28TH ANNUAL
MOLECULAR PARASITOLOGY & VECTOR BIOLOGY SYMPOSIUM

THE GEORGIA CENTER FOR CONTINUING EDUCATION
ATHENS, GEORGIA
THURSDAY, APRIL 26, 2018

Center for Tropical & Emerging Global Diseases
UNIVERSITY OF GEORGIA
Global Health Through Research

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Program

8:15 AM Registration and Poster Set-up

9:00 AM Opening Remarks: Dennis Kyle, Director of CTEGD

SESSION 1 — Msano Mandalasi & Ruby Harrison

9:10 AM **Stephen Vella**, Center for Tropical & Emerging Global Diseases, UGA
Calcium Signaling and *Toxoplasma* Motlity

9:30 AM **Brian S. Mantilla**, Center for Tropical & Emerging Global Diseases, UGA
InsP7 Protein Targets Reveal Distinct Roles in Proliferative Stages of *Trypanosoma cruzi*

9:50 AM **Catherine D. Morffy Smith**, CTEGD and Dept. of Infectious Diseases, UGA
Composition of the Gut Microbiota Influences Infection Severity and Pregnancy Outcome in *Plasmodium chabaudi*-infected Pregnant Mice

10:10 AM **BREAK — POSTER VIEWING**

SESSION 2 — Stephen Vella & Catherine Smith

10:50 AM **Wei Wang**, Center for Tropical & Emerging Global Diseases, UGA
First Glimpse at a High-quality, Evidence-based and Chromosome-level Reference Genome Assembly for *Trypanosoma cruzi*

11:10 AM **Elisabet Gas Pascual**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
A Glycobiology Toolkit for Parasitologist: *Toxoplamsa gondii* Glycogene Genome Editing System

11:30 AM **Peiwei Liu**, Dept. of Cellular Biology, UGA
The Bardet-Biedl Syndrome Protein Complex is an Adapter Expanding the Cargo Range of Intraflagellar Transport Trains for Ciliary Export

11:50 AM **Heather M. Kudyba**, CTEGD and Dept. of Cellular Biology, UGA
PfGRP170 is an Essential ER Protein in the Human Malaria Parasite, *Plasmodium falciparum*

12:10 PM **LUNCH — POSTER VIEWING**

SESSION 3 — Karla Marie Marquez-Nogueras & Manuel Fierro

1:30 PM **Brock Thornton**, Dept. of Biological Sciences, Clemson University
Altered Physiology and Function of the Vacuolar Compartment Ultimately Lead to Reduced Invasion and Microneme Secretion within *Toxoplasma gondii* Parasites

1:50 PM **Christine M. Reitmayer**, Dept. of Infectious Diseases, UGA
Female Mate CHoice Impacts Offspring Immune Performance in *Aedes aegypti* Mosquitoes

2:10 PM **Msano Mandalasi**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
A Glycogenin Homolog in *Toxoplasma gondii* Glycosylates an E3 Ubiquitin Ligase and Controls Parasite Growth

2:30 PM **BREAK — POSTER VIEWING**

SESSION 4 — Evgeniy Potapenko & Molly Bunkofske

3:00 PM **Rodrigo P. Baptista**, CTEGD and Institute of Bioinformatics, UGA
Consistent, Comparative and Evidence-based Genome Annotation and Re-annotation for the Closely-related Species, *Cryptosporidium parvum*, *C. hominis* and *C. tyzzeri*

3:20 PM **Amanda Hott**, CTEGD, UGA and National Institutes of Health
Drug Surveillance Study in Mali Reveals Emergence of Quinine Resistance

3:40 PM **INTRODUCTION OF THE KEYNOTE SPEAKER**

3:45 PM **Patricia Johnson**, UCLA Molecular Biology Institute
Trichomonas vaginalis: Human Host and Parasite Interactions

Poster Presentations

P1 **Viktória Čabanová**, Institute of Parasitology, Slovak Academy of Sciences
PCR Xenomonitoring of Filarial Parasites in Mosquitoes from Cryptic Groups *Anopheles maculipennis* and *Culex pipiens*

P2 **Natacha Karambizi**, EPIC and Dept. of Biological Sciences, Clemson University
Role of eIF2-alpha Kinases in *Entamoeba histolytica* Stress Control

P3 **Flavia M. Zimbres**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Polyisoprenoid Metabolism in *Plasmodium falciparum*

P4 **Susanne Warrenfeltz**, Center for Tropical & Emerging Global Diseases, UGA
EuPathDB: Free, Online Omics Resources for Eukaryotic Pathogens

P5 **Alona Botnar**, Center for Tropical & Emerging Global Diseases, UGA
Arrested Development: Gibberellin and Its Mechanism of Action on Dormant *Plasmodium* Parasites

P6 **Katherine L. Floyd**, Dept. of Biological Sciences, UGA
Function of Protoporphyrinogen IX Oxidase (PPO) of *Toxoplasma* in the Pathogenesis of Toxoplasmosis

P7 **Hannah P. McQueen**, Dept. of Biochemistry & Molecular Biology, UGA
Microscopy Analysis of Trypanosome Nanotubes and Extracellular Vesicles

P8 **Ciro Cordeiro**, CTEGD and Dept. of Cellular Biology, UGA
Identification and Characterization of Glycosomal and Cytosolic Nudix Hydrolases with Polyphosphate Hydrolyzing Activity in *Trypanosoma brucei*

P9 **Logan Crowe**, EPIC and Dept. of Genetics & Biochemistry, Clemson University
Characterization of TbPex13.2 and Its Role in Glycosome Protein Import

P10 **Tessa Berrafato**, Dept. of Infectious Diseases, UGA
The Effects of Ivermectin and Moxidectin on Canine Leukocyte Attachment to Drug-resistant and –sensitive Strains of *Dirofilaria immitis*

P11 **Manuel Fierro**, CTEGD and Dept. of Cellular Biology, UGA
An ER-resident Calcium Binding Protein is Required for Egress and Invasion of *Plasmodium*

P12 **Nicolle Barbieri**, Dept. of Population & Health, College of Veterinary Medicine, UGA
Bacterial Endosymbionts in *Acanthamoeba* Isolates from the Nasal Mucosa and Cutaneous Lesions of Dogs

P13 **Benjamin I. Hoffman**, CTEGD and Dept. of Cellular Biology, UGA
Kinetoplast Scission is Regulated by Accessory Proteins in the African Trypanosome

P14 **Christina Wilkinson**, Dept. of Genetics & Biochemistry, Clemson University
Mapping the Trafficking Route of a Trypanosome Peroxin

P15 **Alicer K. Andrew**, CTEGD and Dept. of Infectious Diseases, UGA
Investigating the Crosstalk Between Host Responses in a Rodent Model of Malaria-induced Pregnancy Loss

P16 **Amrita Sharma**, Dept. of Cellular Biology, UGA
Efficacy and Mode of Action of NEU-4438, a Lead Drug for Human African Trypanosomiasis

P17 **Jillian Milanes**, Eukaryotic Pathogens Innovation Center, Clemson University
Targeting the *Naegleria* Glucokinase as a Therapeutic Target: an Amoeba Achilles Heel?

P18 **Heather A. Walters**, EPIC and Dept. of Biological Sciences, Clemson University
Evaluation of eIF2-α Phosphorylation in *Entamoeba histolytica* in Response to Nitrosative or ER Stress

P19 **Alec T. Thompson**, Southeastern Cooperative Wildlife Disease Study, UGA
The New Rat Lungworms?: The Occurrence OF *Physaloptera hispida* and a *Mastophorus* sp. in Pulmonary Vessels of the Hispid Cotton Rat (*Sigmodon hispidus*) from Georgia, USA

P20 **Lisa M Shollenberger**, Center for Vaccines and Immunology, UGA
Optimizing VacSIM® Delivery of Malaria CelTOS and CSP Antigens to Enhance Vaccine Efficacy

P21 **Maren Smith**, School of Chemical & Biomolecular Engineering, Georgia Institute of Technology and MaHPIC, Emory University
Network Analysis of *Plasmodium cynomologi* Infection and Re-infection Challenge

P22 **Jessica Jones**, Eukaryotic Pathogen Innovation Center, Clemson University
Exploring the Role of a Potential Glucose Sensor in *Trypanosoma brucei*

P23 **Kerri Miazgowicz**, CTEGD, Dept. of Infectious Diseases, and Center of the Ecology of Infectious Diseases, UGA
Rate Summation Fails to Estimate *Anopheles stephensi* Trait Performance under Thermal Fluctuation, Which Alters Predictions of Malaria Transmission

P24 **Christian Cochrane**, Clemson University
Putative Lysosomal Chloride Transporters in *Toxoplasma gondii*

P25 **Rachel Hannah**, Dept. of Genetics & Biochemistry, Clemson
TrypSpotting: Identifying Lipid Droplet Proteins in *Trypanosoma brucei*

P26 **Beatrice L. Colon**, CTEGD and Dept. of Infectious Diseases, UGA
Repurposing FDA-approved Compounds to Identify a Treatment Against the Brain-eating Amoeba

P27 **Pablo D. Jimenez Castro**, Dept. of Infectious Diseases, UGA and Grupo de Parasitolgía Veterinaria, Universidad Nacional de Colombia
Macrocyclic Lactone (ML) Anthelmintics Lack Meaningful in vitro Activity Against L3 and L4 Stages of Both ML-susceptible and ML-resistant *Dirofilaria immitis*

P28 **Anat Florentin**, CTEGD and Dept. of Cellular Biology, UGA
A Bacterial Complex is Required for Plastid Integrity in *P. falciparum*

P29 **O. Agata Walkowiak**, EPIC and Dept. of Genetics & Biochemistry, Clemson University
Effect of Fatty Acid Synthesis Inhibitor Cerulenin on Bloodstream Form *T. brucei*

P30 **Joshua H. Butler**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Natural Products as a Source to Discover Novel Drug Targets in *P. falciparum*

P31 **Ana Lisa Valenciano**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Metabolic Dependency of Chorismate in *Plasmodium falciparum*

P32 **Rudo Kieft**, Dept. of Biochemistry & Molecular Biology, UGA
Identification of a Novel Protein Complex Involved in RNA Polymerase II Transcription Termination in Kinetoplastids

P33 **Justin Wiedeman**, CTEGD and Dept. of Cellular Biology, UGA
A Fixable probe for Visualizing Flagella and Plasma Membranes of the African Trypanosome

P34 **Nicole Holderman-Munro**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Using High-resolution Mass Spectrometry to Decipher the Isoprenome in *Plasmodium falciparaum*

P35 **Blanka Tesla**, CTEGD and Dept. of Infectious diseases, UGA
Impacts of Temperature on Zika Virus Transmission Potential

P36 **Ruby Harrison**, CTEGD and Dept. of Entomology, UGA
The Gut Microbiota is Required for Normal Egg Formation in the Yellow Fever Mosquito, *Aedes aegypti* L. (Diptera: Culicidae)

P37 **Nuria W. Negrão**, CTEGD and Dept. of Cellular Biology,UGA
Characterization of a Phospholipase C-like Protein (TbPI-PLC2) from *Trypanosoma brucei*

P38 **David W. Cobb**, CTEGD and Dept. of Cellular Biology, UGA
An ER-resident Hsp40 is Required for the Asexual Development of the Malaria Parasite *P. falciparum*

P39 **Scott B. Green**, Dept. of Natural Sciences, University of South Carolina - Beaufort
Structure-activity Relationship (SAR) Investigation of Monosaccharide Derivatives: Discovery of Biologically Active and Competitive *Trypanosoma cruzi* Glucokinase Inhibitors

P40 **Angel Padilla**, Center for Tropical & Emerging Global Diseases, UGA
Spontaneous Dormancy Protects *Trypanosoma cruzi* During Extended Drug Exposure

P41 **Yiran Li**, Institute of Bioinformatics, UGA
Strand-specific RNA Sequencing in Zoonotic Protozoan Pathogen *Cryptosporidium parvum* Suggests Widespread and Developmentally Regulated Long Non-coding RNA Transcription

P42 **Kyle Pazzo**, Dept. of Genetics & Biochemistry, Celmson University
Dramatic Morphological Changes in *T. brucei* upon Over-expression of Lipid Droplet Targeting Proteins

P43 **Nicole M. Arroyo Diaz**, Dept. of Infectious Disases, UGA
Development of Parainfluenza Virus 5 (PIV5) Based Zika Virus Vaccine

P44 **Christopher A. Rice**, CTEGD and Dept. of Cellular Biology, UGA
Discovery and Development of Trophocidal and Cysticidal Compounds for the Treatment of *Acanthamoeba* infections

P45 **Kathryn Purple**, Comparative & Experimental medicine, University of Tennessee
Investigating the Molecular Epidemiology and Transmission Potential of *Trichomonas* spp. from Hunter-killed Columbiformes in California

P46 **Nikki M. Meyer**, Dept. of Infectious Diseases, UGA
Expression, Purification of Recombinant Guinea Pig Cytokines and Chemokines for Monoclonal Antibody Production

P47 **Jocelyn Sotolongo Gomez**, Southeastern Cooperative Wildlife Disease Study, UGA
Protective Neutralizing Antibodies to Highly Pathogenic Avian Influenza H5N8 and H5N2 in Blue-winged Teal (*Anas discors*)

P48 **BreeAnna Dell**, College of Veterinary Medicine, Univeristy of Tennessee
Retrospective Investigation of Translocated Elk in Tennessee (USA) and Examination of Canid Definitive Hosts for *Echinococcus granulosus*

P49 **Carlie A. Neiswanger**, Center for Vaccines & Immunology, UGA
Pathogenesis of a Novel Avian Influenza Virus, A/New York/108/2016 (H7N2)

P50 **Miryam A. Hortua Triana**, Center for Tropical & Emerging Global Diseases, UGA
Phosphoinositide Phospholipase C and Calcium Signaling in *Toxoplasma gondii*

P51 **Natasha Perumal**, Center for Tropical & Emerging Global Diseases, UGA
The Impact of STING Pathway Activation During *Trypanosoma cruzi* Infection

