deficient in a subunits and by monitoring internal pH recovery in T. gondii parasites after stress with propionic acid. The V-H\(^+\)-ATPase is important for maintaining membrane potential and responsible for pumping protons across the plasma membrane. The V-H\(^+\)-ATPase also plays a role in the secretion and maturation of microneme proteins and the maturation of cathepsin L protease. The acidification role of the V-H\(^+\)-ATPase is important for controlling the levels of cytosolic Ca\(^{2+}\). The function of the pump at the plasma membrane and/or the PLV could contribute to calcium homeostasis. Extracellular calcium enters parasites in a highly regulated manner and parasites only allow for nM levels in their cytoplasm. This is accomplished by a pump at the plasma membrane, that pumps calcium out and by a highly sensitive and active SERCA localized to the endoplasmic reticulum. When the activity of the V-H\(^+\)-ATPase is downregulated the cytosol of these parasites show an uncontrolled increase in Ca\(^{2+}\) levels. This could be because they are unable to pump Ca\(^{2+}\) out or because the intracellular storage organelles are less efficient at controlling cytosolic calcium levels. In summary, our data indicates that the V-H\(^+\)-ATPase is involved in the acidification of an intracellular compartment (likely the PLV), which uses the generated proton gradient to transport calcium into the organelle. The microneme maturation defect of the mutants could be the result of the defective acidification of the compartment where maturation of microneme happens or because of a general unhealthy parasite intoxicated by Ca\(^{2+}\) overload.

**P2. Dual-Guide CRISPR/Cas9 Construction to Knock out Toxoplasma gondii Glycogenes**

Izzy Serji, Travis Ichikawa, & Christopher West

Biochemistry and Molecular Biology, University of Georgia

The genes involved in glycan synthesis in Toxoplasma gondii have yet to be identified. Thus, the roughly forty predicted biosynthetic glycogenes were disrupted by genome editing and then studied to uncover their biochemical and biological functions. This process is done by using dual-guide CRISPR/Cas9 as a guide RNA-directed nuclease mechanism to cut parasite DNA in two places. Through NHEJ, a new piece of DNA that encodes a drug resistance marker can also be inserted into the break, which will disrupt the gene and allow for selection of genetically modified parasites. Disruption of the DNA will be confirmed via Polymerase Chain Reaction (PCR). Once established, the mutant strains are analyzed using glycomic-profiling methods. The long-term goal is to identify enzyme targets to direct new drugs against Toxoplasmosis.
**P3. Characterization of a Phospholipase C-Like Protein (TbPI-PLC2) from \textit{Trypanosoma brucei}**

Núria W. Negrão¹, Sharon King-Keller¹, Guozhong Huang¹, & Roberto Docampo¹
¹CTEGD

\textit{Trypanosoma brucei} is the causative agent of African Trypanosomiasis, a deadly disease affecting humans and cattle. There are very few drugs to treat the disease and evidence of mounting resistance raises the need for new drug development. The inositol 1,4,5 triphosphate/diacylglycerol (IP3/DAG) pathway regulates important processes in many organisms. \textit{T. brucei} has an active IP3 receptor, localized to the acidocalcisome, that is essential for infection in mice. In previous work (King-Keller et al., \textit{Eukaryot. Cell} 14:486-494, 2015) we characterized a phosphoinositide phospholipase C (TbPI-PLC1, Tb927.11.5970) from \textit{T. brucei} that contains a domain organization characteristic of PI-PLCs, such as X and Y catalytic domains, an EF-hand calcium-binding motif, and a C2 domain, but lacks a pleckstrin homology (PH) domain. In addition, TbPI-PLC1 contains an N-terminal myristoylation consensus sequence only found in trypanosomatid PI-PLCs. Here we report the presence of a second PI-PLC-like protein (TbPI-PLC2, Tb927.6.2090) that is very similar to TbPI-PLC1 but lacks the Y catalytic domain and the C2 domain and possesses instead a PDZ domain. Recombinant TbPI-PLC2 hydrolizes neither phosphatidylinositol (PI) nor phosphatidylinositol 4,5-bisphosphate (PIP2), and does not modulate TbPI-PLC1 activity. However, knockdown of TbPI-PLC2 expression alone or together with downregulation of TbPI-PLC1 expression by RNAi resulted in growth inhibition. This is in contrast with the lack of effect of downregulation of expression of TbPI-PLC1 alone. TbPI-PLC2 has a plasma membrane and intracellular localization and it might be involved in IP3 binding as has been reported for the phospholipase C-related catalytically inactive protein 1 (PRIP-1) of mammalian cells (Uji et al., \textit{Life Sci} 72:443-453, 2002). The PDZ domain could be involved in this binding and this is being investigated.

**P4. Blood Stream Form \textit{Trypanosoma brucei} Regulates Intracellular Glucose Levels in a Density Dependent Manner**

Yijian Qiuᵃ, Charles Voytonᵇ, Kenneth A. Christensenᵇ, & James C. Morrisᵃ
ᵃEukaryotic Pathogens Innovation Center, Department of Genetics and Biochemistry, Clemson University, Clemson SC 29634 ᵇDepartment of Chemistry and Biochemistry, Brigham Young University, Provo UT 84604

The African trypanosome, \textit{Trypanosoma brucei}, has evolved mechanisms to communicate with the environment and regulate metabolic responses to reflect the differences in available nutrients in the mammalian host and tsetse fly vector. In the mammal, the blood stream form (BSF) depends on glycolysis to generate ATP. Two families of glucose transporters, trypanosome hexose transporter 1 (THT1) and THT2, have been described and members of each family have been subject to studies scoring kinetic behavior and gene expression patterns. Several important questions remain unresolved. First, localization studies in \textit{Leishmania} suggest flagellar and surface localization of glucose transporters. It is unknown if the African trypanosome THTs are similarly distributed. Second, the THT1 and 2 families have multiple members, and the role of these additional THT members is unknown. Third, while it is known that the THT1s are predominant in BSF parasites while THT2 are thought to be insect stage transporters, changes in the expression of these in response to environmental glucose has not been considered in BSF parasites. Last, it remains unresolved how the regulation of the different THTs is connected to environmental cues and intracellular glucose, and the feedback from those inputs. Here we describe the localization of the \textit{T. brucei} THTs, assessment of expression of all of the family members, and explore the kinetics of glucose uptake by the transporters under different conditions using a glucose-sensitive probe in live parasites. Our findings suggest that BSF modulate intracellular glucose levels in a cell density-dependent fashion that is likely through altered THT expression patterns, a mechanism that points at a sophisticated regulatory mechanism.
P5. Calcium Signaling and Motility of Toxoplasma gondii
Stephen Vella1, Christina Moore1, Mojtaba Fazli2, Shannon Quinn2, & Silvia Moreno1
1CTEGD, University of Georgia 2Department of Computer Science, University of Georgia

*Toxoplasma gondii*, a global pathogen, is the causative agent of disseminated toxoplasmosis. Calcium signaling, governed by fluctuations in cytosolic calcium (Ca^{2+}) concentrations, is utilized universally and regulates many cellular processes. For a successful lytic cycle, *T. gondii* must traverse biological barriers and invade host cells; and as such, motility is critical for virulence. When a *T. gondii* tachyzoite exits from its host cell, it is exposed to an enormous Ca^{2+} concentration gradient (~20,000-fold) (100nM in the host cytosol, vs. ~1.8mM in the extracellular space). Previous studies in Apicomplexa have shown that Ca^{2+} signals are decoded to modulate invasion, egress, and gliding motility, but the exact mechanism(s) involved are unknown. Within a host cell parasites are stationary and non-motile, and Ca^{2+} levels appear stable. Activation via an unknown agonist induces a Ca^{2+} signaling pathway, resulting in Ca^{2+} oscillations that ultimately stimulate motility and egress. We constructed parasites expressing the Genetically Encoded Calcium Indicator, GCAMP6f, which allowed us to study Ca^{2+} dynamics in real time. We found a direct correlation between Ca^{2+} peak intensity with both distance and duration traveled by motile parasites. Ca^{2+} influx played an important role in enhancing these features and also in altering the motility pattern (drifting to gliding). To investigate the mechanism and the threshold of Ca^{2+} required for the stimulation of motility we developed an algorithm to track parasites and quantify Ca^{2+} signals simultaneously. We studied the role of Ca^{2+} entry vs. Ca^{2+} release from intracellular stores and with respect to motility. Our project seeks to address the direct relationship between cytosolic Ca^{2+} oscillations and motility and its impact in each step of its lytic cycle.

P6. Investigating the Effects of Bordetella bronchiseptica Putative Effector Proteins BB1629 and BB1639 on Yeast Growth
Nathalie Carla Thezan1 & Vincent J. Starai1
University of Georgia, Department of Microbiology, Athens, GA

*Bordetella bronchiseptica* is a pathogenic proteobacterium that colonizes the respiratory tract and causes chronic respiratory infection in small animals. *B. bronchiseptica* infects many non-human mammals, causing atrophic rhinitis in pigs, kennel cough in dogs, snuffles in rabbits and bronchopneumonia in guinea pigs. *B. bronchiseptica* is related to *Bordetella pertussis*, the human pathogen for whooping cough. Although the major hosts of *B. bronchiseptica* include non-human mammals, preliminary studies have examined the potential of *B. bronchiseptica* to act as a human pathogen and cause pertussis-like symptoms. It is known that *B. bronchiseptica* uses a type III secretion apparatus to deliver effector proteins directly into the host, which play critical roles in bacterial intracellular persistence, virulence, and in the development of disease states in the host. While the molecular targets and activities of these effectors remain largely unknown, a deeper understanding of these effector proteins will provide essential insight into the molecular mechanisms of *Bordetella pathogenesis*. The budding yeast *Saccharomyces cerevisiae* has long been used as an effective surrogate eukaryotic host cell, as it shares critical physiological pathways with many higher eukaryotes. Therefore, candidate *B. bronchiseptica* effector protein genes (BB1629 & BB1639) were cloned into yeast expression vectors to analyze the effects of individual *B. bronchiseptica* proteins on yeast physiology. Understanding the mechanisms by which BB1629 and BB1639 may alter yeast physiology can help elucidate host-pathogen interactions and may lead to the development of novel *Bordetella* treatments.
P7. Characterization of an Essential Secreted Effector Binding Protein of *Toxoplasma gondii*

Menna Etheridge, Brent Jenkins, Grant Butschek & R. Drew Etheridge
Department of Cellular Biology and CTEGD, University of Georgia

One of the most conserved features of Apicomplexan parasite biology is the presence of an elaborate endomembrane and secretion system, which includes the endoplasmic reticulum, Golgi, inner membrane complex and three morphologically distinct secretory organelles: micronemes, rhoptries, and dense granules (DG). These secretory organelles are of central importance in Apicomplexans, as they are the source of the parasite’s ability to move, invade host cells, form their parasitophorous vacuole (PV), and inject effector proteins to manipulate host cellular processes. In *Toxoplasma gondii*, the highly regulated secretion of micronemes and rhoptries is essential for parasite motility, invasion, and PV formation whereas the constitutively secreted DG’s have only recently emerged as an important reservoir for host-modulating effectors. A central question remains as to how *T. gondii* effectively sorts DG proteins to generate these distinct secretory organelles? Despite an expanded endomembrane and secretion system, Apicomplexans have not increased their repertoire of identifiable trafficking proteins. The only cargo receptor identified in *T. gondii*, to date, is responsible for the sorting of proteins destined for microneme and rhoptry organelles. In our interaction studies, we have identified an essential hypothetical protein of *Toxoplasma* that binds to the DG secreted effector TgIST. We have successfully TY-epitope tagged this secreted effector binding protein, hereafter referred to as SEB1, and demonstrated that it is processed and localized to the parasite Golgi apparatus. Our IP/mass-spectrometry experiments have shown that SEB1 interacts with proteins of the basal secretory machinery, multiple previously characterized secreted DG proteins (including TgIST) and a host of previously uncharacterized hypothetical proteins. We successfully epitope-tagged and assessed the localization of several of these hypothetical proteins and demonstrated that they are new members of the parasite’s secretome. Due to its Golgi localization and predicted type I transmembrane topology we hypothesize that SEB1 functions as the long sought after cargo receptor for DG secretory organelles.

