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 pipientis* Using the Surrogate Eukaryotic Host, *Saccharomyces cerevisiae*
11:10 AM	**Satyanarayana Lagishetty,** 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	**Dadín Moore,** CONICET, Argentina Delayed Type Hypersensitivity Reaction in Bovine Neosporosis
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 Piperaquine Resistance and Dihydroartemisinin-Piperaquine Treatment Failure in Cambodia
4:50 PM	**ADJOURN**

Poster Presentations

P1 **Andrew J. Stasic**, CTEGD and Dept. of Microbiology, UGA
Dual Role of the Vacuolar H^+-ATPase as a Guardian Against Ion Stress and Maturation of Secretory Proteins

P2 **Izzy Serji**, Dept. of Biochemistry & Molecular Biology, UGA
Dual-Guide CRIPSR/Cas9 Construction to Knock out *Toxoplasma gondii* Glycogenes

P3 **Núria W. Negrão**, Center for Tropical & Emerging Global Diseases, UGA
Characterization of a Phospholipase C-Like Protein (TbPI-PLC2) from *Trypanosoma brucei*

P4 **Yijian Qiu**, EPIC and Dept of Genetics & Biochemistry, Clemson University
Blood Stream Form *Trypanosoma brucei* Regulates Intracellular Glucose Levels in a Density Dependent Manner

P5 **Stephen Vella**, Center for Tropical & Emering Global Diseases, UGA
Calcium Signaling and Motility of *Toxoplasma gondii*

P6 **Nathalie Carla Thezan**, Dept. of Microbiology, UGA
Investigating the Effects of *Bordetella bronchiseptica* Putative Effector Proteins BB1629 and BB1639 on Yeast Growth

P7 **R. Drew Etheridge**, CTEGD and Dept. of Cellular Biology, UGA
Characterization of an Essential Secreted Effector Binding Protein of *Toxoplasma gondii*

P8 **Mattie C Pawlowic**, Center for Tropical & Emerging Global Diseases, UGA
Adapting the TIR-Auxin Conditional System to Study Essential Genes in *Cryptosporidium*

P9 **Tequila S. Porter**, Post-Baccalauarate Research Education Program, UGA
Expression Level and Chemokine Binding Differences of the Duffy Antigen Receptor for Chemokines (DARC/ACKR1) Amongst Various Ethnic Groups

P10 **Anat Florentin**, CTEGD and Dept. of Cellular Biology, UGA
Bacterial Complex is Required for Apicoplast Function in Malaria Parasites

P11 **Molly Bunkofske**, Center for Tropical & Emerging Global Diseases, UGA
Prediction and Validation of CD8+ T Cell Epitopes from the Flagellar Proteins of *Trypanosoma cruzi*

P12 **Paige Teehan**, Dept. of Biological Sciences, Clemson University
Ablation of Chloroquine Resistance Transporter (TgCRT) in *Toxoplasma gondii* Yields Decreased Invasion

P13 **Smriti Hoda**, PREP and CTEGD, UGA
Zinc Transporters in *Trypanosoma cruzi*: Gene Complementation in *Saccharomyces cerevisiae*, and CRISPR/Cas9-Dependent Knockout and Endogenous Tagging

P14 **Joseph W. Walker**, Odum School of Ecology and Center for Ecology of Infectious Diseases, UGA
Modeling Hotspots of Schistosomiasis in Mozambique with Machine Learning

P15 **Ana Lisa Valenciano**, Dept. of Biochemistry & Molecular Biology, UGA
Metabolic Recovery as a Tool to Discover New-Targeted *Plasmodium falciparum* Inhibitors from Natural Products

P16 **Diego M. Moncada**, Universidad del Quindio, Colombia
In silico Identification and Expression Profiling of the Protein Disulfide Isomerase Gene Family in *Toxoplasma gondii*

P17 **Chelsea E. Gunderson**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Investigating the Role of Metabolic Cross-Talk Between *P. falciparum* and Syncytiotrophoblast in Placental Malaria

P18 **Jillian Milanes**, Dept. of Genetics & Biochemistry, Clemson University
Targeting the *Naegleria* Glucokinase as a Therapeutic Target: an Amoeba Achilles Heel?