P52 **Karla M. Márquez Nogueras**, Center for Tropical & Emerging Global Diseases, UGA
Regulation of Calcium Entry by Calcium-binding Proteins

P53 **Margot P. Palmer**, Dept. of Biochemistry & Molecular Biology, UGA
Extracellular Vesicles Produced by African Trypanosomes a Potential Tool for Diagnostics

P54 **Juan M. Bustamante**, Center for Tropical & Emerging Global Diseases, UGA
Curative Effect of Modified Benznidazole Dosing Regimens in Chronic *Trypanosoma cruzi* Infection

P55 **Logan Ballard**, Dept. of Biochemistry & Molecular Biology, UGA
Developmental Changes in Extracellular Vesicles from African Trypanosomes (*Trypanosoma brucei brucei*)

P56 **Edwin Pierre Louis**, CTEGD and Dept. of Cellular Biology, UGA
Role of a Secreted Effector of *Toxoplasma gondii* in Modulating the Host Cell Cycle

P57 **Trisha Dalapati**, Center for Tropical & Emerging Global Diseases, UGA
Effects of *Plasmodium falciparum* on Placental Expression of Inflammatory and Coagulation Factors

P58 **Abigail Calixto**, CTEGD and Dept. of Microbiology, UGA
Using Forward Genetics to Identify Calcium Channels in *Toxoplasma gondii*

P59 **Alma G. Mendoza**, Dept. of Infectious Diseases, UGA
Identification of Macrophage Cell Surface Receptors with Binding Affinity for the Helminth Glycan, LNFP3

Oral Presentations

Calcium Signaling and *Toxoplasma* Motility

Vella, A. S.[1], Moore, C.[1], Fazli, M.S.[2], Potapenko, E.[1], Quinn, S.[2], and Moreno, S. N. J.[1]
[1]Center for Tropical and Emerging Global Diseases [2]Department of Computer Science, University of Georgia

Calcium signaling is utilized universally across life, as binding of Ca^{2+} to signaling effectors induces a cascade of downstream processes. Fluxes in basal cytosolic Ca^{2+} levels ($[Ca^{2+}]i$) serve as the basis for signaling, as mechanisms to increase or decrease cytosolic Ca^{2+} are intricately balanced. *T. gondii*'s pathogenesis and lytic cycle are linked, as calcium oscillations originating from extracellular influx and/or release from intracellular stores precede the induction of the lytic cycle processes of motility, invasion, and egress. For a successful lytic cycle, *T. gondii* must traverse biological barriers and invade host cells, and motility is essential for both. Using genetically encoded calcium indicators (GECI's), in particular GCaMP6f (GFP based calcium indicator) expressing parasites and host cells transiently expressing red GECI's, we can track Ca^{2+} dynamics of both the parasite and host cell in real time. Within a host cell *T. gondii* resides within a specialized intracellular vacuole that functions as a sieve to passively permit for the exchange of small molecules; thus, the surrounding milieu of intracellular parasites is likely in equilibrium with the host cytoplasm. Therefore, intracellular parasites are liable to the same fluxes of host cytosolic ionic composition that occur throughout a host cell signaling event. During intracellular growth, an unknown signal induces calcium oscillations that precede motility and egress, yet we believe this Ca^{2+} signal must meet a threshold for egress. Post achievement of a Ca^{2+} threshold, activation of an unknown agonist induces a signaling pathway, resulting in Ca^{2+} oscillations and is enhanced by later downstream extracellular calcium flux, culminating in motility stimulation and egress. By using whole-cell patch clamp, we delivered exact concentrations of $[Ca^{2+}]i$ to the cytosol of infected host, to determine the level necessary for egress. Our data established the role of Calcium influx from the host cytoplasm and its impact on motility and egress.

InsP7 Protein Targets Reveal Distinct Roles in Proliferative Stages of *Trypanosoma cruzi*

Brian S. Mantilla[1], Nathaniel Brown[2], Dorothea Fiedler[2] and Roberto Docampo[1]
[1]Center for Tropical and Emerging Global Diseases, University of Georgia, [2]Department of Chemical Biology, Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany.

Inositol pyrophosphate (InsP7) or diphosphoinositol pentakisphosphate (PP-IP5) is considered a messenger metabolite involved in the regulation of several cellular processes. The most studied one is the isoform 5-InsP7 that contains a pyrophosphate group at the fifth carbon. In higher eukaryotes and trypanosomes, InsP7 synthesis takes place in the cytosol and is catalyzed by inositol hexakisphopshate kinase (IP6K), which phosphorylates InsP6 producing InsP7 and ADP. It has been reported that InsP7 can modulate protein activity through binding to specific protein domains, recently described as SPX in humans and plants, or by forming a pyrophosphate group in a phosphoserine residue. This latter process is dependent of Mg^{2+}, but InsP7 interaction may also occurs under metal restriction. Making use of affinity reagents containing a non-hydrolyzable InsP7 analogue (PCP-InsP5) and phosphate beads (as control) along with protein mass spectrometry identification and GO-enrichment analysis, we have identified the protein targets of InsP7 in both amastigote and epimastigote stages of the human pathogenic protozoan parasite *Trypanosoma cruzi*. In epimastigote lysates prepared under metal restriction, we have identified 85 protein targets predicted to be nuclear, cytosolic and constituents of microbodies. GO-enrichment analysis revealed roles in biological processes such as: RNA processing, vesicle-mediated transport, ribosome biogenesis and protein translation. In the presence of Mg^{2+}, it was found a reduced cohort of 31 proteins, from which only 5 were redundant in both stages. GO-terms analysis suggested a role in tRNA amino acylation, phosphate homeostasis, lipids and glucose catabolism and cell division. In amastigote forms, our data revealed a role for InsP7 in protein phosphorylation, nucleosome assembly, cell redox homeostasis, purine metabolism, parasite-surface components and exocytosis when compared to epimastigotes (insect stage). Validation of some of these InsP7-mediated protein substrates has been approached using C-terminally tagged parasites and immunoprecipitation experiments. Our data revealed a developmental role for InsP7 in *T. cruzi*.

Composition of the Gut Microbiota Influences Infection Severity and Pregnancy Outcome in *Plasmodium chabaudi*-infected Pregnant Mice

Catherine D. Morffy Smith[1], Caitlin A. Cooper[1], Alicer K. Andrew[1] & Julie M. Moore[1]
[1]CTEGD & Department of Infectious Diseases, University of Georgia

Placental malaria, a severe clinical manifestation of *Plasmodium falciparum* infection, is a major cause of pregnancy loss, neonatal mortality, and severe maternal illness. We have developed a novel mouse model for pregnancy maintenance during maternal malaria infection utilizing outbred Swiss Webster mice. When infected with *Plasmodium chabaudi* in early gestation, several inbred mouse strains will abort their pregnancies at mid-gestation. However, outbred Swiss Webster mice infected with *P. chabaudi* in early gestation carry their pregnancies to term, allowing us to explore the immunological balance between parasite clearance and pregnancy success. The composition of the gut microbiota may alter this balance. As described by Villarino et al., C57BL/6 mice sourced from different vendors display gut microbiota-dependent differences in *Plasmodium* infection severity resulting in a susceptible or resistant phenotype. Similarly, *P. chabaudi chabaudi* AS-infected pregnant Swiss Webster mice exposed to susceptibility-conferring fecal microbes develop higher parasite burdens than mice exposed to resistance-conferring fecal microbes following the disruption of the native gut microbiota by broad-spectrum antibiotic treatment. The microbiota-mediated reduction of parasite burden is associated with reduced maternal morbidity and improved fetal outcomes. Specifically, fetal viability and weight at gestational term are increased in infected dams exposed to resistance-conferring fecal microbes relative to infected dams exposed to susceptibility-conferring fecal microbes. To assess post-natal growth, pups from infected and uninfected dams exposed to resistance- and susceptibility-associated fecal microbes were delivered by caesarean section and placed with a foster dam. Although pup growth prior to weaning is not significantly influenced by either infection status or fecal microbe exposure, pups produced by highly susceptible dams displayed a statistically significant reduction in survival in the first days of life, suggesting that these pups may be less fit than pups born to dams with lower parasite burdens.

First Glimpse at a High-quality, Evidence-based and Chromosome-level Reference Genome Assembly for *Trypanosoma cruzi*

Wei Wang[1], Rodrigo P. Baptista[1,2], Duo Peng[1,3], Todd Mining[1], Jessica C. Kissinger[1,2,4] and Rick L. Tarleton[1,3]
[1]Center for Tropical and Emerging Global Diseases, [2]Institute of Bioinformatics, [3]Department of Cellular Biology, 4Department of Genetics, University of Georgia, Athens, GA, USA.

Trypanosoma cruzi is the causative agent of Chagas disease which affects millions of people primarily in Latin America. Its genomic studies are greatly hampered by the lack of an acceptable reference genome. This is due to the inability of short read sequencing platforms used for generating the current reference genome to handle the highly repetitive DNA content (50%) in *T. cruzi*, as well as the extreme genome-wide heterozygosity and genetic mosaicism in the hybrid reference strain CL-Brener, which resulted in thousands of gaps and regions of assembly collapse. In order to regenerate a more suitable reference genome, two homozygous *T. cruzi* isolates, Brazil (TcI) and Y strain (TcII) were selected for whole-genome sequencing, not only because their genomes are non-mosaic, but also because they represent the two ancestral lines that may have given rise to all other genetic types present in the species. A Single-molecule, long-read sequencing platform (SMRT sequencing) was used to better resolve the genome complexity. Assembled by SMRT Link, 667 and 351 contigs were formed with N50 at 227kb and 410 kb for Brazil and Y strain, respectively. Compared to over 8000 contigs generated for CL-Brener with N50 at only 26 kb, genomes of Brazil and Y strain have substantially improved contiguity. Furthermore, we performed Chicago and Dovetail Hi-C assays to capture the chromosomal conformation of the parasite genome based on chromatin interactions to scaffold one of the strains, Brazil, which achieved a 4-fold increase in N50 and 407 total scaffolds. Strikingly, we found telomeric patterns on both ends of several scaffolds, indicating that we have obtained, for the first time, a chromosome-level assembly. Further work will be conducted to complete de novo assembly and annotation for both strains, and a comparison of the TcI and TcII genomes will evaluate the initial diversity in this species.

A Glycobiology Toolkit for Parasitologist: *Toxoplasma gondii* Glycogene Genome Editing System

Elisabet Gas-Pascual[1,2], H. Travis Ichikawa[1,2], M. Izzy Serji[1,2], M. Osman Sheikh[1,3], Bowen Deng[1,2], Msano Mandalasi[1,2], Giulia Bandini[4], John Samuelson[4], Lance Wells[1,3], and Christopher M. West[1,2]

[1]Department of Biochemistry and Molecular Biology, University of Georgia, [2]CTEGD, University of Georgia, [3]Complex Carbohydrate Research Center, University of Georgia, [4]Department of Molecular and Cell Biology, Boston University Goldman School of Dental Medicine.

Protozoan parasites plague tens of millions of humans but their drug treatments 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 has made it possible to test virtually every gene in any given genome, allowing us now to test the proposed roles of glycans in *Toxoplasma* genetically. However, glycobiology is a specialty area that has required previous knowledge to succeed in the field. To overcome this difficulty, we have developed a resource for parasitologists that identifies the putative glycogenes contributing to the assembly of the parasite glycans and provides an easy-to-implement protocol for disrupting them. We describe here a web-based guide RNA selection tool to help identify relevant gRNAs. We have successfully edited 17 of 23 *T. gondii* glycogenes 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, encourages glycogene disruption, is amenable to multiple rounds of disruption, and is applicable to wild-type strains with intact non-homologous end joining. To assess glycomic consequences, we have consolidated and tested protocols for sample preparation, glycan release, permethylation, and LC and direct infusion MS/MS analysis, 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).

The Bardet–Biedl Syndrome Protein Complex is an Adapter Expanding the Cargo Range of Intraflagellar Transport Trains for Ciliary Export

Peiwei Liu[1] and Karl F. Lechtreck[1]

[1]Department of Cellular Biology, University of Georgia, Athens, GA 30602

Bardet-Biedl syndrome (BBS) is a ciliopathy resulting from defects in the BBSome, a conserved protein complex. BBSome mutations affect ciliary membrane composition and impair parasite-host communication, which is a new target for abolishing parasite virulence. The mechanism by which the BBSome regulates ciliary membrane content, for example, lipid composition, remains unknown. *Chlamydomonas* bbs mutants accumulate phospholipase D (PLD) in the ciliary membrane. Single particle imaging revealed that PLD co-migrates with BBS4 by intraflagellar transport (IFT) while IFT of PLD is abolished in bbs mutants. BBSome deficiency did not alter the rate of PLD entry into cilia. Membrane-association and the N-terminal 58 residues of PLD are sufficient and necessary for BBSome-dependent transport and ciliary export. The replacement of PLD's ciliary export sequence (CES) caused PLD unable to interact with BBSomes and accumulate in cilia of cells, which finally impaired phototaxis signaling, revealing that PLD is a negative regulator in phototactic signaling. In all, we showed that BBSomes interact with their cargos and export cargos from cilia to maintain the signaling transduction.