P8. Adapting the TIR-Auxin Conditional System to Study Essential Genes in *Cryptosporidium*

Mattie C Pawlowic1, Emily K Myers1, Caleb Studstill1, Gillian T Herbert1, Carrie F Brooks1, & Boris Striepen12
1CTEGD, University of Georgia, Athens, GA 2Department of Cellular Biology, University of Georgia, Athens, GA

*Cryptosporidium* is the second leading cause of diarrheal disease in children, and also causes disease in immunocompromised adults. There are no vaccines available, and the only drug approved to treat cryptosporidiosis is not effective in immunocompromised patients—the group that needs drug therapy the most. Lack of continuous culture and other technologies have hindered our understanding of this important pathogen. Recent development of genetic tools and an animal model allow us for the first time to directly dissect gene function. One particular area of interest is the structure and composition of the oocyst wall. The oocyst is the transmitted form of the parasite, and is the product of the sexual stage of the life cycle. Infection with *Cryptosporidium* occurs upon ingestion of food or water contaminated with oocysts. Oocysts are hardy, resilient shells protecting the parasite from most disinfectants, including common water treatment methods i.e. chlorination. Little is known about how the oocyst is constructed and why it is so resilient. We are using genetic tools to interrogate candidate genes and describe their functional role in oocyst wall formation and *Cryptosporidium* transmission. Genes predicted to function in oocyst development have proven refractory to knockout, necessitating a conditional gene expression system. Additionally the expression of two candidate genes, polyketide synthase (PKS) and trehalose-6-phosphate synthase (T6PS), was localized to the females, the life cycle stage responsible for synthesis of the oocyst. Here we report progress towards adapting the TIR-Auxin system to control protein stability in *Cryptosporidium* with the goal of understanding the role of PKS and T6PS in oocyst synthesis and development.

Tequila S. Porter1, Rachel N. Martini, Brittany D. Jenkins, & Melissa B. Davis1,2

University of Georgia, PREP@UGA, NIH

One of the leading causes of death among American women is breast cancer. African-American women are 25% more likely to develop breast cancer, and 50% more likely to die from this disease. This could be due to the fact that this group disproportionally presents with the most aggressive subtype of breast cancer, triple negative breast cancer (TNBC), which is defined by the lack of estrogen receptors (ER), progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2) expression on the surface of the tumor. Currently, there is no single subtyping marker that has been identified to predict tumor immunogenicity in African-American women with breast cancer. However, there is one possible marker that is a chemokine receptor with ancestry-specific alleles, the Duffy Antigen Receptor for Chemokines (DARC). We know genetically, there are two isoforms of DARC, whose proteins differ in their extracellular binding regions. Since DARC is able to bind two structurally distinct classes of chemokines, an atypical feature of a chemokine receptor, it is possible that each isoform binds a distinct structural chemokine class. In addition to the two isoforms, DARC also harbors numerous ancestry-specific mutations, one of which is fixed in some African populations, and is phenotypically known as Duffy-Null. The objective of this work is to determine any functional differences between the DARC isoforms and their ligands in individuals who are Duffy-Null compared to those who are not by completing analyses in a panel of ancestry-specific cell lines. Answering these questions will aid in the formation of novel therapies using DARC as a target, to help improve clinical treatments and outcomes for patients with aggressive breast cancers.

P10. Bacterial Complex is Required for Apicoplast Function in Malaria Parasites

Anat Florentin1,2, David W Cobb1, Jillian D Fishburn1, Paul S Kim2, Manuel A Fierro1, Vasant Muralidharan1,2

1Department of Cellular Biology 2Center for Tropical and Emerging Global Diseases University of Georgia, Athens, Georgia, United States of America

Apicomplexan parasites such as Plasmodium falciparum, the causative agent of malaria, contain a non-photosynthetic plastid known as the apicoplast that functions to produce essential metabolic compounds. It was previously reported that several members of the prokaryotic Clp family of chaperones and proteases localize to the apicoplast. In bacteria and in chloroplasts these proteins form complexes that degrade proteins in a proteasome-like manner to regulate key cellular processes, but their function in the apicoplast is completely unknown. In this study, we generated conditional mutants of the P. falciparum apicoplast-targeted pfclpc and pfclpp genes and found that under normal conditions they localize to the apicoplast. Conditional inhibition of the PfClpC chaperone results in growth arrest, morphological defects, and apicoplast breakdown. In a series of cellular assays, we showed that it is required for sorting of functional apicoplast into daughter cells. Addition of IPP, an essential apicoplast metabolite, completely restores mutants growth, indicating that the only essential function of PfClpC is linked to the apicoplast. Conditional inhibition of the PfClpP protease did not produce the expected phenotype due to incomplete protein knockdown. However, using a double-mutant pfclpc; pfclpp parasite line, we were able to show that PfClpC activity is required for the processing of the PfClpP protease into its active form, suggesting a functional interaction between the two. We combine these genetic studies with a chemical biology approach, by testing a variety of small molecules inhibitors that were developed for the inhibition of bacterial Clp homologs. This study elucidates the molecular mechanism of Plasmodium Clp proteins, and place them, similar to their bacterial homologs, as potential drug targets.
P11. Prediction and Validation of CD8+ T Cell Epitopes from the Flagellar Proteins of *Trypanosoma cruzi*

Molly Bunkofske¹ & Rick Tarleton²
¹,²CTEGD, University of Georgia

Cytotoxic CD8+ T cells are critical for immune control of *Trypanosoma cruzi* infection but are heavily skewed toward recognition of epitopes encoded by the highly variable trans-sialidase family of genes, making them less than ideal effectors. Our lab has recently shown that during host cell invasion, *T. cruzi* trypomastigotes discard their flagella as part of the conversion to amastigotes, releasing flagellar proteins into the cytosol. At least one of these conserved flagellar proteins contains several CD8+ T cell epitopes recognized by infected mice. In this study, we used an immunoinformatic approach to predict additional epitopes within known flagellar proteins and tested these peptides for recognition by CD8+ T cells from *T. cruzi* infected mice. For each of the 21 most abundant flagellar proteins, 5-10 peptides were selected for synthesis based upon predicted binding to class I MHC: H-2Kb or H-2Db. The immunogenicity of these peptides was assessed by the ability of lymphocytes from mice with chronic *T. cruzi* infections to produce IFN-γ in response to peptide stimulation. Responses to approximately 40 epitopes from 10 different flagellar proteins were detected, with varying magnitudes. Peptides from proteins Par1, Par2, GB4, Faz1, and FS179 were well-recognized, eliciting strong responses, while proteins PFC16, Gim5a, Kap3, and FCaBP elicited less robust responses. Despite high abundance in the flagellum, none of the tested peptides from Par3, Calreticulin, and PFC1 proteins elicited responses during *T. cruzi* infection. Future studies will assess the role that recognition of this newly discovered and extensive set of highly conserved “early” antigens plays in control of *T. cruzi* infection and how such responses might be enhanced.

P12. Ablation of Chloroquine Resistance Transporter (TgCRT) in *Toxoplasma gondii* Yields Decreased Invasion

Paige Teehan¹ & Zhicheng Dou¹
¹Department of Biological Sciences, Clemson University, Clemson, SC 29634-0314

Chloroquine resistance transporter (CRT) mutations have been shown to facilitate the expulsion of chloroquine from *Plasmodium falciparum* digestive vacuoles. This resistance has made chloroquine ineffective as an antimalarial drug. *Toxoplasma gondii* expresses an ortholog of CRT, named TgCRT, associated with a lysosome-like digestive organelle known as the vacuolar compartment or plant-like vacuole (VAC/PLV), indicating that the VAC/PLV is an equivalent digestive vacuole structure in *Toxoplasma* parasites. TgCRT-null mutants displayed the VAC/PLV swollen approximately 20-fold larger in volume than that in wild type parasites. In addition, the ablation of the TgCRT gene resulted in a reduction of parasite invasion and secretion of microneme proteins Mic2 and M2AP. This study plans to evaluate the changes in pH within the VAC/PLV of TgCRT-null mutants and the maturation of cathepsin L-like protease (CPL) in relation to the secretion of microneme proteins.
P13. Zinc Transporters in Trypanosoma cruzi: Gene Complementation in Saccharomyces cerevisiae, and CRISPR/Cas9-Dependent Knockout and Endogenous Tagging
Smriti Hoda, Teresa Cruz Bustos, Nathan M. Chasen, & Roberto Docampo
NIH-PREP and Center or Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30602

Zinc is an essential element whose concentration must be tightly regulated because its deficiency or accumulation can lead to severe defects including cell death. The homeostasis of zinc within eukaryotic cells is controlled by two main transporters: ZnT and ZIP. In Trypanosoma cruzi, a protozoan parasite responsible for Chagas disease, three transporters named TcZIP30, TcZnT50, and TcZIP30/50 were identified as highly up-regulated in the intracellular form of the parasite (amastigote). Our goal is to investigate whether these up-regulated transporters play a crucial role in zinc homeostasis in these intracellular parasites. To functionally validate these transporters we are complementing mutant Saccharomyces cerevisiae deficient in zinc transport. To investigate the localization and function of these transporters we are applying the CRISPR/Cas9 technique that we recently developed in our laboratory to do endogenous tagging and knockout of the respective genes. These studies will reveal the essentiality of these transporters and whether they could be used as potential targets for drug development against Chagas disease.

P14. Modeling Hotspots of Schistosomiasis in Mozambique with Machine Learning
Joseph W. Walker1,2 & John M. Drake1,2
1Odum School of Ecology, University of Georgia 2Center for Ecology of Infectious Diseases, University of Georgia

Schistosomiasis is a water-borne parasitic disease caused by flatworms of the genus Schistosoma. These parasites infect over 200 million people each year, mostly children living in rural Africa. Although Schistosomiasis is usually not lethal, chronic infection can stunt physical and cognitive development and hinder productivity at school and work. In 2010, SCORE* began conducting longitudinal studies in Tanzania, Niger, Kenya and Mozambique on the efficacy of Mass Drug Administration (MDA) for controlling the parasite. Investigators observed that some “responder” villages were able to significantly reduce the prevalence of Schistosomiasis by the end of the study period, while other “hotspot” villages experienced comparatively little change in prevalence. The goal of our work was to identify which factors influence the change in Schistosomiasis prevalence among Mozambique study villages. To accomplish this, we trained boosted regression trees on environmental and survey data associated with 120 of the 150 SCORE study villages in Mozambique. We then applied the resulting model to the remaining 30 villages in order to evaluate our prediction ability. Although our model was relatively successful at explaining the change in prevalence for the training villages (Spearman R-squared of 0.66), we were unable to make accurate predictions on the withheld test villages (Spearman R-squared of 0.02). *Schistosomiasis Consortium for Operational Research and Evaluation
P15. Metabolic Recovery as a Tool to Discover New-Targeted *Plasmodium falciparum* Inhibitors from Natural Products

Ana Lisa Valenciano¹, Maria Leonor Fernandez-Murga¹, & Maria Belen Cassera¹

¹Biochemistry and Molecular Biology, University of Georgia, Athens, GA

Human malaria accounts for approximately 80,000 deaths a year, with *Plasmodium falciparum* being the most deadly of the five species that infects humans. Artemisinin combination therapies (ATCs) are the frontline treatment for malaria, however resistance to artemisinin has been already confirmed and is increasing in prevalence. Therefore, there is an urgent need to identify novel inhibitors to overcome parasite resistance, especially inhibitors safe for use in pregnant women and children. Artemisinin was originally identified as a natural product, and natural products in general have long been the basis for the identification of antimalarial drugs. Our overarching goal is to identify novel inhibitors with novel mechanisms of action from plants with ethnomedical history of use as antimalarials as well as from synthetic libraries. Historically, inhibition of growth has been used as phenotypic screening to select potential new antimalarials independently of the mechanism of action where identification of the molecular target is not always possible. We used reversal of growth inhibition in order to select active compounds that specifically target the Shikimate pathway and its end-products. Using this approach, one compound was identified to specifically target one end-product of chorismate, p-Aminobenzoate, an indispensable metabolite in the production of folate. This pathway is essential for parasite survival and it is absent in humans. This inhibitor has a cytotoxic effect on the parasite, specifically affecting the transition of trophozoite to schizont stages. Further studies are being performed in order to identify the molecular target to aid future medicinal chemistry.