P19 **Catherine D. Smith**, CTEGD and Dept. of Infectious Diseases, UGA
Development of a Novel Mouse Model for Pregnancy Maintenance During Maternal Malaria Infection

P20 **Benjamin I. Hoffman**, Department of Cellular Biology, UGA
A Kinesin-Like Protein Regulates Endocytosis in the African Trypanosome, *Trypanosoma brucei*

P21 **Amrita Sharma**, CTEGD and Dept. of Cellular Biology, UGA
NEU-4438, a New Lead for Human African Trypanosomiasis Drug Development

P22 **Vivian Padín-Irizarry**, Center for Tropical & Emerging Global Diseases, UGA
Elucidate Artemisinin Resistance in vitro Phenotypes Using *Plasmodium falciparum* GFP-Expressing Parasites

P23 **Jayesh V. Tandel**, CTEGD and Dept. of Cellular Biology, UGA
Molecular Dissection of the Lifecycle of *Cryptosporidium parvum*

P24 **Sandra Y. Mendiola**, Southeastern Cooperative Wildlife Disease Study, UGA
Variation in EHDV-2 Infection Rates in *Culicoides sonorensis*

P25 **Logan Crowe**, EPIC and Dept. of Genetics & Biochemistry, Clemson University
Glucose Regulation of Glycosome Protein Expression in *Trypanosoma brucei*

P26 **Hetty Swan**, University of Bath, United Kingdom
Does a Potential Endocytic Motif in the Calcium Activated Potassium Channel SLO-1 of Clade III Nematodes, Affect its Localization and Function?

P27 **Connor Wallis**, University of Bath, United Kingdom
Does a Potential Endocytic Motif in a Truncated Form of the *C. elegans* Levamisole Sensitive Acetylcholine Receptor Subunit, UNC29, Alter Receptor Function and Localization?

P28 **David Cobb**, CTEGD and Dept. of Cellular Biology, UGA
Determining the Roles of Exported and ER-Resident Chaperones in *Plasmodium falciparum* Protein Trafficking

P29 **Yiran Li**, CTEGD and Institute of Bioinformatics, UGA
Transmembrane Transporter Proteins in *Cryptosporidium*

P30 **Fernando Sánchez-Valdéz**, Center for Tropical & Emerging Global Diseases, UGA
Detection of Rare Non-Proliferating *Trypanosoma cruzi* Amastigotes in vivo and in vitro

P31 **Raquel S. Negreiros**, Center for Tropical & Emerging Global Diseases, UGA
Inorganic Polyphosphate Interacts with Nucleolar and Glycosomal Proteins in Trypanosomatids

P32 **Srinivasan Ramakrishnan**, Center for Tropical & Emerging Global Diseases, UGA
Elucidating the Role of Membrane Contact Sites in Ca^{2+} Signaling Pathway of *Trypanosoma brucei*

P33 **Alicer K. Andrew**, CTEGD and Dept. of Infectious Diseases, UGA
A Plan for Investigating the Influence of Gut Microbiota on the Innate Immune Response of Monocytes as It Relates to Improved Cerebral Malaria Outcomes

P34 **Maren Smith**, School of Chemical & Biomolecular Engineering, Georgia Institute of Technology
Plasma Metabolomics Analysis During Time-Course *P. cynomologi* Infection in Non-Human Primates Reveals Disruption of Leukotriene Metabolism and Hormone Biosynthesis

P35 **Rodrigo P Baptista**, Center for Tropical & Emerging Global Diseases, UGA
Enhanced *Cryptosporidium* Reference Genome Sequence Resources

P36 **Manuel Fierro**, CTEGD and Dept. of Cellular Biology, UGA
An ER-Resident Calcium Binding Protein is Required for Egress and Invasion of Merozoites in *P. falciparum*

P37 **Mary Shoup**, Dept. of Biology and Center for One Health, Berry College
In vitro Assays Confirm in silico Predictions for REL-1 Binding by Naphthalene-Based Compounds

P38 **Jennifer Dumaine**, CTEGD and Dept. of Cellular Biology, UGA
Identification of Exported Protein Candidates in *Cryptosporidium parvum*