PfGRP170 is an Essential ER Protein in the Human Malaria Parasite, *Plasmodium falciparum*

Heather M. Kudyba, David W. Cobb, Anat Florentin, Manuel Fierro, José L. Rodriguez, Rajani Ravishankar, and Vasant Muralidharan
CTEGD, Department of Cellular Biology, University of Georgia

The vast majority of malaria mortality is attributed to one parasite species: *Plasmodium falciparum*. Asexual replication of the parasite within the Red Blood Cell (RBC) is responsible for the pathology of the disease. There are currently no effective vaccines against *P. falciparum* and drug resistance has emerged for all clinically available drugs, thus making malaria research extremely important. In *Plasmodium*, the endoplasmic reticulum (ER) is a uniquely complex, poorly understood organelle. We are therefore interested in uncovering proteins which regulate and maintain ER homeostasis in the parasite. One group of proteins possibly governing these processes are ER chaperones. In other eukaryotes, ER chaperones assist with protein folding and unfolding, the crossing of biological membranes, ER stress, lipid metabolism, and protein trafficking. We know very little about the roles that ER chaperones play in *Plasmodium*, with most of the information we have based on sequence homology to other organisms. We have generated conditional mutants for PfGRP170, a previously uncharacterized *P. falciparum* ER chaperone, allowing us to interrogate its biological function. These conditional mutants were isolated using flow cytometry, and we have confirmed that PfGRP170 localizes to the ER in all stages of asexual development The protein is essential for asexual survival, with knockdown resulting in parasite death in early schizogony. The protein is also required for surviving a brief heat shock, suggesting this protein may be essential during febrile episodes in the host. Proteomic analysis using mass-spectroscopy revealed that PfGRP170 interacts primarily with proteins exported to the host RBC; however, no clear defects in trafficking of exported proteins have been observed during knockdown. GRP170 in other eukaryotic organisms functions as an important co-chaperone of ER chaperone BiP, and in *Plasmodium* we have also confirmed an interaction with the *Plasmodium* homolog. Additionally, during PfGRP170 knockdown we see phosphorylation of EIF2-alpha, an Unfolded Protein Response protein, which is an indicator of ER stress. The inner workings of the *P. falciparum* ER are largely unknown, but we have shown that the chaperone PfGRP170 is an essential protein that contributes to key ER functions in the clinically relevant asexual stages.

Altered Physiology and Function of the Vacuolar Compartment Ultimately Lead to Reduced Invasion and Microneme Secretion within *Toxoplasma gondii* Parasites

Brock Thornton[1], Paige Teehan[2*], Amy Bergmann[1], Zhicheng Dou[1]
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Toxoplasma gondii is an obligate intracellular protozoan parasite, which currently infects approximately one-third of the human population. The lysosome-like vacuolar compartment/plant-like vacuole (VAC/PLV) plays multiple functions in *Toxoplasma* infection, such as degrading ingested host proteins and turning over autophagosomes to maintain chronic infection. We have previously reported that *Toxoplasma* encodes an ortholog of the *Plasmodium falciparum* chloroquine resistance transporter (CRT) and that TgCRT spans the membrane of the VAC. In this project, we deleted *Tgcrt* and complemented the resulting Δ*crt* with the wild-type *Tgcrt* coding sequence. In the absence of a functional TgCRT, the parasites displayed a swollen VAC that was approximately 20-fold larger in volume than that of WT parasites. The Δ*crt* also displayed reduced invasion and microneme secretion. We hypothesize that the swollen VAC is caused by the accumulation of small solutes, such as amino acids, which results in increased osmotic pressure and further influx of water into the VAC. The significant swelling of the VAC could alter its physiology and function. Currently, I am investigating the regulation of the proteases and transporters residing in the parasite's endolysosomal system by quantitative RT-PCR. Furthermore, a pH-sensitive fluorescent protein has been introduced to the VAC of *Toxoplasma* to quantify its acidity. Understanding the mechanism of how *Toxoplasma* controls the size of its digestive vacuole will help reveal the native function of TgCRT and also provide novel strategies for clinical management of toxoplasmosis.

Female Mate Choice Impacts Offspring Immune Performance in *Aedes aegypti* Mosquitoes

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Aedes aegypti is capable of transmitting several diseases to humans, such as dengue, chikungunya, and Zika. In order to control mosquito populations more effectively, there has been a flood of investment in the development of novel mosquito control technologies. While these approaches seem promising, field trials demonstrate that modified mosquitoes do not spread the desired traits as quickly or efficiently throughout the population as initially predicted. Current, as well as future novel mosquito technologies will continue to spread inefficiently or fail in field trials because we do not know what females find attractive in their male mates and what implications female mate choice has for their offspring. Recent studies have shown, that females choose their mating partners based on signals derived from an acoustic interaction during courtship. We performed acoustic signaling assays with *Ae. aegypti* and investigated whether those signals convey information reflected in offspring immune performance. We used a panel of immune markers to assess offspring immune performance including their melanization response and anti-bacterial defense. A critical barrier to the implementation of novel insect control technologies that rely on the reproductive success of released males is a lack of knowledge of the mechanisms and traits involved in female choice. Our results provide information on female mate choice in an important disease vector, and give insight into whether females might be able to detect traits introduced into released males with the purpose of negatively impacting offspring immune performance. In addition, our ongoing work will help us understand more about the implications of female mate choice on offspring susceptibility to arboviruses.

A Glycogenin Homolog in *Toxoplasma gondii* Glycosylates an E3 Ubiquitin Ligase and Controls Parasite Growth

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Skp1, a subunit of E3-SCF (Skp1/Cullin-1/F-box protein) ubiquitin ligases, is uniquely modified in protists by an O2-sensing prolyl hydroxylase and five glycosyltransferase activities. The first five enzymes contribute to optimal growth of the parasite *Toxoplasma gondii* in fibroblasts, suggesting that the glycan has a specialized function in toto. To test the importance of the full glycan, we identified the glycosyltransferase that follows Glt1, which adds the fourth sugar, to complete formation of the glycan. Gat1 is required for pentasaccharide formation in cells and catalyzes the addition of an alpha-galactose in 3-linkage to the subterminal alpha-glucose residue in vitro. The strong selectivity of Gat1 for Skp1 in extracts is consistent with prior evidence that Skp1 is the sole target of the Skp1 glycosyltransferases. gat1-disruption resulted in slow growth similar to disruption of glt1. The importance of the terminal sugar was reinforced by molecular dynamics simulations showing its hydrogen bonding to the polypeptide, reminiscent of a similar association in the amoebozoan *Dictyostelium* where a different glycosyltransferase assembles a distinct terminal disaccharide. The crystal structure of Gat1 from the plant pathogen *Pythium ultimum* revealed a striking similarity to glycogenin, the alpha-glucosyltransferase that initiates glycogen synthesis in yeast and metazoa. Though Gat1 exhibited low alpha-glucosyltransferase activity, autoglycosylation was not detected and gat1-disruption revealed no effect on starch accumulation. A phylogenetic analysis of the sequences suggests that glycogenin was originally a Skp1 glycosyltransferase that acquired a novel function in glycogen formation following the ancestral disappearance of the underlying Skp1 glycosyltransferase Glt1 prior to amoebozoan evolution.

Consistent, Comparative and Evidence-based Genome Annotation and Re-annotation for the Closely-related Species, *Cryptosporidium parvum*, *C. hominis* and *C. tyzzeri*

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Genome sequences for the genus *Cryptosporidium* are currently being generated with regularity. However, because of insufficient biological material for clinical isolates and the experimental resources needed for validation, fundamental gaps remain in the sequence assemblies and annotation. Currently, there are a few draft genome sequence assemblies for some *Cryptosporidium* species, but they lack experimentally-confirmed genome annotation. Our goal 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*. A manual curation of all genes in the context of existing molecular evidence and synteny was performed by using a local installation of the WebApollo2. In comparison to the previously available *C. parvum* IOWA annotation, > 1,500 annotation alterations were made. All genome sequences including the new *C. tyzzeri* genome sequence had a similar number of single-copy annotated elements, but some genes have become pseudogenes in one species or another, this can be due given the gene family variation or genome sequence misassembly. The new functional analysis is also improved by adding motif, signal peptide, transporter and transmembrane information to some of the uncharacterized predicted proteins. These improvements allowed us to revisit the metabolic pathways of the parasite and look for choke points and potential new drugs targets based on orthology to known drug targets in public databases. Comparative approaches across these closely-related genome sequences facilitated the identification of both conserved and novel features, including copy number variation and a putative rearrangement. The new annotations are available at CryptoDB and GenBank for access by the research community. Additional experimental data are essential for bettering our understanding of *Cryptosporidium*.

Drug Surveillance Study in Mali Reveals Emergence of Quinine Resistance

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Artemether-lumefantrine (AL) has replaced artesunate + amodiaquine as the frontline artemisinin combination therapy (ACT) for uncomplicated *Plasmodium falciparum* malaria in Mali. A standard World Health Organization (WHO)- recommended study was conducted in Kenieroba, Mali to monitor for the emergence of AL resistance after implemented as the frontline ACT and patients were followed for 28 days. Of the 185 patients enrolled, 177 completed the study (95.7%) and adequate clinical and parasitological response (ACPR) was 81.6%. When PCR-corrected for new infections APCR was 100%. Drug susceptibility was measured in ex vivo blood samples to dihydroartemisinin (DHA), lumefantrine (LUM), amodiaquine (AQ), chloroquine (CQ), mefloquine (MQ), piperaquine (PPQ), and quinine (QN). Geometric mean (GM) IC50was calculated (nM): DHA (0.97, 0.34-5.97), LUM (10.83, 0.82-50.82), AQ (23.35, 5.73-88.00), CQ (44.29, 4.9-724.3), MQ (13.35, 1.82-36.16), PPQ (16.25, 3.72-40.41), and QN (224.2, 9.78-1567.00). Parasites remained susceptible to all antimalarials tested except quinine and chloroquine. This is the first report of reduced susceptibility to quinine in this population and the largest range observed compared to other published works. A wide range of quinine susceptibility was discovered within the population suggesting quinine resistance is emerging in Kenieroba, Mali. Quinine susceptibility was significantly correlated with amodiaquine susceptibility; the use of amodiaquine in seasonal malaria chemoprophylaxis (SMC) could be contributing to the quinine resistance observed in the population. In Mali, sulfadoxine/pyrimethamine + amodiaquine (SP+AQ) is given to children 3 to 59 months of age at monthly intervals during the transmission season as part of the SMC program. While artemether is the frontline therapy for severe malaria, quinine is commonly used due to accessibility, and furthermore, the frontline therapy for pregnancy malaria. Additional research is needed to determine the cause of recently discovered quinine resistance in the village, its association with amodiaquine susceptibility, and if it observed elsewhere.

Poster Presentations

P1. PCR Xenomonitoring of Filarial Parasites in Mosquitoes from Cryptic Groups *Anopheles maculipennis* and *Culex pipiens*

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Taxonomy of *Anopheles maculipennis* and *Culex pipiens* complexes constitutes demanding challenge in mosquito research. Recently, new tools have emerged to distinguish members of these cryptic groups. In Europe, molecular identification methods have now begun to be used in field research and data about vector competence of individual mosquito taya are still very limited. In this paper, the preliminary results of the first PCR xenomonitoring of dirofilariosis in mosquitoes from cryptic groups in Slovakia (central Europe) are presented. In total 117 females of *An. maculipennis* and 18 females of *Cx. pipiens* groups were captured using Biogents traps enriched with carbon dioxide in Bratislava, capital city of Slovakia. Individual mosquitoes were identified by multiplex PCR and subsequently, filarioid DNA was detected by means of conventional PCR assays. During the study, two members of maculipennis complex were identified. *An. daciae* (n=56), the new-found species in Slovakia, and *Anopheles messeae* (n=61), previously incriminated malaria vector in the country. DNA of *D. repens* was detected in four *An. messeae* individuals. Within the second group, only biotypes of *Cx. pipiens* were trapped. Interestingly, co-occurrence of pipiens (n=13) and molestus (n=5) biotypes was found in urban habitat of Bratislava city centre. In the era of frequent WNV outbreak in Europe, their co-existence and possible hybridization in highly urbanized area is alarming and represents a serious risk for residents and their pets. Surprisingly, single *D. immitis* infection was detected in pipiens biotype that prefers feeding on birds. This finding can bring new insight into epidemiology of dirofilariosis, especially in central Europe, where *D. immitis* occurs only sporadically and mono-infections with the species are seldom. The study was supported by the Slovak Research and Development Agency, project APVV-15-0114 and by the Research Grant Agency VEGA project No. 2/0018/16.

P2. Role of eIF2-alpha Kinases in *Entamoeba histolytica* Stress Control

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In well-studied systems (i.e., mammalian), stress activates kinases that phosphorylate a serine residue of the eukaryotic initiation factor (eIF2-alpha). This consequently reduces the level of protein translation and permits the system to only focus on the expression of specific genes that are crucial to overcome stress. Our lab has previously shown that the level of phospho-eIF2-alpha increases when *Entamoeba histolytica* is stressed. Presumably, stress-activated kinases are responsible for phosphorylating eIF2-alpha; however, the identification and characterization of such eIF2-alpha kinases has never been undertaken in this organism. We isolated the genes encoding eIF2-alpha and the two putative eIF2-alpha kinases (EHI_109700, EHI_035950), herein referred to as eIF2ks, from *E. histolytica* via PCR. Next, we will express eIF2-alpha and these kinases in *E. coli*. We will then track phosphorylation of recombinant eIF2-alpha by recombinant eIF2-alpha kinases. To determine if these kinases are necessary for stress response, DNA encoding the kinase domains of the two eIF2ks were sub-cloned into “trigger” vectors, which may be used to knockdown expression of genes in *E. histolytica*, and transfected the vectors into *E. histolytica*. In parallel, we will use commercial eIF2-alpha kinases inhibitors to inhibit the activity of the kinases. *E. histolytica* cell lines with reduced expression of endogenous eIF2ks or *E. histolytica* cells treated with inhibitors will then be exposed to different to stress conditions (serum starvation, heat shock, and oxidative stress) and cell viability will be tracked.

P3. Polyisoprenoid Metabolism in *Plasmodium falciparum*

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Malaria continues to be one of the deadliest diseases worldwide, and the emergence of drug resistant parasites is a constant threat. *Plasmodium falciparum* is the most lethal species among the five species that cause human malaria. Due to the capacity of the parasite to develop drug resistance, there is an urgent need for new therapies with novel mechanisms of action. The isoprenoid biosynthetic pathway is a promising source of malaria-specific targets for several reasons. It occurs through the methylerythritol phosphate (MEP) pathway in the apicoplast of malaria parasites, an organelle that is absent in humans. Moreover, isoprenoid products differ from those in the human host and are involved in a wide variety of vital biological functions. Nonetheless, our knowledge of polyisoprenoid function is very limited and many aspects of its biosynthesis, distribution and interaction with other cellular structures remain unexplored. In the present work, we aimed to study polyisoprenoid metabolism with a focus on polyprenols and dolichols which are required for protein glycosylation, GPI-anchor biosynthesis and protein dolichylation. We demonstrate that polyprenols are present in the malaria parasite and differ from those present in human hosts. In addition, the function of a putative polyprenol reductase (PfPPR: PF3D7_1455900) in *P. falciparum* was confirmed by a yeast complementation assay where PfPPR was able to restore protein glycosylation and dolichol biosynthesis in the dfg10 mutant yeast strain. In addition, chemical inhibition and genetic manipulation of the pathway was also assessed. Overall, our results indicate that polyprenols are the precursors for dolichol biosynthesis in *P. falciparum* and that the pathway is essential for the parasite's survival.