P16. In silico Identification and Expression Profiling of the Protein Disulfide Isomerase Gene Family in *Toxoplasma gondii*

Diego M. Moncada¹, Karen J. Gonzalez¹, Yi H. Yan², Elizabeth D. Trippe², Juan B. Gutierrez², & Jorge E. Gomez¹

¹Universidad del Quindio (Colombia) .²University of Georgia

With one third of the world’s population infected by *Toxoplasma gondii*, opportunistic infections caused by this parasite are a frequent cause of clinical complications in immunocompromised individuals. Current treatments are focused on acute infections and are not effective against the many stages of the parasite life cycle. To investigate new pharmacological targets, we began with a comparison of multistage gene expression levels over the life cycle of the parasite using in silico approaches. Comparison with other coccidian species, *Neospora caninum*, *Sarcocystis neurona*, *Hammondia hammondi* and *Eimeria tenella* revealed that the protein disulfide isomerase (PDI) family might be an important target for these parasites. We used a combination of approaches to evaluate PDIs as a viable target for pharmaceutical intervention. We identified 20 proteins in the proteome of *T. gondii* ME49, 20 with synteny in *N. caninum*, *S. neurona*, *H. hammondi* and 3 in *E. tenella*. The same domain architecture is shared among these parasites. These proteins contain at least one thioredoxin domain, as is characteristic of the PDI family, with variable numbers of thioredoxin domains in different architectures and combinations. Also we identified proteins in the *T. gondii* life cycle that are expressed only during specific stages (tachyzoite and cat enterop epithelial stages). We found that the protein TGME49_211680 has multistage expression and is the most expressed in the archetypal strains in both human cells and mice in chronic and acute stages. Invasion assays in human foreskin fibroblast cells with bacitracin, a known inhibitor for PDI proteins, showed reduced invasion percentages of tachyzoites. These results suggest that the PDI family has a role in tachyzoite invasion and could be a target for new therapeutic interventions. Furthermore, due to the high sequence conservation between coccidians, PDIs could also be targets for coccidiosis.
**P17. Investigating the Role of Metabolic Cross-Talk between *P. falciparum* and Syncytiotrophoblast in Placental Malaria**

Chelsea E. Gunderson\(^1,2\), M. Belen Cassera\(^1,2\) & Julie M. Moore\(^1,3\)

\(^1\)Center for Tropical and Emerging Global Diseases \(^2\)Department of Biochemistry & Molecular Biology, \(^3\)Department of Infectious Diseases University of Georgia, Athens, GA, United States

Placental malaria is a major public health concern which results in poor pregnancy outcomes such as maternal anemia and morbidity, low birth weight, stillbirth and abortion. This severe form of malaria occurs due to parasite sequestration to the placenta during infection, which helps the parasites to evade maternal immune screening. *Plasmodium falciparum* parasites readily hide in the placenta by use of antigenic variation, which allows the infected red blood cells to bind preferentially to the syncytiotrophoblast. While this mechanism is widely studied, there may be other important host-pathogen interactions at play that contribute to placental malaria pathogenesis. It has been observed that *Plasmodium* parasites exhibit metabolic behaviors similar to those of cancer cells, and as such may take advantage of the nutrient-rich environment of the placenta or possess an ability to modulate the metabolic output of host tissues. Therefore, we are investigating the possibility of metabolic cross-talk between *P. falciparum* and the syncytiotrophoblast and the underlying mechanisms involved during placental malaria infection.

**P18. Targeting the *Naegleria* Glucokinase as a Therapeutic Target: an Amoeba Achilles Heel?**

Jillian Milanes\(^\), Taylor Werner, Will Newton, & James Morris

Department of Genetics and Biochemistry, Clemson University, Clemson, SC

The free-living amoeba, *Naegleria fowleri*, resides in warm freshwater or soil. It is the cause of the rare and fatal disease called primary amoebic meningoencephalitis (PAM). Infection occurs when contaminated water is introduced to the nasal passage, delivering trophozoites that migrate to the host’s brain. Current treatments for this disease are limited. Death usually occurs within days, so effective and fast acting therapeutics are needed. Glycolysis and the pentose phosphate pathway are likely required metabolic pathways for the amoeba, so targeting important enzymes in these pathways could lead to successful treatment of PAM. Here we aim to target the enzyme that is responsible for the first step of both pathways, glucokinase, which converts glucose to glucose-6-phosphate. The *N. fowleri* glucokinase (NfGlck) gene was cloned into pQE30 for homologous expression in *E. coli* and purified. Assay conditions were optimized and known inhibitors of *Plasmodium falciparum* and *Trypanosoma brucei* hexokinases were tested against the protein. From these inhibition assays, an IC\(_{50}\) value, or concentration of drug that inhibits NfGlck 50%, was found. Promising compounds were then used against amoeba to assess toxicity to parasites. The initial studies were performed using a non-pathogenic relative, *Naegleria gruberi*, and an optimized cell titer blue viability assay. The effects of different therapeutic compounds on the growth of the pathogen were measured after 72 hours and EC\(_{50}\) values, the concentration at which the compound reduced growth by 50%, was determined for each drug. Promising leads will be discussed.
P19. Development of a Novel Mouse Model for Pregnancy Maintenance During Maternal Malaria Infection

Catherine D. Smith¹, Caitlin A. Cooper¹, & Julie M. Moore¹
¹CTEGD and Department of Infectious Diseases, University of Georgia

Placental malaria, a severe clinical manifestation of Plasmodium falciparum infection observed in pregnant women, is a major cause of pregnancy loss, neonatal mortality, and severe maternal illness. Mouse models for malaria infection during pregnancy are vital to understanding the mechanisms underlying these outcomes. Here, we describe a novel mouse model for pregnancy maintenance during maternal malaria infection utilizing outbred Swiss Webster mice. When infected with Plasmodium berghei or Plasmodium chabaudi in early gestation, most mouse strains will abort their pregnancies at mid-gestation. However, outbred Swiss Webster mice infected with P. chabaudi AS in early gestation carry their pregnancies to term, providing a model for pregnancy maintenance during maternal malaria infection. As previously observed in non-pregnant mice, the gut microbiota of pregnant Swiss Webster mice influences the severity of malaria infection. Mice with ‘susceptible’ gut microbiota develop higher parasite burdens compared to mice with ‘resistant’ gut microbes. Despite the severe infections observed in ‘susceptible’ mice, these mice carry their pregnancies to term with no significant reduction in the number of viable pups per litter. Overall, this model provides a tool for exploring the mechanisms of pregnancy survival during maternal malaria infection, as well as the influence of the gut bacterial community on the severity of malaria infection in the context of pregnancy.

P20. A Kinesin-Like Protein Regulates Endocytosis in the African Trypanosome, Trypanosoma brucei

Benjamin I. Hoffman¹ & Kojo Mensa-Wilmot¹
¹Department of Cellular Biology, University of Georgia, Athens, Georgia 30602

The parasite Trypanosoma brucei obtains some nutrients from its mammalian host by endocytosis. Thus, identifying targets for blockage of endocytosis represents a major opportunity for anti-trypanosome drug development. Screening in vitro targets of the regulatory kinase GSK3β identified a kinesin-like protein Tb427.05.2410. Knockdown of this protein upregulates bulk phase endocytosis of bovine serum albumin. Knockdown of Tb427.05.2410 additionally compromises proliferation of the parasite. Tb427.05.2410 has been localized along the axis of the trypanosome flagellum. Studies are in progress to fully characterize the biological functions of the putative kinesin.
P21. NEU-4438, a New Lead for Human African Trypanosomiasis Drug Development

Amrita Sharma¹, Lori Ferrins², Michael Pollastri², & Kojo Mensa-Wilmot¹
¹CTEGD-Department of Cellular Biology, University of Georgia, Athens, GA 30602 USA ²Department of Chemistry & Chemical Biology, Northeastern University, Boston, MA 02115 USA

Trypanosoma brucei, a haemoprotozoan parasite, causes the fatal disease Human African Trypanosomiasis (HAT), in regions of sub-Saharan Africa. Current drugs for treatment of HAT have undesirable properties, so there is a need to identify safer alternatives. We are using a “lead repurposing” approach in which a drug chemotype developed for one indication is optimized by reiterative medicinal chemistry approaches for efficacy against T. brucei. In preliminary work, the 4-anilinoquinazoline drug lapatinib cured 25% trypanosome-infected mice. Extensive modifications of the anilinoquinazoline scaffold yielded hits, some of which were nanomolar inhibitors against the parasite, though were plagued by poor physicochemical features (e.g., NEU-617). Matched sidechain comparisons after exchange of the scaffolds showed that quinoline cores coupled with piperazine or homopiperazine substituents improved physiochemical, as well as absorption and metabolic properties. NEU-4438 is a potent hit (GI50 = 13 nM) with a selectivity index of $2 \times 10^3$ over human cells, low plasma protein binding, and high aqueous solubility. In a mouse model of HAT, orally administered NEU-4438 reduced parasitemia 104-fold, establishing this compound as a lead with great potential for advancement towards a drug for HAT. We will present a structure-activity analysis of the compound series leading to NEU-4438, and describe physiological pathways disrupted by NEU-4438 in the trypanosome.