P39 **Troy M. King Jr.**, Center for Vaccines & Immunology and Dept. of Infectious Diseases, UGA
Investigation of *Bordetella bronchiseptica* Genes Associated with Virulence in Blood

P40 **Zhu-Hong Li**, CTEGD and Dept. of Cellular Biology, UGA
Synergistic Activity Between Statins and Bisphosphonates Against Acute Experimental Toxoplasmosis

P41 **Belen Molina**, PREP and Dept. of Infectious Diseases, UGA
Lysozyme Activity in Regenerative and Non-Regenerative Mammals

P42 **Guozhong Huang**, CTEGD and Dept. of Cellular Biology, UGA
Discovery of Novel Subunits in the Mitochondrial Calcium Uniporter Complex of *Trypanosoma brucei*

P43 **Trisha Dalapati**, Center for Tropical & Emerging Global Diseases, UGA
Effects of *Plasmodium falciparum* Derived Hemozoin on Expression of Inflammatory and Coagulation Factors in BeWo Cells

P44 **H. Travis Ichikawa**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Systematic Glycogene Disruption in *Toxoplasma gondii* with Dual-Guide CRISPR/Cas Genome Editing Tool

P45 **Kellie Soafer**, Dept. of Biology and Center for One Health, Berry College
Prevalence of *Trypanosoma cruzi* Among Rodent Populations in Northwest Georgia

P46 **Ciro Cordeiro**, CTEGD and Dept. of Cellular Biology, UGA
Control of Acidocalcisome Phosphate Transport by 5-Diphosphoinositol Pentakisphosphate (IP7) in *Trypanosoma brucei*

P47 **Alexis Gibson**, CTEGD and Dept. of Cellular Biology, UGA
Genome Wide CRISPR Cas9 Knockout Screen Identifies Genes Important for Susceptibility to *Cryptosporidium parvum* Infection

P48 **Taryn McLaughlin**, Emory Vaccine Center, Emory University
CD4+ T cell Lineage Commitment During *Mycobacterium tuberculosis* and *Schistosoma mansoni* Co-Infection

P49 **Heather M. Bishop**, CTEGD and Dept. of Cellular Biology, UGA
A *Plasmodium* ER Chaperone is Essential for Parasite Growth

P50 **Edwin Pierre Louis**, Dept. of Biochemistry & Molecular Biology and Dept. of Infectious Diseases, UGA
Investigating the Molecular Mechanism of CRISPR-Cas Adaptation in *Streptococcus thermophilus*

P51 **José L. Rodriguez**, Center for Tropical & Emerging Global Diseases, UGA
Characterizing in *P. falciparum* ER Chaperone Network

P52 **Shubham Basu**, CTEGD and Dept. of Genetics, UGA
Improving the Genome Sequence and Assembly of *Toxoplasma gondii* ME49

P53 **Lilith South**, University of Georgia
Investigating CRISPR/Cas9 as a System for Gene Editing in *Trypanosoma cruzi*

P54 **Susanne Warrenfeltz**, Center for Tropical & Emerging Global Diseases, UGA
EuPathDB: Powerful Data-Mining Tools for Exploring the Biology of Host-Pathogen Interactions

P55 **Kerri L. Miazgowicz**, CTEGD, Dept. of Infectious Diseases, and Center for the Ecology of Infectious Diseases, UGA
Resolving Temperature-Driven Malaria Transmission Models

P56 **Karla M. Márquez Nogueras**, Center for Tropical & Emerging Global Diseases, UGA
The Role of Calmodulin-Like Proteins in *Toxoplasma gondii* Calcium-Signaling

Oral Presentations

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

Beatrice L. Colon[1], Christopher A. Rice[2], Abdelbasset A. Farahat[3], David W. Boykin[3], R. Kiplin Guy[4] & Dennis E. Kyle[2]

[1]Morsani College of Medicine, University of South Florida, Tampa, Florida [2]College of Public Health, University of South Florida, Tampa, Florida [3]Department of Chemistry, Georgia State University, Atlanta, Georgia [4]St 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 Savadelis[1], Michael Dzimianski[1], Amanda Coleman[1], Gregg Rapoport[1], Ajay Sharma[1], Cameon Ohmes[2], Joe Hostetler[2], Andrew Moorhead[1]