P4. EuPathDB: Free, Online Omics Resources for Eukaryotic Pathogens

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The Eukaryotic Pathogen Database (EuPathDB, http://eupathdb.org) is a free, online data mining resource supporting over 190 organisms within Amoebazoa, Apicomplexa, Chromerida, Diplomadida, Trichomonadida, Kinetoplastida and numerous phyla of oomycetes and fungi. EuPathDB facilitates the discovery of meaningful biological relationships from large volumes of data by integrating pre-analyzed Omics data with advanced search capabilities, data visualization and analysis tools. The intuitive graphic interface allows users to take full advantage of the data without the need for computational training. EuPathDB integrates a wide range of data including genome sequence and annotation, transcriptomics, proteomics, epigenomics, metabolomics, population resequencing, clinical data, and host-pathogen interactions. Data are analyzed using standard bioinformatics workflows and an in-house analysis pipeline generates data including domain predictions and orthology profiles across all genomes. EuPathDB offers several perspectives for data mining – record pages which compile all data for genes, pathways, study subjects, etc; a genome browser for visualizing sequence data aligned to a reference genome; a search strategy system for querying pre-analyzed data to find genes or features that share biological characteristics, and a private workspace for analyzing primary data (via a Galaxy interface) and viewing the results in context with public data already integrated into EuPathDB. This free, comprehensive data mining resource easily merges evidence from diverse data types and across organisms to place the power of bioinformatics with the entire scientific community. EuPathDB's active user support offers an email help desk, social media, a You Tube channel and a worldwide program of workshops.

P5. Arrested Development: Gibberellin and Its Mechanism of Action on Dormant *Plasmodium* Parasites

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Malaria is a mosquito-borne infectious disease caused by the protozoan of the *Plasmodium* genus. It is a major crisis that affects 3.2 billion people across the globe. Current antimalarial treatments target the asexual blood and liver stages, as well as transmission in the mosquito vector. However, these treatments are ineffective in addressing significant problems at the stages of development at which the parasite enters a dormant period and evades these antimalarial treatments. The mechanism by which the parasite enters dormancy and later recrudesces to continue development is currently unknown. A promising method to elucidate the mechanism by which *Plasmodium* enters and exits dormancy is through the use of a plant hormone (phytohormone) called Gibberellic Acid. Exploring plant biology offers an alternative route to further understand a survival mechanism that is shared amongst organisms that may have been evolutionarily passed down. Without confirmation that the same metabolites exist in *Plasmodium*, we investigated the possible effects of supplementation of Phytohormones on dihydroartemisinin-induced dormant *P. falciparum* parasites. Gibberellic Acid (GA) promotes germination of the seed in plants and acts to release them from dormancy. We discovered that GA-treated parasites showed initial signs of normal morphology 48 hours earlier than our control, non-treated parasites. Furthermore, this effect was achieved using 10mM GA, making it extremely potent. Very little work has been published investigating gibberellins with Apicomplexans, leaving a gap in the field. The investigation of the mechanism of action of Gibberellin on *Plasmodium* will facilitate the identification of targets that could revert or activate the dormant phenotypes. This will be important for the development of novel drugs that will complement dihydroartemisinin treatment. Our current work aims at identifying the target and mechanism of action of Gibberellin using chemical biology approaches, and investigating the effects of Phytohormones on other stages of arrested development.

P6. Function of Protoporphyrinogen IX Oxidase (PPO) of *Toxoplasma* in the Pathogenesis of Toxoplasmosis

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Toxoplasma gondii is an obligate unicellular eukaryotic parasite that causes toxoplasmosis. Toxoplasmosis can be fatal to the immunocompromised population. Exploration of differences in the nutrient metabolism pathway between the host and *T. gondii* will lead to better clinical treatments for toxoplasmosis. *T. gondii* has an intact heme biosynthesis pathway. There is a knowledge gap of understanding the role of de novo heme production in the pathogenesis of toxoplasmosis. Among the eight reactions residing within the heme biosynthesis pathway of *Toxoplasma*, the enzyme catalyzing the second last reaction, protoporphyrinogen IX oxidase (TgPPO), attracts our attention, because the small chemical inhibitors have been developed against this enzyme to control weeds. We genetically ablated the *Tgppo* gene by using the latest genome editing tool, CRISPR-Cas9. The strain of *T. gondii* lacking the PPO enzyme formed smaller plaques than WT parasites and replicate at half the speed of WT parasites. Strikingly, the TgPPO-deficient parasites lost its virulence in a murine model. The potency of 11 PPO inhibitors on parasite growth is currently under investigation by using a luciferase-based growth assay. In addition, we will evaluate the toxicity of these inhibitors on host cells. My research will establish a foundation for testing the efficacy of these inhibitors on parasite's virulence in the future.

P7. Microscopy Analysis of Trypanosome Nanotubes and Extracellular Vesicles

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African Trypanosomes (*Trypanosoma brucei* spp.) are the causative agents of both African Sleeping Sickness in humans and Nagana in cattle. Extracellular vesicles (EVs) have previously been shown by the Hajduk laboratory to be crucial elements in cellular communication, host immune modulation, transfer of virulence factors, differentiation, disease progression, and pathology in trypanosomes. My studies built upon and advanced prior research from the Hajduk laboratory that discovered that EVs fuse with mammalian erythrocytes and cause host anemia, a main cause of morbidity in cattle suffering from veterinary trypanosomiasis (Nagana) and a contributor to morbidity in human infection. Using DIC and fluorescence microscopy with both live cells and fixed cells, I have been able to visualize formation of plasma membrane derived nanotubes that range from 2–20 µm and give rise to free-EVs. Nanotubes can be better visualized using the membrane probe mCLING and are estimated to occur on 80-90% of wild type cells, suggesting this is a high frequency event in culture. The role of GAPDHl, a putative fusogenic protein, may play a role in nanotube formation, structure, and stability and this is being investigated using super-resolution microscopy. There is an enormously broad range of applications to which a furthered understanding of EVs could contribute, including the formation of new treatment approaches, toward vaccinations, and also toward diagnostic tools for otherwise difficult to diagnose conditions (namely, African Sleeping Sickness).

P8. Identification and Characterization of Glycosomal and Cytosolic Nudix Hydrolases with Polyphosphate Hydrolyzing Activity in *Trypanosoma brucei*

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Trypanosoma brucei is a protozoan parasite that causes the neglected tropical disease African trypanosomiasis, also known as sleeping sickness. *T. brucei* infection in humans leads to death if untreated and in cattle causes disease called Nagana, which has considerable socioeconomic impact in developing nations of sub-Saharan Africa. *T. brucei* is well adapted to fight the host immune response as well as various stress conditions it endures in its various hosts. The parasite relies only on glycolysis for ATP production when inside the mammalian host. It activates oxidative phosphorylation once in the fly. We are interested in understanding the metabolism and functions of the inorganic phosphate polymer polyphosphate (polyP) in trypanosomes. Previous work from our laboratory reported the importance of polyP for parasite homeostasis and survival. In this work, we identified and characterized the activity of two Nudix hydrolases, NH2 and NH4 that can degrade polyP. We found that NH2 is an exopolyphosphatase with higher activity on short chain polyP, while NH4 is an endopolyphosphatase that has similar activity on polyP of various chain sizes. Both enzymes have higher activity at around pH 8.0. NH2 has a glycosomal targeting signal and it was found previously in the proteome of glycosomes, peroxisome-related organelles where glycolysis occurs. We confirmed NH2 localization using specific antibodies while endogenously tagged NH4 was localized to the cytosol. We also found that only NH2 can dephosphorylate ATP and ADP. However, NH2 has a higher affinity for polyP, which suggests that polyP could be relevant for glycosomal function. Additionally, NH2 could also participate in regulation of ATP/ADP levels. Furthermore, using the polyP-binding domain (PPDB) of yeast exopolyphosphatase we localized long chain polyP to the glycosomes of trypanosomes. Our results suggest that Nudix hydrolases can participate in polyP homeostasis and therefore may help control polyP levels in glycosomes and cytosol.

P9. Characterization of TbPex13.2 and Its Role in Glycosome Protein Import

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Kinetoplastid parasites include *Trypanosoma brucei*, *Trypanosoma cruz*i and *Leishmania*. All kinetoplastids harbor unique organelles known as glycosomes, which are evolutionarily related to peroxisomes. Glycosomes are unique in that compartmentalize much of the glycolytic and gluconeogenic pathways. While these organelles are essential to parasite viability, little is known about the molecular mechanisms that regulate glycosome dynamics. Glycosome/peroxisome biogenesis is mediated by proteins called peroxins that facilitate organelle formation, proliferation and degradation and import of proteins housed therein. Import of matrix proteins occurs via one of two pathways that are dictated by their peroxisome targeting sequence (PTS). In PTS1 import a tripeptide sequence, SKL, is recognized by the soluble receptor Pex5. In PTS2 import, a less conserved N-terminal sequence is recognized by the soluble receptor Pex7. The soluble receptors deliver their cargo to the import channel consisting minimally of Pex13 and 14. Kinetoplastids are the only organisms to have two TbPex13s, TbPex13.1 and TbPex13.2. GFP-tagged TbPex13s co-localized with glycosome proteins and that TbPex13 silencing in bloodstream form parasites impaired glycosome protein import and slowed parasite growth (Verplaetse et al. 2009). While these findings suggest TbPex13s are involved in protein import, the mechanism by which they function is unknown and it is unclear why kinetoplastids would require two Pex13s. In this work, we demonstrate that TbPex13.2 is associated with the glycosome membrane with its N-terminus facing the cytoplasm. Reduction of TbPex13.2 expression by 90% did not impact cell growth, but did cause a significant disruption in the import efficiency of some glycosome matrix proteins.

P10. The Effects of Ivermectin and Moxidectin on Canine Leukocyte Attachment to Drug-resistant and –sensitive Strains of *Dirofilaria immitis*

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Heartworm disease is a significant threat to dogs and cats in the United States. The disease is caused by the parasite *Dirofilaria immitis*, a filarial worm that is spread through mosquito vectors. There has been a recent increase in parasites resistant to the macrocyclic lactone drugs, found in all commercial preventatives. We studied five different strains of *D. immitis* microfilaria (Mf): Missouri, Georgia 2, JYD-27, Yazoo-2013 and Metarie-2014. Missouri and Georgia 2 are susceptible to Ivermectin, and JYD-27, Yazoo-2013 and Metaire-2014 are resistant. The Mf were cultured with differing drug concentrations of either ivermectin or moxidectin, naïve dog serum, and canine PMN, PBMC or PMN and PBMC. PMN plates were evaluated at 24 hours for cell attachment to the parasites, PBMC and PMN/PBMC plates were evaluated after 40 hours. As previously reported, ivermectin increased attachment of both cell types to susceptible Mf at concentrations of 1-3 nM and higher. Moxidectin gave very similar results, indicating that these effects may be common to the macrocyclic lactone anthelmintics. With Mf from resistant strains, the concentrations of ivermectin required to produce a significant increase in cell attachment over controls rose to at least 100nM; moxidectin increased cell attachment to these strains at 10-30nM. Moxidectin was equally effective as ivermectin in promoting cell attachment to susceptible Mf. In contrast, moxidectin was more effective than ivermectin in promoting attachment to resistant Mf. These data suggest that the phenomenon of drug-promoted cell attachment in vitro is relevant to the in vivo activity of the anthelmintics, since the effect was abrogated in known drug-resistant parasites. They may also suggest that moxidectin is more effective than ivermectin against the resistant strains, though more work will be required to confirm this possibility.

P11. An ER-resident Calcium Binding Protein is Required for Egress and Invasion of *Plasmodium*

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Plasmodium is the causative agent of malaria, a disease that continues to be a large burden in susceptible regions of the world. In recent years, the secondary messenger Calcium (Ca^{2+}) has been shown to be required for the progression of the intraerythrocytic lifecycle of *Plasmodium*. In the genome of *Plasmodium*, there is one protein with identifiable Ca^{2+}-binding domains in the lumen of the ER called the Endoplasmic Reticulum-resident Calcium Binding Protein (ERC). PfERC has 5 predicted EF-hands and previous studies have shown its localization to the ER, as well as its ability to bind Ca^{2+}. We employed a conditional knockdown system using the glmS ribozyme to determine the role of PfERC in the biology of *P. falciparum*. Our results show that PfERC is essential for asexual growth of parasites as knockdown results in death of the parasites. Furthermore, knockdown of PfERC delays the egress of merozoites from erythrocytes and inhibits their subsequent invasion into uninfected erythrocytes. Additionally, knockdown of PfERC does not lead to a difference in the amount of Ca^{2+} stored in the parasite, suggesting that PfERC may be involved in Ca^{2+} signaling rather than Ca^{2+} storage. These results demonstrate that the parasite's ER regulates key steps in egress and invasion of *Plasmodium*.