P22. Elucidate Artemisinin Resistance in vitro Phenotypes Using Plasmodium falciparum GFP-Expressing Parasites

Vivian Padín-Irizarry, PhD¹, Amanda Hott, PhD³, Debora C. Livorsi², Shulin Xu², Amy Conway², John H. Adams, PhD², & Dennis E. Kyle, PhD¹
¹Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, Georgia, US ²Global Health, College of Public Health, University of South Florida, Tampa, Florida, US ³Morsani College of Medicine, University of South Florida, Tampa, Florida, US

Emergence of artemisinin (ART) resistant P. falciparum threatens the existing methods to control malaria. Recently, polymorphisms in the Kelch “K13-propeller” protein was associated in the loss of effectiveness of ART drugs. However, the mechanism of drug resistance is not understood. Herein, we use piggyBac (pB) transposon mutagenesis to express GFP in clones of ART- resistant (ART-R) parasites to define in vitro phenotypes. Previous results from our laboratory showed that clones of stably transfected parasites W2 wild type, and the ART-R parasites ARCo8-22 (4G), and PLo8-09 (5C) entered ring-stage dormancy when treated with 700nM dihydroartemisinin (DHA). GFP expression was observed in DHA-induced ring-stage dormant parasites and parasites that recrudesced localized in the cytoplasm of parasites. In the absence of drug pressure, GFP transfected parasites was unaffected and ART-R growth phenotypes were maintained. In this study, we used a small library of artemisinin derivatives to profile GFP expressing pB mutants’ responses to drugs in a modified ³H-hypoxanthine assay. Also, we used a novel device developed in our laboratory that allows for isolation and live imaging of individual parasitized erythrocytes in microwells. This device will enhance the quantitative and temporal assessment of dormancy and survival rates by using novel single cell analysis. Our studies will provide insights in the phenotypes associated with ART-R and improve these for high content imaging assays for chemogenomic profiling screening.
P23. Molecular Dissection of the Lifecycle of Cryptosporidium parvum
Jayesh V. Tandel¹,², Adam Sateriale¹, Brittain Pinkston¹, Mattie C. Pawlowic¹, Carrie F. Brooks¹ & Boris Striepen¹,²
¹CTEGD, University of Georgia, Athens, GA ²Department of Cellular Biology, University of Georgia, Athens, GA

Cryptosporidium is one of the leading causes of diarrhea-induced mortality in children and immuno-compromised individuals. Currently, there are no efficacious drugs or vaccines against the parasite, and this might be attributable to our paucity of biological insights over the parasite. Cryptosporidium has a single-host lifecycle, and it undergoes asexual and sexual development in the same host. Our preliminary data suggest that the parasite undergoes obligate sexual differentiation after limited rounds of asexual replication to produce oocysts. Oocysts can sustain autoinfection in the same host or can be transmitted to infect a new host. Thus blocking sexual development might be an attractive strategy to prevent autoinfection and parasite transmission. Hence, we aim to understand molecular programming of the lifecycle of C. parvum. Our primary approach is to define molecular repertoires of asexual and sexual stages of the parasite. We have identified several stage specific markers that allow us to fluorescently label different stages of the parasite. We aim to use these stage-specific fluorescent reporter strains to sort different stages and to define their transcriptomes and to eventually identify genes that are differentially expressed between different stages. A subset of identified stage-specific markers belong to ApiAP2 DNA binding family. ApiAP2 DNA binding proteins act as master regulators in development of different stages of Plasmodium. We hypothesize that CpApiAP2s might have synonymous developmental roles. We aim to leverage our DiCre-based conditional KO technology and Auxin-Inducible protein Degradation (AID) system to disrupt the function of CpApiAP2 genes and to study its subsequent effect on the progression of lifecycle of C. parvum. We have recently identified a female-specific ApiAP2 (AP2-F) protein that might be involved in early or late development of females. If this is true then conditional disruption of AP2-F should allow us to directly test the effect of impairment of sexual development on infection and transmission of the parasite in an experimental mice model.

P24. Variation in EHDV-2 Infection Rates in Culicoides sonorensis
Sandra Y. Mendiola¹, Mark G. Ruder¹, & David E. Stallknecht¹
Southeastern Cooperative Wildlife Disease Study, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA

Epizootic hemorrhagic disease (EHD) virus (EHDV) is transmitted by biting midges of the genus Culicoides and infects a wide range of wild and domestic ruminants, producing a spectrum of clinical outcomes. In North America, EHDV is associated with significant morbidity and mortality in white-tailed deer. Outbreaks of EHD range from localized, isolated events to large-scale epizootics that span large geographic areas. A limited understanding of EHDV’s epidemiology has hindered our ability to predict and control outbreaks. Few studies have addressed the vector-host interactions that drive EHDV transmission, diminishing the power of predictive models, which are contingent on the existence of well-defined parameters, to assess the risk of EHD infection. The objective of this research is to characterize the variation in EHDV-2 infection rates in Culicoides sonorensis feeding on infected white-tailed deer over the course of their viremia. To do this, we experimentally infected white-tailed deer with EHDV-2 and allowed colonized C. sonorensis midges to obtain a blood meal at several time points during their viremia. We performed virus isolation and titration on blood samples taken from deer as well as blood-fed midges at each time point. Our preliminary results indicate that high EHDV-2 infection rates in C. sonorensis coincide with peak viremias in deer (about 5 – 7 days post infection). Further work will yield more precise information on how the kinetics of EHDV-2 infection in white-tailed deer affect the infection rates of C. sonorensis and facilitate the calculation of a transmission efficiency parameter that can be used to model future EHDV outbreaks.
P25. Glucose Regulation of Glycosome Protein Expression in *Trypanosoma brucei*

Logan Crowe1, Sarah Bauer1, Kathleen Nicholson1, Terral Patel1, Kelly McQueeny1, Emma Arigi2, Igor C. Almeida2 & Meredith Morris1

1Eukaryotic Pathogens Innovation Center, Department of Genetics and Biochemistry, Clemson University 2The Border Biomedical Research Center, Department of Biological Sciences, University of Texas at El Paso

*Trypanosoma brucei* is the parasite responsible for human African trypanosomiasis and is passed to its mammalian host via the tsetse fly insect vector. Glycosomes are essential organelles that house a number of metabolic pathways. *Trypanosomes* are unique in that they compartmentalize a majority of the glycolytic pathway within these organelles. During the lifecycle, *T. brucei* experiences dramatic changes in extracellular glucose levels. In the mammalian bloodstream glucose levels are relatively high and constant at 5mM. In the tsetse fly midgut, glucose levels are undetectable with the exception of short time intervals immediately after a bloodmeal. Much of our knowledge about the processes that regulate glycosome biogenesis and composition come from studies in parasites grown in high glucose media (SDM79). Here we show how glycosome morphology and expression levels of different glycosome proteins varies in low glucose conditions.

P26. Does a Potential Endocytic Motif in the Calcium Activated Potassium Channel SLO-1 of Clade III Nematodes, Affect its Localization and Function?

Swan, H.1,2,3, Wolstenholme, A.J.1,2, & Reaves, B.J.1,2

1Department of Infectious Diseases, University of Georgia, Athens, GA, USA 2CTEGD, University of Georgia, Athens, GA, USA 3University of Bath, United Kingdom

Emodepside is a relatively new anthelmintic compound, and the only octadepsipeptide currently available. At this time it is used exclusively in small animal medicine, but is being considered for use in the human disease onchocerciasis. Though there is some confusion in the literature, it has become accepted that emodepside acts at SLO-1 potassium channels. Inconsistencies may be due to a different subcellular localization of SLO-1 between species. A potential endocytic di-leucine motif has been identified in the *Slo-1* sequence of clade III nematodes that is absent in clade V nematodes. By expressing and then comparing the localization of the clade III *Brugia malayi* SLO-1 and clade V *Haemonchous contortus* SLO-1 in a polarized cell line, PC-12, we will be able to observe differences in subcellular localization that might result from the presence/absence of this motif. Additionally expression of *B. malayi* and *H. contortus* with the di-leucine motif removed and inserted respectively will demonstrate if any difference in localization is due to the di-leucine motif. We have created plasmids that direct the expression of epitope-tagged versions of the parasite SLO-1 channels in cultured cell lines and have shown that we can detect expression of Hco-SLO-1 in transfected A549 cells. We are currently optimizing the transfection protocols for these cells and for PC12 cells. Expression of the wild type and the mutated *Slo-1* constructs in *C. elegans slo-1* knockout worms will test the effect of the di-leucine motif in vivo. Acquiring a greater understanding of localization and function of SLO-1 channels between specific species of parasitic nematode, will further our understanding of the mode of action of emodepside. This will aid the development of further octadepsipeptide compounds and their use as broad spectrum anthelmintic drugs.
P27. Does a Potential Endocytic Motif in a Truncated Form of the C. elegans Levamisole Sensitive Acetylcholine Receptor Subunit, UNC29, Alter Receptor Function and Localization?

Wallis, C.1,2,3, Neveu, C.4, Wolstenholme, A.J.2,3 & Reaves, B.J.2,3

1Department of Infectious Diseases, University of Georgia, Athens, GA 2CTEGD, University of Georgia 3University of Bath, United Kingdom 4Université François Rabelais de Tours, France

With resistance to anthelmintic drugs becoming increasingly common, understanding the mechanisms behind this resistance and its impact on treatment and prevention programs is essential. Nicotinic acetylcholine receptors (nAChRs) are an essential component of the neuromuscular system across many species, including nematodes. This, and their variable pharmacology, makes them important targets for drug development. Levamisole is an established anthelmintic, acting as an agonist at specific levamisole sensitive nAChRs (L-AChRs). Resistance to levamisole is common in GI parasites of livestock. Whilst the structure of C. elegans L-AChRs have been characterized, we recently discovered a novel truncated form of the UNC-29 nAChR subunit, UNC-29T. This C-terminal truncated subunit terminates with an intracellular YxxΦ peptide sequence, indicative of an endocytic signalling motif. We have demonstrated that co-expression of UNC-29T with the wild type L-AChR causes a significant reduction in whole cell currents, suggesting competition between subunits for incorporation into the final protein. Overexpression of UNC29-T in C. elegans results in a severe phenotype with sharply reduced motility. We have also shown that the addition of this YxxΦ motif to the C-terminus of a CD8 reporter protein causes a distinct alteration of in vitro localization, reducing cell-surface expression and increasing intracellular accumulation of the protein. Surprisingly, mutation of the aromatic amino-acids in the motif to alanine residues increases the severity of the phenotype associated with UNC-29T expression, both in vitro and in vivo, and appears to result in CD8 accumulating in the endoplasmic reticulum. We hypothesise that UNC29-T alters receptor function by reducing the number of nAChRs at the cell surface, either by inhibiting transport to the plasma membrane or by mediating receptor uptake into the endocytic pathway. We will further investigate this via in vivo expression in C. elegans, measuring localization and trafficking when this YxxΦ motif is added to the C terminus of a CD9 reporter protein.

P28. Determining the Roles of Exported and ER-Resident Chaperones in Plasmodium falciparum Protein Trafficking

David Cobb¹, Anat Florentin¹, Baylee Bruton¹, Michelle Krakowiak¹, & Vasant Muralidharan¹

¹University of Georgia, CTEGD, Department of Cellular Biology

Infection of erythrocytes by the eukaryotic pathogen Plasmodium falciparum results in the most severe manifestations of malaria. In order to survive within the terminally differentiated erythrocyte, P. falciparum parasites renovate their host cells, a process which requires trafficking of proteins from the parasite endoplasmic reticulum (ER) to the host cell. To investigate the roles of P. falciparum chaperones in this export process, cell lines were generated to study two parasite chaperones. The first, PfHsp70x, is the only parasite Hsp70 exported to the host cell. The glmS ribozyme was used to conditionally knockdown PfHsp70x, but export of parasite proteins was unaffected by the reduction of this chaperone. Confirming these results, protein export was not disturbed in pfhsp70x-knockout parasites. A second chaperone—the Hsp40 PfJ2—localizes in the ER. The TetR-DOZI system was used to tag and conditionally knockdown PfJ2, and we found that, unlike PfHsp70x, PfJ2 is essential for parasite proliferation in the erythrocyte. With this PfJ2 mutant in hand, we will monitor the ability of the parasites to export proteins during PfJ2 knockdown. Protein export is an essential process for P. falciparum survival, and a better understanding of the biology behind P. falciparum protein export is invaluable in the fight against this deadly parasite.
P29. Transmembrane Transporter Proteins in Cryptosporidium

Yiran Li¹,² & Jessica C. Kissinger¹,²,³
¹Institute of Bioinformatics ²Center for Tropical and Emerging Global Diseases & ³Department of Genetics, University of Georgia, Athens, GA 30602

Cryptosporidium is a genus of obligate protozoan parasites that infect both human and animals, causing moderate-to-severe diarrhea and death in some cases. Their genomes are highly-reduced and streamlined leading to a > 50% reduction in metabolic pathways relative to their free-living ancestors. Transporters, which are a type of transmembrane protein that exchanges materials between intracellular and extracellular spaces, have been reported to play an essential role in parasite survival as they can provide essential, missing compounds to the organism. As such, transporters can be important therapeutic targets. In this project, we utilized computational approaches, combining sequence similarity, protein structure and machine learning methods to predict transporter proteins in several Cryptosporidium species and compared the results to other apicomplexans. A notable improvement was made in the number of transporters detected in the newly-annotated genomes of Cryptosporidium parvum and hominis. The phylogenetic pattern of transporter presence and absence may shed light on parasitic evolutionary strategies for survival.