[1]University of Georgia College of Veterinary Medicine [2]Bayer 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 microfilaremia 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'Antonio[1,a],* Gustavo F. Mercaldi[2,a], Ana Rodriguez[3] & Artur T. Cordeiro[2]
[1]Department of Natural Sciences, University of South Carolina Beaufort, [1]University Boulevard, Bluffton, South Carolina 29909, United States of America [2]Brazilian Biosciences National Laboratory, Brazilian Center for Research in Energy and Materials, Campinas, SP, Brazil [3]Department of Microbiology, New York University, School of Medicine, 341 East 25th Street, New York, New York 10016, United States of America
[a]Authors 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 (*Tc*GlcK). *Tc*GlcK 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 *Tc*GlcK 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 pipientis* Using the Surrogate Eukaryotic Host, *Saccharomyces cerevisiae*

Emily M. Carpinone[1] & Vincent J. Starai1,[2]
Departments of [1]Microbiology and [2]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 pipientis* (*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 Lagishetty[1,2] & Kerry Smith[1,2]
[1]Eukaryotic Pathogen Innovative Center [2]Genetics 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. Yan[1], Elizabeth D. Trippe[1], Diego M. Moncada[2], Mary R. Galinski[3], Alberto Moreno[3], Jessica C. Kissinger[1], Rabindra Tirouvanziam[3], Juan B. Gutierrez[1], & the MaHPIC Consortium
[1]University of Georgia, [2]Universidad del Quindio (Colombia), [3]Emory 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. HHSN272201200031C] 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 Dameron[1] & Zhicheng Dou[1]
[1]Clemson 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. Maclean[1,2], Walter W. Lorenz[3], Christopher J. Anna[4], Michael T. Dzimianski[2], & Adrian J. Wolstenholme[1,2]
[1]CTEGD, University of Georgia, [2]Department of Infectious Diseases, University of Georgia, [3]Institute of Bioinformatics, University of Georgia, [4]Perthera, 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*.

Delayed Type Hypersensitivity Reaction in Bovine Neosporosis

Franco Fiorani[1], Joaquín Armendano[2], Yanina Hecker[1], Ignacio Gual[1], María Leunda[3], Susana Pereyra[3], Anselmo Odeón[3], & Dadín Moore[1]
[1]CONICET, Argentina [2]Facultad de Ciencias Agrarias, UNMDP, Argentina [3]INTA, EEA Balcarce, Argentina

Neosporosis is a parasitic disease caused by the protozoan *Neospora caninum*, which is closely related to *Toxoplasma gondii*. *N. caninum* is a major concern in cattle industry and economic losses due to abortions have been estimated in over 1 billon dollars worldwide. Little information is available regarding to a delayed type hypersensitivity (DTH) reaction in bovine neosporosis. In this study, we examined the elicitation of a DTH reaction in cattle naturally and experimentally infected with *N. caninum* by intradermal inoculation with a soluble tachyzoite lysate at different concentrations: 0.5, 1 y 2mg/mL and mock inoculums. Four experimental groups of 5 cows each one were as follow: seronegative (SN), seropositive (SP), inoculated with live tachyzoites of *N. caninum* (LNc) and inoculated with soluble tachyzoite lysate plus adjuvant (Sol-Adj). The intradermal reactions were measured at 0, 24, 48, 72 and 96 hours post inoculation (hpi) in all groups. Development of specific antibodies were assessed by in house indirect ELISA. The intradermal reaction was statistically analyzed considering groups, inoculums and time as the main explicative variables. Noteworthy, DTH were observed in all groups except in animals from SN group. The intradermal reactions were observed and recorded between 24 and 96 hpi, but maximum increases were observed at 48 and 72hpi in animals from groups SP and LNc. Interestingly, no development of specific antibodies were observed in any cows from the SN group, which is highly desirable since the only way to identify infected cattle is by performing serological tests. These results indicate that cows either naturally or experimentally exposed to *N. caninum* produced a DTH reaction, which is a good indicator of the development of type 1 immune responses, which is frequently associated to protection.