P12. Bacterial Endosymbionts in *Acanthamoeba* Isolates from the Nasal Mucosa and Cutaneous Lesions of Dogs

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Acanthamoeba free-living amoebae (FLA) are ubiquitous and have been isolated from various natural sources. *Acanthamoeba* can infect the nasal mucosa, cutaneous lesions and the brain of humans and animals, but it is more frequently associated with keratitis caused by the use of contact lenses. *Acanthamoeba* can be hosts for antimicorbial resistant bacteria, while most of the bacteria internalized by amoebae are rapidly digested through the phagocytic process, some bacteria have acquired elaborate ways to survive this predation. Most of these bacteria are pathogens of humans and animals, such as *Legionella pneumophila*, *Mycobacterium* spp., *Pseudomonas* spp., *Enterobacteriaceae*, *Vibrio cholera*, *Escherichia coli*, *Listeria*, *Campylobacter*, *Pseudomonas*, *Helicobacter*, *Staphylococcus aureus*, and the harboring of these pathogens within protozoa has been associated with increased survival and persistence in environment. In this study, 14 samples of *Acanthamoeba* of the genotypes T3, T4, T5 and T16 were investigated for bacterial presence and genes of antimicrobial resistance. All isolates of *Acanthamoeba* contained at least 1 bacterial endosymbiont, and one isolate contained 5 endosymbionts. We found that these isolates of *Acanthamoeba* hosted *Enterobacteriaceae* (80%), *L. pneumophila* (47%), *Mycobacterium* spp. (20%), *Pseudomonas* (60%), *Staphylococcus* spp. (53%) and *V. cholerae* (27%). Antimicrobial resistance genes were identified in some *Acanthamoeba* isolates including: mecA (Methicillin 33%), blaTEM (Ampicillin 40%), aph(3)ia (Gentamicin 7%), dfr 17 (Trimethoprim 7%), tetC (Tetracycline 7%), silP (Silver 40%), pcoD (Copper 33%), sul1 (Sulfonamides 7%), qacEa1 (Quaternary ammonium 40%). This is the first report of the isolation and characterization of *Acanthamoeba* as a host of antimicrobial resistant bacteria and associated with multi drug resistance in isolates from clinical samples of dogs.

P13. Kinetoplast Scission is Regulated by Accessory Proteins in the African Trypanosome

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The mitochrondrial genome of *Trypanosoma brucei* sp., causative agent of Human African trypanosomiasis (HAT), is comprised of a network of catenated maxicircles and minicircles (kDNA) organized as nucleoid and termed the kinetoplast. A cytoplasmic basal body (nucleation center for flagellar microtubules) is found near the kinetoplast. After duplication, basal bodies move to opposite ends of a kinetoplast. Many aspects of the pathway for division of a kinetoplast are not understood, and the mechanism of kDNA division has not been solved. Two hypotheses have been advanced to explain division of the kDNA network after/during synthesis of kDNA. In the first, movements of basal bodies are a mechanism for segregation of the mitochrondrial genome. A second hypothesis posits passive division of kDNA: minicircles are removed from the network's center, replicated, and reattached at opposite ends of the kinetoplast. However, these models cannot explain recent documentation of failed kinetoplast division when separation of basal bodies was successful. We propose a new kinetoplast division factor (KDF) hypothesis for cleavage of kDNA, based on inhibition of kDNA division by either (i) knockdown of casein kinase TbCK1.2, or (ii) treatment with small molecules NEU-617 and SB-431542. We propose that movement of basal bodies to opposite ends of the kinetoplast "licenses" the nucleoid for division and promotes recruitment of KDFs. kDNA scission occurs in G2 phase of the cell cycle. After division, the two kinetoplasts are inherited, after capture by a tripartite attachment complex, by daughter trypanosomes during cytokinesis. We predict that during knockdown or inhibition of KDF: 1) Failed kinetoplast division occurs 2) kDNA synthesis occurs. 3) Basal bodies and flagella are duplicated. 4) Movement of basal bodies to ends of kinetoplast occurs. Data on a prototypical KDF will be presented. Experiments to discover new KDFs using chemical tools and genetic probes are ongoing.

P14. Mapping the Trafficking Route of a Trypanosome Peroxin

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Trypanosoma brucei is the causative agent of human African trypanosomiasis, which threatens over 70 million people in sub-Saharan Africa. Current drugs are inadequate and the search for new drug targets essential. *T. brucei* harbors microbodies called glycosomes that are evolutionarily related to peroxisomes and harbor a number of essential biochemical pathways. Glycosome and peroxisome dynamics are regulated by proteins called peroxins that are involved organelle biogenesis, proliferation, degradation and the post-translational import of matrix proteins from the cytoplasm. Peroxisome biogenesis occurs via de novo biogenesis from the ER or division of existing organelles. During de novo biogenesis, specific peroxins localize to subdomains of the ER and bud into vesicles that eventually mature into functional organelles. De novo biogenesis has never been demonstrated in *T. brucei*. We recently detected the peroxin, TbPex13.1 in the ER and hypothesize that it traffics through the ER during de novo biogenesis. Currently, we are taking several approaches to test this hypothesis. First, we are scoring the degree to which a TbPex13.1 variant containing a glycosylation sequence is glycosylated. As glycosylation occurs in the ER, the addition of sugar moiety to the protein would indicate ER transit. Additionally, we are measuring the extent to which a TbPex13.1 variant containing the N-terminal signal peptide from the ER protein BiP (BiPNTbPex13.1) localizes to glycosomes. Identification of BiPNTbPex13.1 in glycosomes would suggest that cells contain the molecular machinery to facilitate this ER-glycosome transition. This work will provide insight into parasite-specific processes that can be exploited for therapeutic development.

P15. Investigating the Crosstalk Between Host Responses in a Rodent Model of Malaria-induced Pregnancy Loss

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Despite an overall reduction in the global malaria burden, placental malaria (PM) is a major complication of *Plasmodium falciparum* malaria that impacts hundreds of thousands of pregnancies annually. Primigravid women living in Sub-Saharan Africa are especially prone to PM and often experience poor maternal-fetal health outcomes through pregnancy loss, preterm delivery and infant low birth weight. Mouse models for malaria infection during pregnancy have been instrumental in our current understanding of PM, but our understanding remains incomplete. In the laboratory of Dr. Julie Moore, we have developed a mouse model of infection during pregnancy using the murine-infective species *P. chaubaudi chaubaudi* AS (PccAS). Infection initiated at gestation day 0 (GD 0) in mice deficient in either tumor necrosis factor (TNF) or tissue factor (TF) function and intact C57BL/6J control mice treated with antioxidant and anticoagulant drugs were used to probe host responses to malaria infection during pregnancy. While unmanipulated control mice lose their pregnancies, TNF and TF-deficient and drug-treated mice experience improved offspring viability at mid-gestation. These preliminary findings suggest that by disrupting the function of key host mediators of severe malaria, it may be possible to mitigate negative pregnancy outcomes associated with malaria. Through strategic use of genetically modified mice, combined with studies of gene expression, tissue staining, and therapeutic intervention, this work aims to investigate the crosstalk between three major host responses to infection - inflammation, coagulation and oxidative stress – and characterize how their interplay may contribute to PM pathogenesis and associated negative pregnancy outcomes.

P16. Efficacy and Mode of Action of NEU-4438, a Lead Drug for Human African Trypanosomiasis

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Trypanosoma brucei, a single cell eukaryote, causes the fatal disease Human African Trypanosomiasis (HAT). Current drugs for treatment of HAT have undesirable properties, so there is a need to identify safer alternatives. We have executed a target class repurposing strategy in a hit-to-lead campaign beginning with the 4-anilinoquinazoline drug lapatinib, which cured 25% of trypanosome-infected mice in an animal model of HAT. Matched sidechain comparisons showed that quinolinimine cores coupled with piperazine or homopiperazine substituents improved aqueous solubility and metabolic properties. NEU-4438 is a potent and trypanocidal hit (GI50 = 13 nM) with a selectivity index of at least 2,000-fold over human cells, low plasma protein binding, and excellent aqueous solubility (882 µM). Compounds were prioritized for testing in a mouse model by consideration of the trypanocidal effect, as well as safety, ADME and physicochemical properties. In a mouse model of HAT, orally administered NEU-4438 decreased parasitemia by 108-fold, establishing it as a lead for HAT drug development. In mode of action studies, NEU-4438 perturbs the proteome of *T. brucei* after a 3h exposure, reducing steady-state quantities of key proteins involved in chromosomal DNA synthesis. The drug increased steady-state amounts of some polypeptides including 10 protein kinases. Physiologically, NEU-4438 disrupted the G1/S transition in the trypanosome cell cycle, consistent with loss of protein involved in DNA replication after exposure to the drug.

P17. Targeting the *Naegleria* Glucokinase as a Therapeutic Target: an Amoeba Achilles Heel?

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The free-living amoeba, *Naegleria fowleri*, is the causative agent of primary amoebic meningoencephalitis (PAM), which while rare is usually lethal. Infection occurs when contaminated water is introduced into the nasal passage, with trophozoites then migrating to the brain. Current treatment options are limited and infection progresses rapidly to death within days. Preliminary experiments suggest glucose plays an important role in the pathogen's success and prolonged survival, as *N. fowleri* trophozoites encyst to their dormant life stage when cultured in media without glucose. Here, we describe work toward characterization of a new potential therapeutic target, the lone glucose phosphorylating enzyme in the parasite's genome, glucokinase(NfGlck). This enzyme is likely responsible for the first step in both glycolysis and the pentose phosphate pathway, both of which utilize the NfGlcK product glucose-6-phosphate. Following cloning and heterologous expression of NfGlck, we have optimized enzyme assay conditions, revealing the enzyme is inhibited by saturating amounts of substrate (ATP) and product (ADP). The protein is predicted to be 49.3kDa and by gel filtration chromatography the protein behaves as a monomer with an apparent molecular mass of 47.7kDa. Last, we have screened a collection of known inhibitors of *Plasmodium falciparum* and *Trypanosoma brucei* hexokinases against the protein. At present, none of the scaffolds tested inhibit the enzyme, suggesting a new screen may be necessary. These findings, along with efforts to genetically validate the target by RNA interference and gene knockout, will be discussed.

P18. Evaluation of eIF2-α Phosphorylation in *Entamoeba histolytica* in Response to Nitrosative or ER Stress

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Entamoeba histolytica is an intestinal parasite infecting over 50 million people worldwide, and is the causative agent of amoebic dysentery and amoebic liver abscess. Infection begins after ingestion of the environmentally-stable cyst, followed by an in vivo transformation to the pathogenic trophozoite. In the human host, *E. histolytica* experiences stress brought on by nutrient availability and the host immune response. In other systems, the stress response may include down-regulation of protein translation, which is regulated by phosphorylation of eukaryotic initiation factor (eIF-2α). Previous work in our lab has demonstrated a similar response in *E. histolytica*, where eIF-2α phosphorylation increased significantly when exposed to long-term serum starvation, oxidative stress, and long-term heat shock; however, the effect of nitrosative and ER stress have yet to be evaluated. Nitrosative stress is part of the host's normal immune response and ER stress has been shown to elicit quality control mechanisms that involve the phosphorylation of eIF-2α. We exposed trophozoites to various nitrosative and ER stress conditions and measured levels of total and phosphorylated eIF-2α. To date, we have found that dithiothreitol, an inducer of ER stress, significantly increases phosphorylation of eIF-2α. Moreover, our lab has generated transgenic cell lines that overexpress wildtype EheIF2α, a non-phosphorylatable variant of eIF-2α in which the phosphorylated Ser was mutated to alanine (EheIF2α-S59A), or a phosphomimetic variant of eIF-2α in which the phosphorylated Ser was mutated to aspartic acid (EheIF2α-S59D). To answer the question about whether phosphorylation is necessary and sufficient to counter nitrosative and ER stress, we will expose these mutants to nitrosative and ER stress and record viability.

P19. The New Rat Lungworms?: The Occurrence of *Physaloptera hispida* and a *Mastophorus* sp. in Pulmonary Vessels of the Hispid Cotton Rat (*Sigmodon hispidus*) from Georgia, USA

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Physaloptera (Nematoda: Physalopteroidea) and *Mastophorus* species (Nematoda: Spiruroidea) are stomach parasites of many wildlife and domestic animals. For the few species with known life cycles, larvae develop in insects which when ingested by the vertebrate host undergo complete development within the stomach. During a survey for *Angiostrongylus cantonensis* (Nematoda: Metastrongyloidae) (Rat Lungworm) in rodents from Piedmont and Coastal physiographic regions of Georgia, USA, a single adult nematode was recovered from the pulmonary vessels of four individual hispid cotton rats (*Sigmodon hispidus*). Two each of these worms were identified as *Physaloptera* or *Mastophorus* sp. by morphology. This is the first report of these parasites in the pulmonary vessels of a definitive host. There was no evidence that these parasites migrated post-mortem. To better characterize these parasites, representatives were collected from cotton rat stomachs and identified morphologically and genetically characterized. Based on partial cytochrome c oxidase subunit 1 (COI) gene sequences, *Physaloptera hispida* from stomachs were identical to the Physaloptera sp. from the pulmonary vessels. The COI sequences from Mastophorus sp. were more variable but confirmed the pulmonary worms were the same species as the stomach worms and that sequences from *Mastophorus* from a coastal site grouped separately from *Mastophorus* from a Piedmont site. Our data show that adult worms recovered from pulmonary vessels of cotton rats could be either *Physaloptera* or *Mastophorus* spp. suggesting that these parasites are not always restricted to the stomach and that worms from pulmonary vessels must be carefully examined and not assumed to be *A. cantonensis*.

P20. Optimizing VacSIM® Delivery of Malaria CelTOS and CSP Antigens to Enhance Vaccine Efficacy

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Globally, malaria infects 220 million people and accounts for approximately 550,000 deaths annually. With half of the world's population at risk for the disease, there is an urgent need to develop an effective vaccine. At this time, we still do not have a highly efficacious malaria subunit vaccine. The most promising malaria vaccines require 3 to 5 vaccinations to induce varying levels of protection. Therefore, the field is looking to advances in adjuvant and/or vaccine delivery methods to increase vaccine efficacy, while reducing the number of doses. VacSIM® (Vaccine Self-assembling Immune Matrix) is a novel, 3-dimensional vaccine delivery method that enhances immune responses to several different vaccine antigens, and likely will enhance efficacy of CelTOS- and CSP-based malaria vaccines. After injection, VacSIM® self-assembles in situ to form a 3-D hydrogel, which allows for antigen persistence and enhanced immune responses. Non-reactogenic, stable without a cold-chain, and a plug-and-play platform, VacSIM® also reduces local and systemic reactogenicity to molecular adjuvants. The results presented here are the first step in the project, optimizing VacSIM® delivery parameters of *Plasmodium berghei* (Pb) CelTOS and CSP in mice. First, we optimized the injection route and the kinetics of immunogen release by changing VacSIM® concentration. Next, using optimal concentration and route parameters, we optimized the prime-boost interval. Our next step will use sporozoite challenge to evaluate efficacy after vaccinating with PbCelTOS and PbCSP in VacSIM® under the optimized conditions. Future work includes testing VacSIM®-delivered *P. falciparum* CelTOS and CSP proteins in mice using optimized delivery conditions. We then hope to extrapolate these results toward the long-term goal of generating a functional subunit human malaria vaccine. Enhancement of vaccines by VacSIM® delivery may represent a fundamental paradigm shift in how vaccines are developed and delivered.