P30. Detection of Rare Non-Proliferating Trypanosoma cruzi Amastigotes in vivo and in vitro

Fernando Sánchez-Valdéz & Rick Tarleton
Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, Georgia, USA

Benznidazole (BZ), the primary drug used to treat Chagas disease, fails to provide parasitological cure in the majority of cases, despite long treatment times (up to 60 days). In a number of infections, drug treatment failures have been linked to the presence of subpopulations of pathogens that become physiologically inactive or dormant. These so-called persister cells may be stochastically generated in pathogen populations and have been hypothesized to serve as a survival mechanism against possible catastrophic events that could eliminate an entire metabolically active population. In our analysis of persisting parasites in mice with chronic T. cruzi infection, we observed the existence of rare amastigotes not undergoing active proliferation, as assessed by the incorporation of the thymidine analog 5-ethynyl-2´-deoxyuridine (EdU) into amastigote DNA during a 24 hr pulse. Exploring this phenomenon further in vitro, we also consistently observed rare, EdU-negative amastigotes in host cells even when the EdU pulse was extended as long as 72 hours. To determine if these EdU-negative parasites were dead rather than just arrested, terminal deoxynucleotidyl transferase dUTP Nick-End Labeling (TUNEL) assays were performed and identified an even rarer subset of dead amastigotes within host cells filled with otherwise healthy, mostly proliferative amastigotes. These results identify a previously unrecognized subset of amastigotes that arrest and occasionally die in host cells both in vitro and in vivo. The cause of this arrest, the ability of arrested amastigotes to resume proliferation or stage transition and the link, if any, between these arrested parasites and drug treatment failures in vivo will be the subject of future studies.
P31. Inorganic Polyphosphate Interacts with Nucleolar and Glycosomal Proteins in Trypanosomatids

Raquel S. Negreiros¹, Noelia Lander¹, Stephanie Smith², James H. Morrissey², Guozhong Huang¹, & Roberto Docampo¹

¹Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA 30602 ²Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801

Inorganic polyphosphate (polyP) is a polymer of three to hundreds of phosphate units bound by high-energy phosphoanhydride bonds and present from bacteria to humans. Most polyP in trypanosomatids is concentrated in acidocalcisomes, acidic calcium stores that possess a number of pumps, exchangers, and channels, and are important for their survival. In this work, using polyP as bait we identified > 50 putative protein targets in cell lysates of Trypanosoma cruzi and T. brucei. Gene Ontology analysis of the binding partners found a significant overrepresentation of nucleolar and glycosomal proteins. Interestingly, using the polyphosphate-binding domain (PPDB) of Escherichia coli exopolyphosphatase we localized long chain polyP to the nucleolus and glycosomes of trypanosomes. A competitive assay based on the pre-incubation of PPBD with exogenous polyP and subsequent immunofluorescence assay of procyclic forms of T. brucei showed polyP dose-dependent and chain length-dependent decrease in the fluorescence signal. Targeting of yeast exopolyphosphatase to the glycosomes of procyclic forms resulted in alteration in their glycolytic flux and increase in the susceptibility to oxidative stress.

P32. Elucidating the Role of Membrane Contact Sites in Ca²⁺ Signaling Pathway of Trypanosoma brucei

Srinivasan Ramakrishnan, Guozhong Huang & Roberto Docampo

Center for Tropical and Emerging Global Diseases, University of Georgia, Athens 30602, GA

Membrane contact sites are regions of close apposition between two organelles typically less than 30 nanometers apart and facilitate transfer of biomolecules. Presence of such contact sites has been demonstrated in yeast, plant and mammalian cells. However, their existence and role in protozoan parasites remains to be investigated. Here we investigated the presence of such contact sites in the protozoan parasite, Trypanosoma brucei. In mammalian cells, ER-mitochondria contact sites facilitate Ca²⁺ uptake by mitochondria. However, acidocalcisomes are the major Ca²⁺ storage site in trypanosomes and they possess an inositol 1,4,5-trisphosphate receptor for Ca²⁺ release. Therefore we hypothesized that in trypanosomes, acidocalcisome-mitochondria contact sites may be involved in Ca²⁺ transfer to the mitochondria. Using super-resolution microscopy we have determined that membrane contact sites exist between the acidocalcisomes and mitochondria of Trypanosoma brucei. Further we have confirmed the close association of these organelles using electron microscopy and proximity ligation assays. To further test Ca²⁺ transfer through these contact sites, we are currently engineering parasite lines expressing genetically encoded Ca²⁺ indicators in mitochondria. Next, we will test if the T. brucei mitochondrial Ca²⁺ uniporter (MCU) accumulates near these acidocalcisome-mitochondria contact sites. Lastly, we plan to identify interorganellar tethers that may facilitate the close association and regulation of acidocalcisome-mitochondria membrane contact sites. Ca²⁺ transfer to the mitochondria is crucial for regulating the activity of mitochondrial enzymes and autophagy. Therefore characterization of these contact sites may be a necessary starting step towards unraveling the role of Ca²⁺ in regulating the overall parasite bioenergetics.
P33. A Plan for Investigating the Influence of Gut Microbiota on the Innate Immune Response of Monocytes as It Relates to Improved Cerebral Malaria Outcomes

Alicer K. Andrew & Julie M. Moore
CTEGD, University of Georgia, Department of Infectious Diseases, College of Veterinary Medicine

Cerebral malaria (CM) is a major complication of Plasmodium falciparum malaria, and is most prevalent in young children in Sub-Saharan Africa. The in vivo Plasmodium berghei ANKA (PbANKA) mouse model is a widely used platform for studying childhood CM pathogenesis. Recent studies have shown that the gut microbiota influences the outcome of PbANKA-mediated CM, but the underlying mechanism has yet to be described. Indeed, the mechanisms of CM pathogenesis in general remain largely undefined, but a dual role for monocytes in protection and pathogenesis has been confirmed. Specifically, the recruitment and subsequent sequestration of monocytes in the brain vasculature is a key feature of CM. My hypothesis is that specific gut bacterial communities can induce reprogramming of monocytes prior to malaria infection, leading to improved CM outcomes. Reprogramming (“trained immunity”) manifests as epigenetic changes in bone marrow monocyte precursors and can be induced by several pathogens. A relationship between specific gut microbiota and trained immunity in monocytes has to our knowledge not been studied. This work will assess whether probiotic Lactobacillus, shown previously to induce resistance to murine malaria, promotes a trained immune response that can protect against CM.

P34. Plasma Metabolomics Analysis During Time-Course P. cynomologi Infection in Non-Human Primates Reveals Disruption of Leukotriene Metabolism and Hormone Biosynthesis

Maren Smith1,2, Shuzhao Li2,3, Dean P. Jones2,3, Mary Galinski2,4, & Mark Styczynski1,2
1School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA 2Malaria Host-Pathogen Interaction Center (MaHPIC), Emory University, Atlanta, GA, USA 3Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, School of Medicine, Emory University Atlanta, GA, USA 4Emory Vaccine Center, Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA

Plasmodium vivax is a significant cause of malaria in over 20 South American and Asian countries, putting an estimated 2.5 billion people at risk. Plasmodium cynomologi infection of Rhesus macaques (M. mulatta) serves an excellent non-human primate model system for P. vivax malaria, enabling us to study in vivo immune and metabolic responses. High-resolution metabolomics provides the means to elucidate many chemical changes that occur in bodily tissues during such malaria infections. We performed high-resolution mass spectrometry (MS) on plasma blood samples from P. cynomolgi-infected Rhesus macaques collected via daily capillary measures and venous blood samples representing baseline, peak blood-stage infection, post-peak infection, relapses, and post relapse infection points. Analysis of this data with repeated measures ANOVA indicated significant variation across infection stages. We then annotated analytes showing significant change using Mummichog. Varying analytes were enriched for metabolites involved in beta-oxidation of saturated fatty acids, bile acid biosynthesis, steroid biogenesis, prostaglandin formation, and leukotriene metabolism. These results are consistent with metabolic analysis of plasma blood from NHPs infected with other malaria strains in experiments also performed by our consortium. These metabolic pathways may, therefore, represent conserved inflammatory processes occurring during blood-stage malaria. In addition, we compared infection time-points to proximate daily measurements as part of an ongoing project comparing the metabolic profiles of capillary sampling and venous blood draws, since differences in analyte profile between blood types have been observed in humans. Our initial results indicate such differences are also present in NHP during malaria infection. These findings have significant implications for malarial clinical research. Future studies will focus on the use of bioinformatics to characterize differences in metabolic profiles between blood sample types, and investigate interactions with processes disrupted by malaria infection.
**P35. Enhanced Cryptosporidium Reference Genome Sequence Resources**

Rodrigo P Baptista1, Yiran Li2, Adam Stareiale1, Boris Striepen1,3 & Jessica C Kissinger1,2,4

1Center for Tropical and Emerging Global Diseases 2Institute of Bioinformatics 3Department of Cellular Biology and 4Department of Genetics and University of Georgia, Athens, GA, USA

Genome sequences for the genus Cryptosporidium are currently being generated with regularity. However, because of insufficient biological material in clinical isolates and the experimental resources needed for validation, fundamental gaps remain in the sequence assemblies and annotation. Currently, there is not a complete genome sequence assembly for either *C. parvum* or *C. hominis*, the species that infect humans. The community also lacks experimentally-confirmed genome annotation. Our aim was to generate the best possible structural assembly and functional genome annotation for three closely-related species of Cryptosporidium, *C. parvum* IOWA, *C. hominis* 30976 and the new crypto mouse model *C. tyzzeri*. Using all available ESTs, cDNA, RNA-Seq, mass spectrometry proteomics and gene orthology information data as evidence, we trained three different gene prediction tools and all data were added as evidence tracks in WebApollo2 for manual curation. In comparison to the previously available *C. parvum* IOWA annotation, we made > 1,500 annotation changes. These improvements were related to gene boundaries, such as adding UTRs, altering the start codon and updating or adding intron features, as well as adding > 50 new RNA-seq supported genes to the annotation. The functional analysis was also improved but~50% of the annotated genes in each species are still hypothetical. Additional experimental data are essential for bettering our understanding of *Cryptosporidium*. Comparative approaches across several closely-related genome sequences facilitate the identification of conserved and novel features, including copy number variation. The new annotations are being submitted to CryptoDB and GenBank for access by the research community.