A Natural Mouse Model for Cryptosporidiosis

Adam Sateriale[1], Jan Slapeta[2], Rodrigo Baptista[1], Jessica Kissinger[1], Carrie Brooks[1], Gillian Herbert[1], Ravi Pulusu[1], & Boris Striepen[1]
[1]CTEGD, University of Georgia [2]Veterinary 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.

Cryo-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.

Poster Presentations

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, 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 VHa1 conditional knockout strain, which shows significant defects in motility, egress, growth/replication, and invasion/attachment. Biochemical function of VHa1 was validated by complementing VHa1 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 CRIPSR/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 *Trypanosoma brucei*

Núria W. Negrão[1], Sharon King-Keller[1], Guozhong Huang[1], & Roberto Docampo[1]
[1]CTEGD

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. *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., *Eukaryot. Cell* 14:486-494, 2015) we characterized a phosphoinositide phospholipase C (TbPI-PLC1, Tb927.11.5970) from *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 hydrolyzes 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., *Life Sci* 72:443-453, 2002). The PDZ domain could be involved in this binding and this is being investigated.

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

Yijian Qiu[a], Charles Voyton[b], Kenneth A. Christensen[b], & James C. Morris[a]
[a]Eukaryotic Pathogens Innovation Center, Department of Genetics and Biochemistry, Clemson University, Clemson SC 29634 [b]Department of Chemistry and Biochemistry, Brigham Young University, Provo UT 84604

The African trypanosome, *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 *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 are connected to environmental cues and intracellular glucose, and the feedback from those inputs. Here we describe the localization of the *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 Vella[1], Christina Moore[1], Mojtaba Fazli[2], Shannon Quinn[2], & Silvia Moreno[1]
[1]CTEGD, University of Georgia [2]Department 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 Thezan[1] & Vincent J. Starai[1]
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[1]. *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[2] 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[3], 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 Pawlowic[1], Emily K Myers[1], Caleb Studstill[1], Gillian T Herbert[1], Carrie F Brooks[1], & Boris Striepen[12]
[1]CTEGD, University of Georgia, Athens, GA [2]Department 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.

P9. Expression Level and Chemokine Binding Differences of the Duffy Antigen Receptor for Chemokines (DARC/ACKR1) Amongst Various Ethnic Groups

Tequila S. Porter[1], Rachel N. Martini[1], Brittany D. Jenkins, & Melissa B. Davis[1,2]
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 Florentin[1,2], David W Cobb[1], Jillian D Fishburn[1], Paul S Kim[2], Manuel A Fierro[1], Vasant Muralidharan[1,2]
[1]Department of Cellular Biology [2]Center 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 Bunkofske[1] & Rick Tarleton[2]
[1,2]CTEGD, 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 Teehan[1] & Zhicheng Dou[1]
[1]Department 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. Walker[1,2] & John M. Drake[1,2]
[1]Odum School of Ecology, University of Georgia [2]Center 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 Valenciano[1], Maria Leonor Fernandez-Murga[1], & Maria Belen Cassera[1]
[1]Biochemistry 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. Moncada[1], Karen J. Gonzalez[1], Yi H. Yan[2], Elizabeth D. Trippe[2], Juan B. Gutierrez[2], & Jorge E. Gomez[1]
[1]Universidad del Quindio (Colombia) .[2]University 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 enteroepithelial 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 IC50 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 EC50 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. Smith[1], Caitlin A. Cooper[1], & Julie M. Moore[1]
[1]CTEGD 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. Hoffman[1] & Kojo Mensa-Wilmot[1]
[1]Department 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 Sharma[1], Lori Ferrins[2], Michael Pollastri[2], & Kojo Mensa-Wilmot[1]
[1]CTEGD-Department of Cellular Biology, University of Georgia, Athens, GA 30602 USA [2]Department 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 x 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, PhD[1], Amanda Hott, PhD[3], Debora C. Livorsi[2], Shulin Xu[2], Amy Conway[2], John H. Adams, PhD[2], & Dennis E. Kyle, PhD[1]
[1]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, Georgia, US [2]Global Health, College of Public Health, University of South Florida, Tampa, Florida, US
[3]Morsani 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 ARC08-22 (4G), and PL08-09 (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 ^{3}H-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. Tandel[1,2], Adam Sateriale[1], Brittain Pinkston[1], Mattie C. Pawlowic[1], Carrie F. Brooks[1] & Boris Striepen[1,2]
[1]CTEGD, University of Georgia, Athens, GA [2]Department 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. Mendiola[1], Mark G. Ruder[1], & David E. Stallknecht[1]
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 Crowe[1], Sarah Bauer[1], Kathleen Nicholson[1], Terral Patel[1], Kelly McQueeny[1], Emma Arigi[2], Igor C. Almeida[2] & Meredith Morris[1]