P21. Network Analysis of *Plasmodium cynomologi* Infection and Re-infection Challenge

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Plasmodium vivax is a significant source of malaria infection in South America and Asia. *Plasmodium cynomologi* infection of Rhesus macaques serves an excellent non-human primate model system for P. *vivax* malaria, enabling us to study in vivo gene expression during infection. High-throughput sequencing technologies and network analysis techniques provide the means to quantify genome-wide expression, and organize patterns within the data gained. Monkeys were infected with harvested *P. cynomologi* sporozoites to induce malarial illness at the Yerkes National Primate Research Center. RNAsequencing was then used to quantify gene expression from peripheral blood samples at several points during the experiment. Weighted Gene Correlated Network Analysis (WGCNA) was used to identify gene expression modules from RNAseq data from a study of *P. cynomologi* infection in malaria naïve macaques, followed by two rounds of re-infection, first with the homologous *P. cynomologi* strain, then with a heterologous *P. cynomologi* strain. To determine whether gene expression patterns are conserved during multiple malaria exposures, modules identified during initial infection, homologous re-infection, and heterologous re-infection were compared for overlap. Finally, the immunological processes represented by each module were assessed using bioinformatics. Bioinformatics results showed enrichment for similar processes across initial infection, homologous reinfection, and heterologous reinfection. Specifically, enrichment was seen for T-cell processes, B-cells, natural killer cells, and cell cycle regulation. These results indicate that both the innate and adaptive immune response may be involved in malaria control. However, more immune processes were enriched in modules identified during initial and heterologous reinfection, indicating that second exposure to the same strain produces a different immune response than a previously seen strain. These results are consistent with lower parasitemia during homologous reinfection. Network analysis, therefore, potentially reveals the adaptive immune response to initial malaria infection, and indicates how genetic diversity of malaria strains help them evade the immune system.

P22. Exploring the Role of a Potential Glucose Sensor in *Trypanosoma brucei*

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Trypanosoma brucei is the eukaryotic pathogen responsible for causing human African trypanosomiasis and a disease called nagana in cattle. *T. brucei* has a complex lifecycle, with different life stages in both the human host and the tsetse fly vector. In the mammalian blood, the parasite utilizes glucose as its sole energy source through the glycolytic pathway. Exploiting our understanding of glucose signaling in yeast and other model systems, we have begun to resolve mechanisms involved in the response of *T. brucei* to glucose. RNAseq analysis revealed that a number of critical pathways are modulated by the parasite in response to growth under minimal glucose conditions. Here we knockdown a potential glucose sensor (PGS) in bloodstream and procyclic life stages and monitored growth of these parasites when cultured in different glucose concentrations. qRTPCR analysis has revealed evidence of reduced gene regulation in response to varying glucose conditions in parasites with the PGS silenced. These data suggests PGS plays an important role in a *T. brucei* glucose signaling pathway.

P23. Rate Summation Fails to Estimate *Anopheles stephensi* Trait Performance under Thermal Fluctuation, Which Alters Predictions of Malaria Transmission

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Vectors, hosts and the diseases they carry exist in a variable world. However, most laboratory studies are conducted at constant temperatures. Rate summation is a computational technique that can be used to infer the performance of traits in a fluctuating environment from performance at constant temperatures. Rate summation has been shown to breakdown at the extreme ends of the thermal ranges for other ectothermic organisms, thus, the accuracy of laboratory studies performed under constant temperatures in recapitulating transmission dynamics for mosquitoes under real-world temperature fluctuation is unknown. Individually-housed *An. stephensi* were placed across five mean temperatures under both a constant and fluctuating temperature regime. We directly measured bite rate, mortality rate, and fecundity daily for each individually-housed mosquito across our temperature treatments. Trait performance under fluctuating conditions were estimated using rate summation and directly compared to empirically derived performance. Furthermore, the implications these deviations have on transmission dynamics were evaluated with a temperature-dependent mechanistic R0 model.

P24. Putative Lysosomal Chloride Transporters in *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular eukaryotic parasite that invades a wide range of mammalian hosts. During the invasion, the parasites secrete proteins from microneme, a unique organelle in *Toxoplasma*. Before the micronemal proteins arrive at microneme, some of them experience the proteolytic cleavage within the parasite's endolysosomal system for optimal efficiency. A previous study has revealed that the late endosome (LE) is the place where micronemal proteins meet the maturase, a cathepsin L-like protease (TgCPL), for trimming. However, the TgCPL self-maturates within the vacuolar compartment (VAC), a lysosome-equivalent structure, in the parasites. Only a minute amount of TgCPL shuffles from the VAC to the LE to conduct micronemal maturation. Since the activity of TgCPL is pH-dependent, the precise control of pH within parasite's endolysosomal system is crucial. To help acidification of the acidic organelles, anion transporters are needed to help neutralize the accumulation of positive charge produced by proton pump. Therefore, we speculate that the endolysosomal anion transporters are involved in parasite invasion. By homolog search, we identified two putative lysosomal chloride transporters by using human lysosomal transporter as a sequence template. These two transporters TGGT1_265500 and TGGT1_290330 (TgClC1 and TgClC2, respectively) were endogenously tagged with epitope tag by using the latest genome editing tool, CRISPR-Cas9. The immunofluorescence assay determined that the TgClC2 localizes in the LE exclusively, while the TgClC1 exists in both of the LE and VAC. I have successfully deleted the TgClC2 in *Toxoplasma*. Currently, I am conducting a series of phenotypic studies to determine whether the loss of an LE-associated chloride transporter can reduce microneme secretion and parasite invasion. In addition, I am testing whether the chemical inhibition of chloride transporters affects maturation of micronemal proteins. Understanding of how *Toxoplasma* regulates pH within its endolysosomal system may provide a new therapeutic strategy for toxoplasmosis.

P25. TrypSpotting: Identifying Lipid Droplet Proteins in *Trypanosoma brucei*

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Trypanosoma brucei, the protozoan parasite that causes African sleeping sickness, alternates between mammalian and tsetse fly hosts, which offer dramatically different environments to which the parasite must adapt in order to survive. The host tissues encountered by *T. brucei* differ in their availability of nutrients, such as fatty acids. In environments where fatty acids are scarce, the parasites will have to rely on fatty acid synthesis and stored fatty acids to meet its needs. A potentially important source of stored fatty acids comes from lipid droplets, which are dynamic organelles involved in lipid storage and homeostasis. Lipid droplets in *T. brucei* are largely uncharacterized. To identify proteins involved in lipid droplet formation and dynamics in *T. brucei* we have screened the TrypTag imaging database for proteins whose GFP-tagged localization resembles the punctate staining pattern expected for lipid droplets. For each candidate lipid droplet protein, we have generated RNA interference cell lines for the inducible knock-down of each gene's expression. For each candidate, we will examine the effect of RNAi-mediated knockdown on (1) cell growth using flow cytometry; and (2) lipid droplet staining patterns using a fluorescent lipid droplet stain, LipidTox. We will also confirm the localization of each candidate protein using an in situ epitope tagging approach independent of that used in the TrypTag database. Candidate genes with a confirmed effect on lipid droplets upon RNAi induction, and/or confirmed lipid droplet localization will be further characterized for their role in *T. brucei* growth and survival in its mammalian and tsetse fly hosts.

P26. Repurposing FDA-approved Compounds to Identify a Treatment Against the Brain-eating Amoeba

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The brain-eating amoeba, *Naegleria fowleri*, is a free-living amoeba that causes the disease primary amoebic meningoencephalitis (PAM). The amoeba can infect healthy individuals and within 2 weeks causes nearly 97% fatality in those infected. Although there have been 3 survivors in recent years, current therapeutics are not effective and current drug discovery efforts are minimal. In this study, we use a high-throughput assay that we previously developed to identify active compounds from St. Jude Children's Research Hospital FDA-approved compound collection and the Medicines for Malaria Venture Pathogen Box. We identified 24 hits from the FDA-approved library and 13 hits from the MMV Pathogen Box that produced > 70% growth inhibition. We confirmed 29 of the 37 single-point hits with dose-response curves. Since the disease progresses rapidly, it was important to identify compounds that quickly inhibit the amoeba. To do so, we developed an assay that measures the relative luminescence units every hour to identify the relative abundance of cells over time. In this assay, we compared hits identified in the screens to current treatments (amphotericin B, fluconazole, azithromycin, and miltefosine). Of the current treatments, azithromycin was the first compound to begin inhibiting the amoeba at 30 hours. In contrast, we found posaconazole, ketoconazole, butenafine and terbinafine to start inhibiting *N. fowleri* trophozoites within 6 to 12 hours. Due to the amount of drugs currently given to patients, it was important to identify potential antagonistic activity with the hits found in this study. We found posaconazole to show additive activity when combined with azithromycin, amphotericin B, and miltefosine. Lastly, we tested posaconazole in our in vivo PAM model. We found that dosing mice with 20mg/kg of posaconazole IV once a day for 3 days after they are infected with *N. fowleri* provides cures in 33% of the mice ($p < 0.05$).

P27. Macrocyclic Lactone (ML) Anthelmintics Lack Meaningful in vitro Activity Against L3 and L4 Stages of Both ML-susceptible and ML-resistant *Dirofilaria immitis*

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Macrocyclic lactone (ML) anthelmintics are the only treatment available for preventing vascular infection with *Dirofilaria immitis* (Di). In recent years, reports of resistance have increased, however, the mechanism of action of ML drugs against Di remains unclear. In this study, we tested the in vitro dose response of both L3 and L4 stages of ML-susceptible (Missouri strain) and ML-resistant (Metairie-2014 strain) Di, using both ivermectin (IVM) and eprinomectin (EPR) at concentrations ranging from 0.625 to 20 µM. L3 were isolated from mosquitoes and used directly, or were cultured for 6 days to obtain L4 before adding drug. Culture media was composed of NI media with 10% FBS and supplemented with antibiotics. Motility measurements were performed every 24 hrs for 4 days using a computer-processed video imaging software (Worminator system), and a nonlinear regression model was fit to the dose-response data. Mean IC50 values for IVM and EPR were 3.33, CNC and 8.93, CNC uM for the susceptible and resistant strains, respectively, for L3, and CNC, 10.73 and 14.47, 9.74 uM, respectively, for L4. Maximal inhibition of motility ranged from 59-92% with no apparent biological differences between strains or larval stages. In vitro exposure to MLs failed to achieve complete inhibition in the ML-susceptible strain, even at the highest concentration tested (20 µM), which is >5,000X times higher than the in vivo plasma concentration following a preventive dose of IVM. There were also no apparent differences in dose-response between ML-susceptible and ML-resistant strains. With regards to the stages of Di targeted by monthly preventive doses of MLs, these data strongly suggest that the mechanism of action and of resistance in Di are not mediated through paralytic effects and that ML drugs do not demonstrate meaningful in vitro activity against Di.

P28. A Bacterial Complex is Required for Plastid Integrity in *P. falciparum*

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The deadly malaria parasite, *Plasmodium falciparum*, contains a non-photosynthetic plastid known as the apicoplast, that functions to produce essential metabolites. Little is known about its biology or regulation, but drugs that target the apicoplast are clinically effective. Previous studies have identified several prokaryotic Clp (caseinolytic protease) genes, encoded by the *Plasmodium* genome. In bacteria, the evolutionary ancestors of the apicoplast, and in plants 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. Using phylogenetic analysis, we identified the Clp members that may form a regulated proteolytic complex in the apicoplast. We genetically targeted members of this complex and generated conditional mutants of the apicoplast-localized PfClpC chaperone, PfClpP protease and PfClpR inactive protease subunit. Conditional inhibition of the PfClpC chaperone resulted in growth arrest and apicoplast loss, and was rescued by addition of the essential apicoplast-derived metabolite, IPP. Moreover, cellular assays suggest that PfClpC inhibition interferes with the ability of the schizont stage parasites to divide and sort functional apicoplast organelles to daughter-merozoites. Using a double conditional-mutant parasite line, we discovered that the chaperone activity is required to stabilize the active protease, revealing functional interactions. Finally, bacterial Clp inhibitors were screened to test selective activity against *Plasmodium* Clp members. These data demonstrate the essential function of PfClpC in maintaining apicoplast integrity and its role in regulating the proteolytic activity of the Clp complex.

P29. Effect of Fatty Acid Synthesis Inhibitor Cerulenin on Bloodstream Form *T. brucei*

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Trypanosoma brucei ssp. are blood-borne parasites transmitted by the bite of a tsetse fly (*Glossina* spp.) that cause Human African Trypanosomiasis (HAT) in humans and Nagana, a wasting disease in cattle. There is no vaccine for HAT because the parasites have evolved strategies to evade the immune system including upregulation of endocytosis and antigenic variation. Central to immune evasion are the Variant Surface Glycoproteins (VSGs), proteins anchored to the plasma membrane by myristate, a 14-carbon fatty acid. T. *brucei* depends on fatty acid synthesis to be able to make myristate, as this fatty acid is scarce in the host. Previously, it was shown that RNAi-mediated reduction of acetyl-CoA carboxylase, the first enzyme in fatty acid synthesis, reduced endocytosis and immune evasion. Here, we examine Cerulenin, a fatty acid synthesis inhibitor previously shown to block production of myristate in vitro in *T. brucei* lysates, but only inhibited growth in culture, rather than killed the parasites (i.e. trypanostatic rather than trypanocidal). In contrast to previous findings, we found that Cerulenin killed bloodstream form *T. bruce*i in culture with an EC50 of 4.64 μM. Currently, we are examining the effect of Cerulenin on fluid-phase endocytosis and myristate production in intact BF *T. brucei* grown in culture. Targeting endocytosis as well as inhibiting the production of myristate in the parasite may lead to novel methods to treat HAT and Nagana.