**P36. An ER-Resident Calcium Binding Protein is Required for Egress and Invasion of Merozoites in *P. falciparum***

Manuel Fierro & Vasant Muralidharan

Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA

*Plasmodium falciparum* is the causative agent of Malaria, a disease that continues to be a large burden in susceptible regions of the world. Calcium (Ca^{2+}) is an important secondary messenger in cells and is required for the progression of the intraerythrocytic lifecycle of Malaria. Genomic analysis shows that *Plasmodium* lacks many of the channels (voltage-gated Ca^{2+} channels, STIM/ORI complex) or signaling cascade components (IP3 receptor, ryanodine receptor) that are essential for regulating Ca^{2+} in other organisms. However, there are many calcium-binding proteins annotated in the genome, some of which reside in the endoplasmic reticulum (ER). One of these is Endoplasmic Reticulum-resident Calcium Binding Protein (ERC). PfERC has 5 predicted EF-hands and previous studies have shown its localization to the ER. We hypothesize that PfERC is an essential protein required for regulating Ca^{2+} homeostasis in the ER. We employed a conditional knockdown system using the ribozyme glmS to determine the role of PfERC in the biology of *P. falciparum*. Our results show that PfERC required for both growth in the blood stages of the parasite and egress and invasion of released merozoites. These results serve to support the role of Ca^{2+} in the progression of the intraerythrocytic life cycle of *P. falciparum*. 
P37. In vitro Assays Confirm in silico Predictions for REL-1 Binding by Naphthalene-Based Compounds

Shoup, Mary V. & Christopher A Hall
Department of Biology, Center for One Health, Berry College, Mount Berry, GA. 30149

The potential use of in silico modeling to predict the binding kinetics of proposed therapeutic molecules with pathogen proteins may offer a cost-effective method for pre-screening prior to in vitro studies. One such in silico study predicted that certain naphthalene-based compounds (NBCs) would bind with high affinity to the ATP-binding site of the REL-1 subunit of the kinetoplast editosome complex. Previously we demonstrated the ability of V4, an NBC, to inhibit the proliferation of Trypanosoma cruzi, the etiologic agent of Chagas disease, in vitro. Here we describe efforts to confirm whether the in silico predictions for binding of REL-1 with NBCs analogs V2 and V3 are reflected in T. cruzi growth curves in vitro. DH-82 canine macrophage cells infected with the Brazil strain of T. cruzi were cultured in the presence of V2 and V3 at 0, 10, 50 and 100 μM (micromolar) concentrations, as previously with V4. T. cruzi proliferation was monitored through hemocytometer counts of emerging blood stream form trypomastigotes (BSF) at 0, 24, 48, and 72 hours. The results were consistent with in silico predictions, with V3, less effective at inhibiting BSF proliferation than V4 under similar conditions. Also in agreement with the model, V2 in vitro demonstrated an increased efficiency in suppressing BSF proliferation. These results also confirmed that, like V4, mammalian DH-82 cell proliferation was not negatively affected by the presence of the NBCs in the media. The experimentally confirmed differences in the inhibitory effects between V2, V3, and V4 are consistent with in silico predictions, supporting the use of such modeling approaches in pre-screening NBCs against T. cruzi. This also offers circumstantial support that the target of the NBCs is the REL-1 ATP binding site.

P38. Identification of Exported Protein Candidates in Cryptosporidium parvum

Jennifer Dumaine1,2, Sarah Hampton1, Amita Reddy1, Adam Sateriale1, & Boris Striepen1,2
1CTEGD, University of Georgia, Athens, GA 2Department of Cellular Biology, University of Georgia, Athens, GA

Cryptosporidium spp. is a major cause of diarrhea worldwide, especially in young children and immunocompromised individuals. Currently there is only one approved drug that can be used to treat cryptosporidiosis; however, the drug cannot be used in the previously mentioned groups. Consequently, in patients, this results in stunted growth, exacerbated malnourishment, and in many cases, death. There is a great need for new drugs to be developed to treat Cryptosporidium spp. infection, but before this can be accomplished, parasite factors involved in the establishment of the host-parasite interaction need to be identified. Once inside the human host, the parasite travels to the small intestine, where it makes a home for itself inside of the parasitophorous vacuole (PV). It is thought that the parasite sends proteins into the host cell to cause changes in the cell shape to make a home for the parasite. In other Apicomplexans, exported proteins are used for this function, as well as for modulation of the host immune response and expression of key virulence factors to promote infection. Because the C. parvum genome encodes proteins with features similar to known exported proteins of other related organisms, we have developed a screening technique utilizing the Cre-Lox system to assay protein candidates identified using a bioinformatics approach. We have identified one strong candidate for export by tagging the endogenous protein with the Cre recombinase and are now working to confirm export status and characterize protein function. By studying protein export in C. parvum, we hope to identify critical effectors needed for host cell infection, such that these interactions can be exploited as potential therapeutic targets.
P39. Investigation of *Bordetella bronchiseptica* Genes Associated with Virulence in Blood

Troy M. King Jr., Monica Cartelle Gestal, Katie Howard, Harry Ma, & Eric T. Harvill  
Center for Vaccines and Immunology, Department of Infectious Diseases, Athens, University of Georgia, Georgia

*Bordetella pertussis* is a Gram-negative respiratory bacterium and the causative agent of whooping cough in humans. There is a growing need for new treatment options due to deficiencies in the current vaccine. To study *B. pertussis* virulence, we use a well-described mouse model utilizing a close-related pathogen, *Bordetella bronchiseptica*. *B. bronchiseptica* can infect animals such as cats, dogs, and most importantly, mice. To generate an effective vaccine for *B. pertussis*, we will utilize *B. bronchiseptica* as a model to determine which genes are involved in virulence. Previous research in the Harvill lab has demonstrated that *B. bronchiseptica* possesses significant differences in gene expression when incubated in sheep blood. We hypothesize that the blood upregulates genes necessary for host colonization and persistence. To determine the function of these genes in host-pathogen interactions, we are targeting the *B. bronchiseptica* genes significantly upregulated in blood for deletion to observe phenotypic differences. The purpose of this study is to determine which genes are necessary for virulence. In order to evaluate the contribution of the selected genes, the generated knockout mutants will be tested for defects in virulence using a cytotoxicity assay and mouse infections. By identifying the genes involved in virulence, we can gain greater insight into potential drug targets in the future vaccines.

P40. Synergistic Activity Between Statins and Bisphosphonates Against Acute Experimental Toxoplasmosis

Zhu-Hong Li, Catherine Li, Sergio H. Szajnman, Juan B. Rodriguez, & Silvia N. J. Moreno  
Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, Georgia, 30602, USA  
Departamento de Química Orgánica and UMYMFOR (CONICET–FCEyN), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, C1428EHA, Buenos Aires, Argentina

Bisphosphonates are widely used for the treatment of bone disorders. These drugs also inhibit growth of a variety of protozoan parasites like *Toxoplasma gondii*, the etiologic agent of toxoplasmosis. Bisphosphonates inhibit the target of the most potent bisphosphonates is the isoprenoid biosynthesis pathway enzyme farnesyl diphosphate synthase (FPPS). Based on our previous work on the inhibitory effect of sulfur-containing linear bisphosphonates against *T. gondii*, we investigated the potential synergistic interaction between one of these derivatives (1-[(n-heptylthio)ethyl]-1,1-bisphosphonate, C7S, compound 6) with statins, which are potent inhibitors of the host 3-hydroxy-3-methyl glutaryl-coenzyme A reductase (3-HMG-CoA reductase). C7S showed high activity against the *T. gondii* bifunctional farnesyl diphosphate/geranylgeranyl diphosphate synthase (TgFPPS), which catalyzes the formation of farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP) (IC50 = 31 ± 0.01 nM), and modest effect against the human FPPS (IC50 of 1.3 ± 0.5 µM). We tested combinations of C7S with statins against the in vitro replication of *T. gondii*. We also treated mice infected with a lethal dose of *T. gondii* with similar combinations. We found a strong synergistic effect in vivo when using low doses of C7S, which was stronger in vivo than when tested in vitro. We also investigated the synergism of several commercially available bisphosphonates with statins both in vitro and in vivo. Our results provide evidence that it is possible to develop drug combinations that act synergistically by inhibiting host and parasite enzymes in vitro and in vivo.
P41. Lysozyme Activity in Regenerative and Non-Regenerative Mammals

Belen Molina1,2, Jennifer Cyr1, Thomas Gawriluk3, John Kimani4, Stephen Kiama4, Ashley Seifert3 & Dr. Vanessa Ezenwa5,6
1Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia 2Post-baccalaureate Research Education Program, University of Georgia 3Department of Biology, University of Kentucky, 4Department of Veterinary Anatomy and Physiology, University of Nairobi 5Odum School of Ecology, University of Georgia 6College of Veterinary Medicine, University of Georgia

While animals like salamanders and fish often display regeneration of injured tissue, this characteristic is rare in mammals. There are only a few mammals known to have true regenerative abilities. African spiny mice (Acomys spp.) have the capacity to regenerate damaged skin, muscle, nerve, and cartilage without scarring. Ongoing research in our laboratory suggests that the regenerative capacity of spiny mice may be tied to a dampened inflammatory response. Acomys serum was found to be 5 to 15 times more effective at killing E. coli in vitro when compared to serum of non-regenerating rodents; however, this enhanced killing is not due to serum complement, a well-known mediator of inflammation. Thus, specific immune molecules associated with this enhanced killing have not yet been identified. The focus of this study was to examine the role that lysozyme, an antimicrobial enzyme thought to have anti-inflammatory properties, might play in the humoral response of Acomys to bacterial infection. To address this question, we quantified lysozyme activity in three Acomys species, four closely related sympatric wild rodent species, and one lab mouse species using a turbidity-based lysozyme assay. Serum samples collected over three years were analyzed and results indicate that Acomys have significantly lower concentrations of lysozyme than non-regenerating rodents. These results suggest that lysozyme is not a major contributor to the enhanced serum bacteria killing ability of Acomys. Further research is needed to pinpoint specific immune molecules that contribute to the heightened serum bacteria killing ability of the spiny mouse. Information gleaned from this study will help elucidate the mechanisms by which tissue-regenerating mammals control bacterial infection.

P42. Discovery of Novel Subunits in the Mitochondrial Calcium Uniporter Complex of Trypanosoma brucei

Guozhong Huang & Roberto Docampo
Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA

The mitochondrial calcium uniporter complex is a highly selective channel that conducts calcium ions across the organelle inner membrane. In Metazoa, the complex consists of the pore forming subunit or mitochondrial calcium uniporter (MCU), its dominant negative paralogue MCUb, the essential MCU regulator (EMRE), and the regulatory subunits, mitochondrial calcium uptake 1 and 2 (MICU1 and 2) and MCU regulator 1 (MCUR1). The genome of Trypanosoma brucei, the causative agent of African trypanosomiasis, has orthologues to metazoan MCU, MCUb, MICU1, and MICU2 but not to EMRE or MCUR1. We previously characterized T. brucei MCU (TbMCU) as an essential component of the MCU complex (MCUC) required for parasite viability and infectivity. In this study we applied a tandem affinity purification (TAP) and mass spectrometry (MS) approach and identified two novel components of the complex that we named TbMCUc and TbMCUd. Interestingly, TbMCUc and TbMCUd, along with TbMCUb, shared 16-19% identity and 28-34% similarity with TbMCU and contained all the conserved domains of TbMCU including a putative mitochondrial targeting signal, 1-2 coiled-coil motifs, two transmembrane domains and one putative Ca2+ selectivity filter. These new MCUC proteins localized to the mitochondria of T. brucei. RNA interference (RNAi) or overexpression of TbMCUc and TbMCUd significantly reduced or enhanced mitochondrial Ca2+ uptake in T. brucei, respectively, without affecting the mitochondrial membrane potential, indicating that they are essential components of the MCUC of this parasite. The specific interactions of TbMCU with TbMCUb, TbMCUb or TbMCUd were confirmed by co-immunoprecipitation (Co-IP) and split-ubiquitin membrane-based yeast two-hybrid (Y2H) assays. In summary, our study has identified two novel essential components of the MCUC of T. brucei, and defined their direct physical interactions that result in a hetero-oligomeric MCU complex.
P43. Effects of *Plasmodium falciparum* Derived Hemozoin on Expression of Inflammatory and Coagulation Factors in BeWo Cells