[1]Eukaryotic Pathogens Innovation Center, Department of Genetics and Biochemistry, Clemson University [2]The 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]

[1]Department of Infectious Diseases, University of Georgia, Athens, GA, USA [2]CTEGD, University of Georgia, Athens, GA, USA [3]University 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]
[1]Department of Infectious Diseases, University of Georgia, Athens, GA [2]CTEGD, University of Georgia [3]University of Bath, United Kingdom [4]Université 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 Cobb[1], Anat Florentin[1], Baylee Bruton[1], Michelle Krakowiak[1], & Vasant Muralidharan[1]
[1]University 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 Li[1,2] & Jessica C. Kissinger[1,2,3]
[1]Institute of Bioinformatics [2]Center for Tropical and Emerging Global Diseases & [3]Department 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. Negreiros[1], Noelia Lander[1], Stephanie Smith[2], James H. Morrissey[2], Guozhong Huang[1], & Roberto Docampo[1]

[1]Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA 30602 [2]Department 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 Ca^{2+} 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 Ca^{2+} uptake by mitochondria. However, acidocalcisomes are the major Ca^{2+} storage site in trypanosomes and they possess an inositol 1,4,5-trisphosphate receptor for Ca^{2+} release. Therefore we hypothesized that in trypanosomes, acidocalcisome-mitochondria contact sites may be involved in Ca^{2+} 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 Ca^{2+} transfer through these contact sites, we are currently engineering parasite lines expressing genetically encoded Ca^{2+} indicators in mitochondria. Next, we will test if the *T. brucei* mitochondrial Ca^{2+} 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. Ca^{2+} 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 Ca^{2+} 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 Smith[1,2,] Shuzhao Li[2,3], Dean P. Jones[2,3], Mary Galinski[2,4], & Mark Styczynski[1,2]
[1]School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA [2]Malaria Host-Pathogen Interaction Center (MaHPIC), Emory University, Atlanta, GA, USA [3]Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, School of Medicine, Emory University Atlanta, GA, USA [4]Emory Vaccine Center, Yerkes National Primate Research Center, Emory University, Atlanta, GA, USA; Department of Medicine, Division of Infectious Diseases, 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 Baptista[1], Yiran Li[2], Adam Stareiale[1], Boris Striepen[1,3] & Jessica C Kissinger[1,2,4]
[1]Center for Tropical and Emerging Global Diseases [2]Institute of Bioinformatics [3]Department of Cellular Biology and [4]Department 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 uM (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 Dumaine[1,2], Sarah Hampton[1], Amita Reddy[1], Adam Sateriale[1], & Boris Striepen[1,2]
[1]CTEGD, University of Georgia, Athens, GA [2]Department 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[a], Catherine Li[a], Sergio H. Szajnman[b], Juan B. Rodriguez[b] & Silvia N. J. Moreno[a]
[a]Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, Georgia, 30602, USA [b]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. 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 Molina[1,2], Jennifer Cyr[1], Thomas Gawriluk[3], John Kimani[4], Stephen Kiama[4], Ashley Seifert[3] & Dr. Vanessa Ezenwa[5,6]
[1]Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia [2]Post-baccalaureate Research Education Program, University of Georgia [3]Department of Biology, University of Kentucky, [4]Department of Veterinary Anatomy and Physiology, University of Nairobi [5]Odum School of Ecology, University of Georgia [6]College 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 (*Tb*MCU) 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 *Tb*MCUc and *Tb*MCUd. Interestingly, *Tb*MCUc and *Tb*MCUd, along with *Tb*MCUb, shared 16-19% identity and 28-34% similarity with *Tb*MCU and contained all the conserved domains of *Tb*MCU including a putative mitochondrial targeting signal, 1-2 coiled-coil motifs, two transmembrane domains and one putative Ca^{2+} selectivity filter. These new MCUC proteins localized to the mitochondria of *T. brucei*. RNA interference (RNAi) or overexpression of *Tb*MCUc and *Tb*MCUd significantly reduced or enhanced mitochondrial Ca^{2+} 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 *Tb*MCU with *Tb*MCUb, *Tb*MCUc or *Tb*MCUd 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 Dalapati[1], Catherine D. Smith[2], & Julie M. Moore[2]
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 Ichikawa[1,2], Elisabet Gas-Pascual[1,2], M. Isabella Serji[1,2,] M. Osman Sheikh[1,3], Lance Wells[1,3], & Christopher M. West[1,2]
[1]Department of Biochemistry & Molecular Biology, [2]Center for Tropical and Emerging Global Diseases, [3]Complex 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, GIPLs, 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 Soafer[1], Christopher Hall[1], & William Nicholson[2]
[1]Department of Biology, Berry College One Health Center, Mt. Berry GA [2]National 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 pathogens 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 Cordeiro[1,2], Evgeniy Potapenko[1], Guozhong Huang[1], Henning J. Jessen[3], Adolfo Saiardi[4], & Roberto Docampo[1,2]
[1]Center for Tropical and Emerging Global Diseases, [2]Department of Cellular Biology, University of Georgia, Athens, Georgia, 30602, USA, [3]Department of Chemistry and Pharmacy, University of Zürich, Zürich, Switzerland, and [4]Department 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 Gibson[1,2], Adam Sateriale[1], & Boris Striepen[1,2]
[1]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA [2]Department 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 McLaughlin[1] & Cheryl Day[1,2]
[1]Emory Vaccine Center [2]Department 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* (*Mtb*) 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 *Mtb* is endemic. As such, we hypothesized that antigen-specific responses will be impaired in the setting of *Mtb* 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 *Mtb* 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 *Mtb* and *S. mansoni* are modulated by the presence of a concomitant infection.