P30. Natural Products as a Source to Discover Novel Drug Targets in *P. falciparum*

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Malaria is a deadly infectious disease that caused an estimated 216 million cases around the world in 2016 according to the World Health Organization. *Plasmodium falciparum* is the parasite responsible for causing the majority of malaria cases in humans. Resistance to all current frontline antimalarial therapies is a growing problem thus, there is an urgent need for new antimalarial drugs with novel targets. Natural products have a lengthy history of utility in drug discovery and remains a great source of new therapeutics. We have screened against *P. falciparum* in vitro cultures over 230 natural compounds isolated from various plants indigenous to the southern parts of China which have long been used in traditional Chinese medicine to treat malaria. This collection presented different degrees of antimalarial activity and toxicity toward mammalian cells. Two sesquiterpenoid dimers that exhibited potent antiplasmodial activities (<100 nM) and low toxicity to mammalian cells were selected to study their mechanism of action in more detail by inducing drug resistance in in vitro culture of *P. falciparum*. We were able to obtain resistant parasites against one of the selected sesquiterpenoid dimers. Clonal subcultures were subjected to next generation Illumina sequencing to identify the potential mechanism of resistance that may lead to the identification of the molecular target. In addition, a subset of similar sesquiterpenoid dimers present in this collection of natural products were screened against the resistant parasites and the analysis has outlined a starting point for structure-activity relationship studies. We have identified a new class of promising antiplasmodial compounds due to their unique scaffold that is different from all currently known antimalarials and is likely acting through a new mechanism of action.

P31. Metabolic Dependency of Chorismate in *Plasmodium falciparum*

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Human malaria accounts for more than 400,000 deaths a year, with *Plasmodium falciparum* being the deadliest of the five species that infect humans. Artemisinin Combination Therapies (ATCs) are the frontline treatment for malaria; however, resistance to artemisinin has been 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. Among the potential metabolic drug targets absent in humans is the shikimate pathway for chorismate biosynthesis, which has been postulated to be essential for *P. falciparum*'s survival. Interestingly, two recent studies indicated that chorismate synthase is not essential in *P. berghei*. Chorismate is a branching metabolite used to synthesize p-aminobenzoic acid (pABA, an intermediate in folate biosynthesis), aromatic amino acids, and p-hydroxybenzoic acid (pHBA, an intermediate in ubiquinone biosynthesis). This study aimed to assess the fate of chorismate and its metabolic dependencies. We have identified an inhibitor that targets the shikimate pathway using reversal of growth inhibition, and it was used as a tool to assess the metabolic dependencies of chorismate in *P. falciparum*. The identified inhibitor is cytotoxic for the malaria parasite, specifically affecting the transition of trophozoite to schizont stage. Our results support that the molecular target is within the 5-step AROM multicomplex of the shikimate pathway inhibiting chorismate's production and that folate biosynthesis is the main metabolic fate of chorismate in *P. falciparum*. Inhibition of chorismate biosynthesis does not affect the synthesis of ubiquinone, indicating that the production of pHBA, the precursor of the benzoquinone ring, may not be derived from chorismate or that alternative sources may be used. Further studies will be needed to elucidate the origin of the ring of ubiquinone in *P. falciparum*.

P32. Identification of a Novel Protein Complex Involved in RNA Polymerase II Transcription Termination in Kinetoplastids

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The nuclear genome of all kinetoplastids contain unusually long Polycistronic Transcription Units (PTUs), which are transcribed by RNA Polymerase II and flanked by O-linked glucosylated thymine residues (called base J). Alterations in base J levels lead to changes in gene expression, suggesting a role of base J in RNA Polymerase II initiation and termination. The first step in base J synthesis is the hydroxylation of thymine residues by the thymidine hydroxylases JBP1 and JBP2, followed by a subsequent glucosylation reaction by the glucosyl transferase JGT. To fully understand the role of base J in transcription, we set out to purify associating proteins for the JBPs and JGT. We have identified a protein complex in kinetoplastids, which, in analogy to the yeast WDR82 and PNUTS/PP1 complex, is involved in termination of RNA Polymerase II transcription. Associated with this complex we have also identified a novel base J binding protein (JBP3). Binding of this protein complex to J containing DNA flanking the PTUs, would be the first example of the direct involvement of base J in transcription termination in kinetoplastids. We are currently investigating the role of the individual components from the complex and their precise role in termination of RNA Pol II transcription.

P33. A Fixable Probe for Visualizing Flagella and Plasma Membranes of the African Trypanosome

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The protozoan *Trypanosoma brucei* sp. cause diseases in humans and animals. Studies of *T. brucei* cell biology have revealed unique features, such as major endocytic events being limited to a single region, and mitochondrial genome segregation mediated via basal bodies. Further understanding of trypanosome cell biology can be facilitated with super-resolution fluorescence microscopy. Lack of a plasma membrane probe for fixed trypanosomes remains a persistent problem in need of a working solution. Herein, we report protocols developed using mCLING in super-resolution structured illumination fluorescence microscopy (SR-SIM). mCLING comprehensively labels flagellar membranes, including nascent intracellular stages. To extend its usefulness for trypanosome biology we optimized mCLING in combination with organelle-specific antibodies for immunofluorescence of basal bodies or mitochondria. Then in work with live trypanosomes, we demonstrated internalization of mCLING into endocytic stations that overlap with LysoTracker in acidic organelles. Greater detail of the intracellular location of mCLING was obtained with SR-SIM after pulsing trypanosomes with the probe, and allowing continuous uptake of fluorescent concanavalin A (ConA) destined for lysosomes. In most cases, ConA and mCLING vesicles were juxtaposed but not coincident. A video of the complete image stack at the 15 min time point shows zones of mCLING staining surrounding patches of ConA, consistent with persistence of mCLING in membranes of compartments that contain luminal ConA. In summary, these studies establish mCLING as a versatile trypanosome membrane probe compatible with super-resolution microscopy that can be used for detailed analysis of flagellar membrane biogenesis. In addition, mCLING can be used for immunofluorescence in fixed, permeabilized trypanosomes. Its robust staining of the plasma membrane eliminates a need to overlay transmitted light images on fluorescence pictures obtained from widefield, confocal, or super-resolution microscopy.

P34. Using High-resolution Mass Spectrometry to Decipher the Isoprenome in *Plasmodium falciparum*

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In 2016, cases of human malaria numbered over 216 million worldwide with over 445,000 deaths due in large part to *Plasmodium falciparum*, the most lethal malaria parasite species. Given the prevalence of malaria around the globe, it is of great importance to understand as much as possible about the metabolic profile of the parasite to discover novel ways to target biosynthetic pathways. High-resolution mass spectrometry (HRMS) is a powerful tool for elucidating the metabolome of biological organisms, and we will be focusing specifically on polyisoprenoids, long chain hydrocarbons synthesized by the methylerythritol 4-phosphate pathway (MEP) in *P. falciparum*. Two subtypes of polyisoprenoids are dolichols and prenols, which differ in the saturation vs. unsaturation of the alpha-terminal carbon, respectively. We first set out to determine if we could detect polyisoprenoids in Escherichia coli labeled with a stable isotope precursor [1-13C]-glucose, a metabolic precursor of isopentenyl phosphate (IPP). IPP is the 5-carbon isoprene unit that joins together to comprise isoprene chains. We were able to visualize a Gaussian distribution on the isotopic envelope for ubiquinone-8 in isotopically labeled *E. coli*. Ubiquinones, which consist of a quinone ring and a polyisoprene chain, are members of the polyprenylbenzoquinones. As expected, undecapolyprenol was not detected in *E. coli*. Upon these encouraging results we moved forward with a labeling of *P. falciparum* with [1-13C]-glucose. While we were unable to locate labeled ubiquinone species, we have detected biosynthesis of dolichols and prenols larger than 11 and 12 isoprenic units in *P. falciparum*. These results support that we can identify isoprenoids via HRMS and are promising for future labeling studies to study biosynthetic pathways of isoprenoids.

P35. Impacts of Temperature on Zika Virus Transmission Potential

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Diseases like Zika, dengue, and chikungunya, which were once considered tropical and sub-tropical diseases, are now threatening temperate regions of the world due to climate change and increasing urbanization. Temperature is a strong driver of vector-borne disease transmission, and characterizing the thermal range and optimum for transmission is crucial for accurately predicting arbovirus emergence and spread. To address the lack of data on the relationship between temperature and key pathogen traits for emerging arboviruses, we conducted a series of experiments to estimate the thermal performance of Zika virus (ZIKV) in field-derived *Aedes aegypti* across eight constant temperatures. We observed strong, unimodal effects of temperature on vector competence, extrinsic incubation period, and mosquito survival. We used thermal responses of these traits to update an existing temperature-dependent R0 (the basic reproductive number) model, to infer how temperature impacts ZIKV transmission. We demonstrated that ZIKV transmission is optimized at a mean temperature of approximately 29°C, and has a thermal range of 22.7°C to 34.7°C. The predicted thermal minimum for Zika transmission is 5°C warmer than for dengue virus which suggests that current estimates on the global environmental suitability for Zika transmission are over-predicting its possible range. Accurately characterizing the unimodal effect of temperature on emerging arboviruses, like ZIKV, is critical for estimating the potential geographic and seasonal range for transmission, and accurately predicting where future climate change will increase, decrease, or have minimal impact on transmission.

P36. The Gut Microbiota is Required for Normal Egg Formation in the Yellow Fever Mosquito, *Aedes aegypti* L. (Diptera: Culicidae)

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The adult yellow fever mosquito *Aedes aegypti* harbors a diverse gut microbiota comprising approximately 100 species. This microbiome is mainly acquired during the juvenile life stage from the mosquito larval aquatic habitat. Previous research from our group showed that mosquito larvae depend on a bacteria-induced hypoxia signal for development, although specific bacterial taxa are not required. Given the indispensable role for bacteria in larval development, we hypothesized that bacteria may also play an essential role in the developmental processes in adults, which principally concern egg formation in females. To test this hypothesis, we developed methods for culturing of axenic (microbe-free) adult *Ae. aegypti*. Here we report that axenic *Ae. aegypti* adults exhibit multiple defects in egg production over successive oogenic cycles. Axenic adults also exhibit defects in lipid transport. Following bloodfeeding, neural lipid droplets accumulate in gut cells and fail to be shuttled to the principle metabolic storage tissue, the fat body. Zhou, Pennington and Wells (2004) previously reported that mosquitoes provision developing eggs primarily using mobilized protein and lipid stores, while ingested blood is invested in replenishing these stores for the following oogenic cycle. Hence, we propose that the gut microbiota serves a critical role in egg formation in adult *Ae. aegypti* due to its importance in bloodmeal nutrient processing and consequent replenishment of metabolic reserves.

P37. Characterization of a Phospholipase C-like Protein (TbPI-PLC2) from *Trypanosoma brucei*

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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. Cell14:486-494, 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.

P38. An ER-resident Hsp40 is Required for the Asexual Development of the Malaria Parasite *P. falciparum*

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Malaria remains a significant global health burden. The parasitic disease kills hundreds of millions of people every year, with infection by *Plasmodium falciparum* associated with the most severe cases of malaria. All of the clinical symptoms of malaria result from the asexual replication of *Plasmodium* parasites within human red blood cells (RBCs); thus, an understanding of the mechanisms used by the parasite to survive within the RBC is critical. The Endoplasmic Reticulum (ER) is an organelle central to parasite biology, and its function is required for parasite survival. The ER serves as the starting point for protein trafficking to other organelles and to the host RBC, and stress response signaling from the ER is associated with parasites surviving treatment with the frontline anti-malarial Artemisinin. ER-resident chaperones support ER function and are therefore ideal candidates for exploring the parasite's biology. To this end, we have generated a conditional knockdown parasite line for PfJ2, a putative ER-resident Hsp40 expressed throughout the asexual cycle. Using this parasite line, we have confirmed that PfJ2 is an ER-resident protein and is essential for parasite survival inside the RBC. Specifically, knockdown of PfJ2 results in delayed parasite development during the trophozoite stage before failure to complete schizogony to form new invasive parasites. ER functions, such as protein trafficking and stress response, will be assayed during PfJ2 knockdown to investigate the chaperone's role in these processes. Additionally, PfJ2 uniquely contains both a J-domain and a thioredoxin-like domain, and we will explore how these domains contribute to PfJ2 function. By elucidating the role this essential chaperone plays in parasite biology, we will gain a better understanding of the mechanisms used by the parasite to survive in the RBC and cause disease.

P39. Structure-activity Relationship (SAR) Investigation of Monosaccharide Derivatives: Discovery of Biologically Active and Competitive *Trypanosoma cruzi* Glucokinase Inhibitors

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There are 6 – 7 million people worldwide affected by Chagas' disease, a neglected tropical disease caused by the protozoan parasite *Trypanosoma cruzi*. Such individuals principally reside in Latin American countries, spanning from Mexico to Argentina, where medical treatment is sparse and outdated. The antichagasic drugs currently available include only benznidazole and nifurtimox and were developed over 35 years ago. Side effects include peripheral neuropathy, vomiting, nausea, and insomnia. Recently, benznidazole was approved for use in the United States but it was not previously accepted due to its poor set of side effects. There is an urgent need to develop alternative drugs to combat this disease. Our laboratory previously demonstrated that *T. cruzi* glucokinase (TcGlcK) could be significantly inhibited with glucosamine-analogue inhibitors and the most potent of the series was carboxybenzyl glucosamine (CBZ-GlcN). TcGlcK is proposed to be an essential drug-target of the protozoan parasite and is situated at the first step of glycolysis, where glucose becomes phosphorylated at the expense of ATP. By inhibiting TcGlcK, the flux of glycolysis becomes diminished and leads to cellular apoptosis. The focus of the SAR investigation was to develop twenty-one monosaccharide derivatives around the design of CBZ-GlcN. As such, these compounds were synthesized, purified, and fully characterized by 1H-NMR, 13C-NMR, and HRMS. The synthesis of each compound involved a one-step hydrolysis reaction of seven different acyl chloride derivatives with D-glucosamine, D-mannosamine, and D-galactosamine. These twenty-one compounds were screened against TcGlcK to assess enzyme inhibition and they were further screened against the *T. cruzi* (Tulahuen strain) infective form (trypomastigote and amastigote life stages).