Trisha Dalapati¹, Catherine D. Smith², & Julie M. Moore²
CTEGD, University of Georgia

*Plasmodium falciparum* infection during pregnancy, which commonly progress into placental malaria (PM), is estimated to cause over 200,000 infant deaths annually. PM is characterized by sequestration of parasite-infected red blood cells (iRBCs) in the maternal space of the placenta. Pathogenesis is additionally marked by aberrant inflammation, adversely affecting nutrient and gas exchange in the syncytiotrophoblast, the outermost multinucleated cell layer of the placenta. Alongside inflammation, increased expression of tissue factor (TF) leads to activation of the extrinsic coagulation cascade and subsequent blood clotting. PM has been marked by excessive clotting, leading to low fetal birth weight and growth restriction. Protease activated receptors including PAR-1 and PAR-2 may be involved in positive feedback of inflammation and coagulation. Although inflammation and coagulation are often viewed separately, activation is concurrent. Because the pathology of PM remains ambiguous, quantifying gene expression of inflammatory and coagulation factors may provide essential mechanistic information. BeWo, a choriocarcinoma cell line, was used as an in vitro model to mimic in vivo host-parasite interactions. After reaching proper confluency, BeWo cells in duplicate samples were syncytialized and stimulated using lipopolysaccharide (LPS) or hemozoin. LPS, the positive control, is found on the outer membrane of gram-negative bacteria and elicits immune response. Hemozoin is the primary parasite by-product from the digestion of hemoglobin. After stimulation, cells were scraped at various time points for RNA isolation, cDNA generation, and real-time PCR. Three genes were chosen for study: TF, PAR-1 and PAR-2. Analysis of gene expression of these targets will provide insight into inflammatory and coagulation responses during PM.

P44. Systematic Glycogene Disruption in *Toxoplasma gondii* with Dual-Guide CRISPR/Cas Genome Editing Tool

H. Travis Ichikawa¹,², Elisabet Gas-Pascual¹,², M. Isabella Serji¹,², M. Osman Sheikh¹,³, Lance Wells¹,³, & Christopher M. West¹,²
¹Department of Biochemistry & Molecular Biology, ²Center for Tropical and Emerging Global Diseases, ³Complex Carbohydrate Research Center, University of Georgia, Athens

Protozoan parasites plague tens of millions of humans and drugs are limited, so new targets for control are urgently needed. A considerable literature implicates glycosylation-related processes in the biology, virulence and persistence of well-known parasites such as *Toxoplasma gondii*, responsible for toxoplasmosis. The advent of CRISPR/Cas9 genome editing makes it possible to incisively test the proposed roles of glycans genetically. Nevertheless, glycobiology is a specialty area that is imposing to non-aficionados. We are developing a resource for parasitologists that i) delineates the framework of glycogenes contributing to the assembly of parasite glycans, ii) provides an easy-to-implement protocol for disrupting the glycogenes and, iii) for select examples, documents the feasibility of disruption and consequences on the cellular glycome. We describe here a web-based guide RNA selection tool to help identify relevant gRNAs. Glycogenes of *T. gondii* are being targeted systematically using double-CRISPR/Cas9 plasmids and a transient generic (no homology ends) floxed-DHFR-resistance amplicon that inserts into the gRNA-directed Cas9-mediated double strand cut site. This strategy has high efficiency, avoids persistence of toxic Cas9, ensures glycogene disruption, is amenable to multiple rounds of disruption, and is applicable to wild-type strains with intact non-homologous end joining. This method has allowed us to disrupt 10 out of 62 predicted glycogenes and 5 more are in the pipeline. To assess glycomic consequences, we are consolidating protocols for sample preparation, glycan release, permethylation, and LC and direct infusion MS/MS analysis, and generating standards, to examine N- and O-glycans, phosphodiester-linked glycans, GPI-anchors, GPIPLs, and other glycolipids. We are expanding existing GRITs platform databases to semi-automate the analyses. With this toolbox, we envision that non-specialists can make connections between their favorite areas of interest and glycan components of the molecules that mediate those functions. This work is supported by NIH 1R21 AI123161 (Common Fund).
P45. Prevalence of *Trypanosoma cruzi* Among Rodent Populations in Northwest Georgia

Kellie Soafer¹, Christopher Hall¹, & William Nicholson²

¹Department of Biology, Berry College One Health Center, Mt. Berry GA ²National Center for Emerging Zoonotic Infectious Diseases, Center of Disease Control, Atlanta GA

An estimated 7 million people are infected with *Trypanosoma cruzi*, the causative agent of Chagas disease. Although largely associated with Latin America, *T. cruzi* is enzootic throughout the southern tier of North America. Unfortunately, little is known of this pathogen’s distribution in natural mammalian reservoir populations in the southeastern United States. As part of a larger survey, rodents were trapped on the Berry College campus. DNA was extracted from spleen tissue and subjected to PCR analysis using the *T. cruzi* specific S35-36 primers. The results showed that 75 of 102 (73.5%) Cotton mice (*Peromyscus gossypinus*) and 28 of 64 (43.8%) of Cotton rats (*Sigmodon hispidus*) tested positive for the presence of *T. cruzi* DNA. In addition, 2 of 9 (22.2%) shrews tested positive, as did the single specimen of chipmunk (*Tamias striatus*) and a flying squirrel (*Glaucomys volans*). This resulted in an overall prevalence of 60.8% among those specimens tested. DNA from 177 rodents was subjected to PCR using the D71-72 primers to determine the strain type of *T. cruzi*. These results showed that all those tested harbored the Type-1 strain of *T. cruzi*, the one most associated with human infections in the U.S. This highlights the broad distribution of *T. cruzi* among rodent populations in the southeastern United States.

P46. Control of Acidocalcisome Phosphate Transport by 5-Diphosphoinositol Pentakisphosphate (IP7) in *Trypanosoma brucei*

Ciro Cordeiro¹,², Evgeniy Potapenko¹, Guozhong Huang¹, Henning J. Jessen³, Adolfo Saiardi⁴, & Roberto Docampo¹,²

¹Center for Tropical and Emerging Global Diseases, ²Department of Cellular Biology, University of Georgia, Athens, Georgia, 30602, USA, ³Department of Chemistry and Pharmacy, University of Zürich, Zürich, Switzerland, and ⁴Department of Cell and Developmental Biology, University College London, London WC1E 6BT, United Kingdom

*T. brucei* is a human and animal parasite transmitted by tsetse flies and found in sub-Saharan Africa. *T. brucei* human infection is called African trypanosomiasis and it leads to death if untreated. In cattle, the parasite causes a disease called Nagana, which has high socioeconomic impact in developing nations affected. *T. brucei* cells are adapted to wrestle the host immune response along with various stress conditions they endure in their various locations in the mammalian and insect hosts. One cellular component extremely important for stress resistance is the acidocalcisome, acidic organelle that stores phosphate, polyphosphate, calcium and other metal ions. Acidocalcisomes are essential for *T. brucei* survival and participate in osmotic control and calcium signaling. We discovered that the physiology of acidocalcisomes is affected by inositol pyrophosphates. Inositol pyrophosphates are metabolites that participate in a range of different cell signaling events. Little is known about these molecules in protist parasites. We characterized the inositol pyrophosphate synthesis pathway in *T. brucei*. Additionally, we observed that *T. brucei*, as other previously characterized organisms, accumulate inositol hexakisphosphate (phytic acid), which is used for synthesis of the 5-diphosphoinositol pentakisphosphate (IP7). Interestingly, *T. brucei* has the sodium/phosphate transporter PHO91 (homologous to the vacuolar PHO91 from yeast) that contains one SPX domain, recently identified as an inositol phosphate sensor sequence. *TbPHO91* is located in the membrane of acidocalcisomes. We characterized the electrophysiological properties of the transporter and we discovered that upon stimulation of the transporter with nanomolar concentrations of IP7, the transporter conductance is enhanced, suggesting that inositol pyrophosphate from trypanosomes are signaling molecules that regulate phosphate metabolism. Our finding uncovers an unknown mechanism of phosphate control in trypanosomes. The understanding of *T. brucei* inositol pyrophosphate and acidocalcisome biology may lead us to further discoveries in cell biology and provide better tools to fight disease.
P47. Genome Wide CRISPR Cas9 Knockout Screen Identifies Genes Important for Susceptibility to Cryptosporidium parvum Infection

Alexis Gibson1,2, Adam Sateriale1, & Boris Striepen1,2
1Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 2Department of Cellular Biology, University of Georgia, Athens, GA

Cryptosporidium parvum is an obligate intracellular parasite and the second leading cause of diarrheal disease worldwide. There is currently only one ineffective drug available to treat cryptosporidiosis. A greater understanding of parasite biology is necessary to develop novel therapeutics. Due to past difficulty maintaining the parasite in the laboratory, little research has been conducted into the host response to infection. We conducted a genome-wide CRISPR Cas9 mediated knockout screen in the HCT-8 intestinal epithelial cell line. Briefly, cells stably expressing Cas9 were transfected with a lentiviral sgRNA library of 80,000 guides, these cells were then subjected to three 72-hour C. parvum infections followed by a period of recovery. After each challenge, cells were removed for gDNA extraction which was sequenced to determine the abundance of each sgRNA. The top 28 enriched genes after three replicates of the screen indicate that type I/III interferons are important for susceptibility to C. parvum infection. In addition, genes in pathways related to glycosaminoglycan synthesis, G protein coupled receptor (GPCR) signaling, and glycosylphosphatidylinositol (GPI) anchors were also amongst the top candidates. Further research is necessary to understand the importance of these pathways in host defense and the role parasite manipulation may play in these responses.