P49. A *Plasmodium* ER Chaperone is Essential for Parasite Growth

Heather M. Bishop[1], David W. Cobb[1], José L. Rodriguez[1], Rajani Ravishankar[1], & Vasant Muralidharan[1]
[1]University 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 Louis[1,4], Yunzhou Wei[1], & Michael Terns[1,2,3]
[1]Department of Biochemistry and Molecular Biology, [2]Department of Genetics, [3]Department Microbiology [4]Department 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. Rodriguez[1], Heather M. Bishop[2], & Vasant Muralidharan[2]
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 Basu[1,2] & Jessica C. Kissinger[1,2,3]
[1]Center for Tropical and Emerging Global Diseases, [2]Department of Genetics [3]Institute 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 Warrenfeltz[1] & Jessica Kissinger[1,2,3] for the EuPathDB Team
[1]CTEGD, University of Georgia, 2Department of Genetics, University of Georgia, Athens, GA 30602, USA [3]Institute 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.

P55. Resolving Temperature-Driven Malaria Transmission Models

Kerri L. Miazgowicz[1,2,3], Jack Owen[1], Temi Alandowa[1], & Courtney Murdock[1,2,3]
[1]Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, 501 D.W. Brooks Drive, Athens, GA 30602 U.S.A. [2]Center for Tropical and Emerging Global Diseases, University of Georgia, 500 D.W. Brooks Drive, Athens GA 30602, U.S.A. [3]Center for the Ecology of Infectious Diseases, Odum School of Ecology, University of Georgia, 140 E. Green Street, Athens GA 30602, U.S.A.

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.

P56. The Role of Calmodulin-Like Proteins in *Toxoplasma gondii* Calcium-Signaling

Karla M. Márquez Nogueras[1] & Silvia N. Moreno[2]
[1]CTEGD, University of Georgia, Department of Microbiology [2]CTEGD, 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.

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