P48. CD4+ T cell Lineage Commitment During Mycobacterium tuberculosis and Schistosoma mansoni Co-infection

Taryn McLaughlin1 & Cheryl Day1,2
1Emory Vaccine Center 2Department of Microbiology and Immunology, Emory University, Atlanta, GA

Research in mice and humans has demonstrated that in the absence of an effective type 1 immune response, individuals infected with Mycobacterium tuberculosis (Mtbi) cannot control the infection. As a result, these individuals develop active disease and often die in the absence of treatment. Helminths stimulate strong type 2 immune responses, which have been shown to antagonize type 1 CD4+ T cells. In fact, the two responses were canonically thought to be mutually exclusive and self-perpetuating due to their ability to antagonize one another. Infections with helminths are common and recurrent in areas where Mtbi is endemic. As such, we hypothesized that antigen-specific responses will be impaired in the setting of Mtbi and S. mansoni co-infection in a cohort of individuals in Kisumu, Kenya. We found an increased risk of being infected with the parasitic worm, S. mansoni, in individuals with active TB disease as compared to latently infected TB (LTBI) individuals and healthy controls (HC). However amongst those infected with S. mansoni, LTBI individuals had a much higher egg burden. To investigate the differential response to both Mtbi and S. mansoni across HC, LTBI and TB individuals, we analyzed CD4+ T cell responses via flow cytometry in individuals from each of these groups with or without coexistent S. mansoni infection. Our data reveal that co-infected individuals have higher general cytokine production as well as type 1 cytokine production, and that co-infected TB individuals also have increased type 2 cytokine production. Our data also demonstrates a difference in type 1 and type 2 lineage marker expression across disease groups. This therefore suggests that responses to both Mtbi and S. mansoni are modulated by the presence of a concomitant infection.
P49. A *Plasmodium* ER Chaperone is Essential for Parasite Growth

Heather M. Bishop¹, David W. Cobb¹, José L. Rodriguez³, Rajani Ravishankar¹, & Vasant Muralidharan¹

¹University of Georgia, CTEGD, Department of Cellular Biology

During the blood stages, the malaria parasite is highly dependent on protein trafficking and export for its survival. Most of the trafficking routes originate in the ER, but how proteins are packaged and sorted to their final destinations within the parasite and to the host cell are important unanswered questions. In other eukaryotes, proteins are recognized and sorted out of the ER by cargo receptors, however no such receptors have been discovered in *Plasmodium*. We hypothesize that ER chaperones lie at the center of protein transport; ensuring the efficient import, folding, and packaging of proteins into vesicles. Chaperones are an attractive class of proteins to act as receptors or adaptors for trafficked proteins as chaperones do not need sequence specificity to bind substrates. We have generated conditional mutants for a previously uncharacterized ER chaperone, PfGRP170, which allows us to interrogate its function. This protein localizes to the ER of the parasite and knockdown of PfGRP170 results in parasite death. Our data suggests that this chaperone is necessary for progression through the trophozoite stage. Proteomic analysis using mass-spectroscopy revealed that PfGRP170 interacts with proteins exported to the host red blood cell, indicating a potential role in protein export. We are currently assessing defects in protein export in response to PfGRP170 inhibition. Loss of PfGRP170 function results in defects in sexual development of the malaria parasite. We will perform mechanistic studies using recombinant PfGRP170 as well as high-resolution complementation. Thus, we will elucidate the essential function of PfGRP170 in the asexual and sexual life-cycle.

P50. Investigating the Molecular Mechanism of CRISPR-Cas Adaptation in *Streptococcus thermophilus*

Edwin Pierre Louis¹⁴, Yunzhou Wei¹, & Michael Terns¹²³

¹Department of Biochemistry and Molecular Biology, ²Department of Genetics, ³Department of Microbiology ⁴Department of Infectious Diseases, The University of Georgia, Athens

Bacteria are constantly challenged by viral infection. In order to combat potentially lethal viruses, bacteria possess adaptive immune defenses called CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-Associated) systems. These systems are RNA-based defense systems that memorize past viral infections to protect prokaryotes like bacteria from future infections. The acquisition of short DNA sequences (spacers) from previously encountered invaders allows bacteria to generate immunity. These systems capture spacers and incorporated them into their CRISPR loci to initialize immunity. Once integrated, spacers are then transcribed and processed into small RNA molecules called crRNAs, which are eventually bound by Cas proteins and form targeting complexes capable of cleaving viral DNA to achieve immunity. Genetic evidence indicates that Cas1, Cas2, Cas9 and Csn2 are each essential for CRISPR adaptation in the Type II-A system of *Streptococcus thermophilus*.[¹] However, the role of each of these proteins in adaptation is not clear. We have systematically constructed single *cas* gene deletion strains. We aim to detect and analyze low level of adaptation events in these genetic backgrounds, and in turn gain insight into the specific roles of each of these proteins. We also plan to apply biochemical approaches using purified proteins to investigate the molecular mechanistic steps of adaptation.
P51. Characterizing in *P. falciparum* ER Chaperone Network

José L. Rodriguez¹, Heather M. Bishop², & Vasant Muralidharan²  
CTEDG, University of Georgia, Athens, GA

In our lab we study the deadly malaria parasite, *Plasmodium falciparum*. This parasite is responsible for almost 450,000 deaths, most of them in children and pregnant women in sub-Saharan Africa. We study asexual replication in the blood stages, which is the source for the entire malaria-associated morbidity and mortality. We are interested in molecular processes that regulate key cellular events in the life cycle of the parasites. Protein trafficking is essential for parasite viability, however the molecular mechanisms, which drive this important process, are unclear. Almost all trafficking pathways begin at a central hub, the parasite ER. There is evidence that molecular chaperones play a role in transport out of the ER. In other eukaryotes, an ER HSP70 chaperone complex is involved in import, trafficking, and stress response. Several homologs of this complex have been identified in the *Plasmodium* genome. We will reveal the mechanism by which these proteins function using two approaches. One will be to complement LHS1, a yeast homolog, with GRP 170 to identify potential evolutionary traits of grp170 protein. The second approach is to express and purify these chaperones using a bacterial system in order to study their activities in vitro. Overall, using these complementary approaches we hope to understand the molecular roles of *Plasmodium* ER chaperones in order to shed light on the complex trafficking processes in the malaria parasite.

P52. Improving the Genome Sequence and Assembly of *Toxoplasma gondii* ME49

Shubham Basu¹,² & Jessica C. Kissinger¹,²,³  
¹Center for Tropical and Emerging Global Diseases, ²Department of Genetics ³Institute of Bioinformatics, University of Georgia, Athens, GA, USA

*Toxoplasma gondii* is a unicellular, tissue-cyst forming parasite that can infect and cause disease in all warm-blooded animals including humans. Previous studies discovered multiple, differential loci that are subject to lineage-specific expansions in *T. gondii*. Often these genes are responsible for encoding pathogenic proteins in the parasite. Many of these genes have been found as tandem copies. Copy Number Variations (CNVs) across lineages highlights their importance in strain-specific divergence that correlates with phenotypes and likely, host-pathogen interactions. CNVs are easily observed when reads from multiple strains of *T. gondii* are mapped to specific locations in the reference genome sequence, which is from strain ME49. These read pile-ups and the presence of Fosmid clones in which only one end can be mapped to the reference genome sequence, highlight the presence of collapsed sequences in these regions. Preliminary data from SNP detection experiments also found an excess of SNPs at low frequency in similar locations reinforcing the notion of compression in reference genome sequence. My research focuses on identification, characterization and correction of these problematic genomic regions. I am taking a Read Depth Coverage (R.D.C) approach. I will transform the pipeline into a user-friendly tool that will correctly detect, quantify and compare repetitive content lost by current short-read NGS-based assemblies. The project also focuses on application of single-molecule long read technologies to manually resolve these compressions and develop a novel pipeline that integrates data from locally-resolved locations with that from existing assemblies to provide an improved genome sequence. The newly-resolved and re-annotated genome of ME49 will be used as framework for improving the genome sequences of other closely-related strains and aid in determination of lineage-specific expansions to better understand the scope and pattern of *T. gondii* evolution.
P53. Investigating CRISPR/Cas9 as a System for Gene Editing in *Trypanosoma cruzi*

Lilith South  
University of Georgia

*Trypanosoma cruzi* is the protozoan responsible for human Chagas Disease. This disease is prevalent in Latin America and there is no vaccine or effective drug treatment. Development of new drugs and vaccines has been hobbled by the difficulties of genetic manipulation which could lead to better understanding of the complex parasite biology. CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR associated gene 9) is a useful genome editing system recently borrowed from bacteria and widely applied to efficiently manipulate the genomes of many diverse species. The system utilizes a nuclease Cas9 protein and a guide RNA which directs Cas9 to cut a target gene. We have previously shown that SpCas9 protein, derived from *S. pyogenes*, can be expressed by *T. cruzi* and used to target parasite genes for editing. Although this method is relatively effective, expression of SpCas9 is toxic to *T. cruzi*. Here we show that the smaller recombinant SaCas9 protein from *Staphylococcus aureus*, but not SpCas9, can be electroporated into *T. cruzi* for effective gene editing. This procedure has shown nearly 100% knockout (KO) efficiency using a fluorescent reporter protein. SaCas9 fused to GFP – thus creating a protein comparable in size to SpCas9 – also fails to achieve reporter gene KO. These experiments demonstrate a new and highly efficient gene editing protocol for *T. cruzi* that depends on the ability of the smaller SaCas9 protein to traverse the *T. cruzi* cell membrane and nuclear envelope that appears impermeable to the larger SpCas9 protein.

P54. EuPathDB: Powerful Data-Mining Tools for Exploring the Biology of Host-Pathogen Interactions

Susanne Warrenfeltz1 & Jessica Kissinger1,2,3 for the EuPathDB Team

1CTEGD, University of Georgia, 2Department of Genetics, University of Georgia, Athens, GA 30602, USA 3Institute of Bioinformatics, University of Georgia, Athens, GA 30602, USA

The Eukaryotic Pathogen Database (EuPathDB, http://eupathdb.org) is a free, online data mining resource that facilitates the discovery of meaningful biological relationships from large volumes of data by integrating pre-analyzed omics data with advanced search capabilities, data visualization, analysis tools and an extensive record system. This comprehensive resource places the power of bioinformatics with the entire scientific community in support of hypothesis driven research and the graphical web-interface does not require prior computer skills. EuPathDB supports over 170 organisms including pathogenic protists (e.g. *Plasmodium spp.*, *Leishmania spp.*, and *Cryptosporidium spp.*) and fungi (e.g. *Aspergillus spp.*, *Cryptococcus spp.*, and *Candida spp.*) as well as related non-pathogenic species. EuPathDB integrates a wide range of omics data, applies standard bioinformatics workflows and creates orthology profiles and domain predictions. This powerful data mining resource offers four general mechanisms for interrogating the data – record page examination, dynamic visualization of sequence based data aligned to the genome, analyses such as GO or metabolic pathway enrichment and Galaxy workflows, and a sophisticated search strategy system that offers over 100 pre-configured searches the query the precomputed data and can associate multiple forms of evidence in support of a hypothesis. With this resource, it is easy to interrogate biological questions such as stage-specific expression, gene model integrity or alternative splice variants, and to compile lists of genes that share multiple biological characteristics such as secreted kinases that are expressed during the gametocyte stage. Recently EuPathDB has expanded to support and integrate data from clinical and systems biology programs such as MaHPIC, PRISM, ICEMR, and MAL-ED. EuPathDB’s active user support offers an email help desk (help@eupathdb.org), social media, a You Tube channel with tutorials and a worldwide program of workshops. Please visit our poster or exhibitor hall booth for a demonstration.
Predicting current and future vector-borne disease (VBD) transmission is a significant challenge. While many factors influence VBD, recent work in a variety of transmission systems highlights the importance of climatic factors in shaping vector-borne disease transmission. In particular, temperature has strong effects on many aspects of mosquito biology involved in transmission including bite rate, mortality rate, fecundity, as well as, the rate of pathogen development within the mosquito vector. These trait by temperature relationships are non-linear, unimodal, and asymmetric. However, the resolution of these trait by temperature relationships is poor as few studies have investigated the variation in these life history traits across the full thermal breadth for a single mosquito species, or how age and temperature integrate to influence trait performance. Transmission models which incorporate these poorly defined relationships with temperature and age can have significant impacts on disease risk predictions, the implementation of intervention strategies, and the evaluation of interventions. To address these gaps, we conducted a study which observed individual *Anopheles stephensi* mosquitoes, the primary malaria vector in India, across six temperature treatments (16°C, 20°C, 24°C, 28°C, 32°C, 36°C) over the duration of their lifespan. We provided a bloodmeal daily and directly measured bite rate, mortality rate, and fecundity for each individually-housed mosquito across our temperature treatments. Temperature effects were observed for each trait, with mosquito age affecting a subset of traits. We also observed interactions between temperature and age for some of these traits, suggesting that warming temperature can accelerate the effects of mosquito age on these traits. These data will be used to parameterize a temperature-dependent R0, which will then be compared to malaria models generated from coarser mix-species data. This study highlights that mosquito thermal performance is likely to vary with mosquito species, temperature, and age suggesting that current transmission models likely fail to capture important variation.