P40. Spontaneous Dormancy Protects *Trypanosoma cruzi* During Extended Drug Exposure

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Microbial dormancy, defined as a state of reduced metabolic activity, is well-recognized in bacterial systems as a mechanism of transient resistance to environmental stresses, including antibiotic treatment. The ability of the Chagas disease agent *Trypanosoma cruzi* to resist extended in vivo exposure to highly effective trypanocidal compounds prompted us to explore the potential for dormancy and its contribution to failed drug treatments in this infection. Using both the incorporation of the nucleotide analogue EdU and the cell proliferation tracking dyes CFSE and CellTrace Violet, we detected non-proliferating amastigotes in vivo in mice and in vitro in cultured host cells. *T. cruzi* amastigotes became dormant in the absence of stressors such as drug selection and were present in host cells that contained substantial numbers of other actively dividing amastigotes. Non-proliferative amastigotes ultimately converted to trypomastigotes and established infections in new host cells. Most significantly, dormant amastigotes were uniquely resistant to extended drug treatment in vivo and in vitro and could re-establish a flourishing infection after as many as 30 days of drug exposure. These results demonstrate a dormancy state in *T. cruzi* that accounts for the failure of highly cytotoxic compounds to completely resolve the infection. The ability of *T. cruzi* to establish dormancy throws into question current methods for identifying curative drugs but also suggests alternative therapeutic approaches.

P41. Strand-specific RNA Sequencing in Zoonotic Protozoan Pathogen *Cryptosporidium parvum* Suggests Widespread and Developmentally Regulated Long Non-coding RNA Transcription

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Cryptosporidium is an apicomplexan unicellular parasite identified as the second most prevalent diarrheal pathogen in the world, infecting both humans and animals. Due to difficulties of working with *Cryptosporidium*, transcriptomic analyses, despite their utility, are still lagging. To date, the regulatory elements orchestrating critical parasite processes remain largely unknown. Yet it is becoming increasingly clear that long non-coding RNAs (lncRNAs) could represent a missing regulatory layer across a broad range of organisms including *Cryptosporidium*. In this study, strand-specific RNA sequence data for both dormant and post-infection stages of *C. parvum* enabled the annotation of many lncRNA candidates of various types. We have performed a global analysis with these candidates and examined their: transcriptional expression periodicity, transcription correlation with neighbor coding genes and intron splicing events. Given the compact genome of *C. parvum*, the analyses revealed that at least 17% of protein-coding genes were complementarily overlapped with possible lncRNAs, indicating the possibility of antisense-like transcription in *C. parvum*. A general positive correlation was observed between lncRNA expression and expression of upstream genes. The existence of intron-containing lncRNAs supported by RNA-Seq reads suggested they may be transcribed and processed like mRNAs with the possible flexibility of functions provided by the intron. This work has contributed to the initial characterization of the *C. parvum* non-coding transcriptome and may facilitate further insights into the potential function of lncRNAs in parasitic development and parasite-host interaction as was recently published by the group of Chen XM, Creighton University in 2017.

P42. Dramatic Morphological Changes in *T. brucei* upon Over-expression of Lipid Droplet Targeting Proteins

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Lipid droplets (LDs) are dynamic organelles formed from the ER involved in lipid storage and metabolism. LD proteins and the roles of LDs in *Trypanosoma brucei* remain poorly characterized. To enable purification of *T. brucei* LDs for proteomic characterization, we generated eYFP-tagged constructs designed to target LDs in *T. brucei*. Two constructs, thuPLIN and thuPLIN-CC, were based on human Perilipin 1, whose amphipathic helix (residues 93-192) was sufficient to target GFP to yeast LDs. Transfection of these over-expression constructs into procyclic cells resulted in dramatic cellular elongation (up to ~40µm), a halt in cell division, and eventual culture death. Moreover, the fluorescence patterns did not resemble the punctate pattern expected for LD localization: both thuPLIN and thuPLIN-CC eYFP fusions showed diffuse cytosolic localization, with thuPLIN also exhibiting point-like structures. The localization became more reticular as the cells elongated, and frequently fluorescence signal was lost. Though the elongated forms resembled epimastigotes, DAPI staining showed no anterior re-positioning of the kDNA (a hallmark of epimastigote differentiation), indicating these are not true epimastigotes. Introduction of elongated cells into extremely low glucose media (SDM79Θ) caused reversion of elongated cells to WT morphology, maintenance of fluorescence as detected by microscopy and flow cytometry, and restoration of cell division. Re-introduction of SDM79Θ-grown cells back into SDM79 recapitulated the lethal elongation and cell division arrest phenotype. Taken together, these results suggest that over-expression of thuPLIN constructs in glucose-abundant media results in lethal elongation that is reversibly abrogated by growth in very low glucose media.

P43. Development of Parainfluenza Virus 5 (PIV5) Based Zika Virus Vaccine

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Zika virus (ZIKV) is a positive sense RNA virus that belongs to the family Flaviviridae. This mosquito borne virus has been linked to birth defects and an autoimmune neurological syndrome. To this date, there is no licensed vaccine available. Parainfluenza virus 5 (PIV5), a paramyxovirus, does not cause any known disease in humans and has been used as a platform for viral vectored-vaccine development. This study is focused on testing PIV5 based vaccine candidates for ZIKV. We made recombinant PIV5 viruses expressing ZIKV genes using the reverse genetics system and subsequently, examined the growth kinetics and protein expression. After generating the vaccine candidates, animal studies were performed on BALB/c mice to determine the neutralizing antibody titer and vaccine efficacy.

P44. Discovery and Development of Trophocidal and Cysticidal Compounds for the Treatment of *Acanthamoeba* Infections

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The pathogenic free-living amoeba, *Acanthamoeba*, cause several diseases including a severe brain disease known as Granulomatous Amoebic Encephalitis (GAE), with a 90% mortality rate. *Acanthamoeba* has also been found to cause Amoebic Keratitis (AK), in association with poor contact lens hygiene, which may result in blindness. The current drug regimens were not originally discovered for *Acanthamoeba* infections but have shown in vitro and in vivo potency against these organisms. The high mortality shows that these compounds are not the best indicative treatment for the patients. New specifically developed drugs for these diseases need to be discovered, developed and implemented into the current drug regimen to give patients a better chance of survival or loss of eyes. Herein, using high-throughput screening methods we screened several large compound libraries from Calibr (11,968 compound ReFrame library), an FDA approved drug library (1,134 drug library - Dr. Kiplin Guy formerly at St. Jude Children's Research Hospital) and Medicines for Malaria Ventures (MMV Malaria Box (400 compounds) and Pathogen Box (400 compounds)) in search for new active chemical scaffolds. We identified 67 compounds to possess sub micro-nanomolar potency against *Acanthamoeba*, confirmed through dose response secondary screening. Fifty two of these compounds are newly described to have any activity against these amoebae, 15 compounds have been previously documented within the literature. Nanomolar potency compounds were tested for cysticidal activity in a newly developed 30 day high-throughput cysticidal screening method developed in our lab. Through collaborations with Dr. David Boykin at Georgia State University, using bis-benzimidazole amidine and diamidine scaffolds through a structure activity relationship (SAR) study we discovered several compounds that possess anti-trophozoite activity. Only the nanomolar inhibitors were further tested for cysticidal activity. Forty two compounds mainly from the SAR studies were discovered to have cysticidal or cystistatic activity against three clinical isolates of *Acanthamoeba*.

P45. Investigating the Molecular Epidemiology and Transmission Potential of *Trichomonas* spp. from Hunter-killed Columbiformes in California

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Avian trichomonosis, a disease caused by flagellated protozoan parasites of the *Trichomonas* genus, can lead to deadly epidemics in wild bird populations. To determine if sympatric mourning doves (*Zeneida macroura*), white-winged doves (*Z. asiatica*), and Eurasian collared-doves (*Streptopelia decaocto*) may be involved in transmission of *Trichomonas* spp. to Pacific Coast band-tailed pigeons (*Patagioenas fasciata monilis*) in southern California, 32 hunter-killed doves were sampled using oral swabs and the *Trichomonas foetus* InPouch™ culture system (BioMed, Inc.) during September, 2015. Our sampling location was located in a neighboring county (Imperial County) to a documented *Trichomonas*-associated mortality event in band-tailed pigeons in San Diego County in January and March, 2015. Inoculated InPouches™ were incubated at 37°C and evaluated for motile trichomonads by light microscopy every other day for 7 days. Trichomonads were detected by culture in 43% (10/23) of mourning doves, 100% (6/6) of white-winged doves, and 33% (1/3) of Eurasian collared-doves for an overall prevalence of 53% (17/32). PCR was performed on each culture-positive sample, targeting the ITS1-5.8S-ITS2 region. Genotypes from mourning doves and white-winged doves most closely aligned with ITS-group genotypes I, J, and L, which were previously isolated from a white-winged dove from Texas, a mourning dove from Texas, and three species (mourning doves, white-winged doves, and Cooper's hawks (*Accipiter cooperii*)) from Arizona, respectively. The single isolate from the Eurasian collared-dove most closely aligned with ITS genotypes C, D, and E, which were from columbids from Colorado, Georgia, Tennessee, Texas and one unknown geographic origin. The *Trichomonas* spp. implicated in the January and March 2015 mortality event, *T. gallinae* ITS-group A, subtype 2, and *T. stablerii,* were not detected in this study. Ongoing investigations of avian host species, trichomonad genotype, and geographic associations among California wild birds continue to improve our understanding of the ecology and epidemiology of avian trichomonosis.

P46. Expression, Purification of Recombinant Guinea Pig Cytokines and Chemokines for Monoclonal Antibody Production

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For over a century, the guinea pig has been one of the most widely used animal infection model systems for studying bacterial, viral and parasitic pathogens. A limitation of the model is the dearth of immunological reagents available against cellular targets; commercially available antibodies targeting guinea pig-specific cytokines and immune cell markers are especially lacking. Without proper reagents, the immune response to infection and protective effects of vaccination using this model cannot be quantified. Accordingly, our goal is to develop murine monoclonal antibodies against guinea pig cytokines IL-4, IL-8, IL-10, and MCP-1; these cytokines have been shown to be crucial in both mice and humans for assessing the efficacy of vaccines, such as the BCG vaccine against Mycobacterium tuberculosis. In our study, we used a stable expression system for the overproduction and purification of Guinea pig cytokines IL-10 and MCP-1 in *Escherichia coli* for murine antibody production. Here we present the challenges encountered with potential solutions in expressing and purifying recombinant guinea pig cytokines and chemokines from a commercially available bacterial expression system for later use in antibody development.

P47. Protective Neutralizing Antibodies to Highly Pathogenic Avian Influenza H5N8 and H5N2 in Blue-winged Teal (*Anas discors*)

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In 2014, highly pathogenic (HP) H5N8 avian influenza viruses were first reported in Eurasia. These HP H5N8 viruses subsequently reassorted with North American low pathogenicity (LP) avian influenza viruses, leading to the emergence of novel HP H5Nx viruses. While wild birds in the order Anseriformes (ducks, geese, swans) are known natural reservoirs for influenza A viruses (IAV), the HP H5Nx viruses were apparently not successfully maintained in North American wild birds. We hypothesize that a possible mechanism for this may be that wild birds had existing immunity to the HP viruses due to previous exposure to a wide-range of LP IAV, including viruses of the H5, N1, N2, and N8 hemagglutinin (HA) and neuraminidase (NA) subtypes, respectively. Serum samples from Blue-winged Teal (BWTE; *Anas discors*) were collected in Louisiana and Texas during the spring, from 2012 to 2017, and screened for antibodies to IAV. Sera were tested by a blocking enzyme-linked immunosorbent assay (bELISA); 58.84% (1518/2580) of the samples were positive for antibodies to the nucleoprotein of IAV. Of the bELISA positive samples, 55.01% (n=835) were screened by a virus microneutralization (MN) assay against two HP (rg-H5N8 and rg-H5N2) and two LP (H5N1 and H5N2) IAV. Sera collected before, during, and after the outbreak tested positive to antibodies against the LP H5N1, H5N2, and HP H5N8 antigens; only sera collected during and after the outbreak tested positive to HP H5N2 antibodies. Of the samples tested, 54.61% (n=456) had neutralizing antibodies to the LPAI viruses, 4.79% (n=40) had neutralizing antibodies to the HPAI viruses, and 3.71% (n=31) were antibody positive for both HP and LP IAV. The samples will be further tested by enzyme-linked lectin and hemagglutination-inhibition assays to try to better understand the role of antibodies to NA and HA, respectively, in the wild bird immune response to HPAI.

P48. Retrospective Investigation of Translocated Elk in Tennessee (USA) and Examination of Canid Definitive Hosts for *Echinococcus granulosus*

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Echinococcus granulosus is a zoonotic cestode parasite that maintains a life cycle in wildlife in North America between ungulates and canids. *E. granulosus* has been described in wildlife in Canada with few reports in the Unites States; however, the geographical distribution of *Echinococcus* species in wild hosts is increasing consequent to human activities. In 2000, the Tennessee Wildlife Resource Agency reintroduced elk sourced from Elk Island National Park in Canada to the North Cumberland Wildlife Management Area in the southeastern US. From 2000 to 2008, 201 elk were released, and the population continues to increase. We investigated the prevalence of *Echinococcus* spp. in the re-established elk and wild canid populations in an area with no previous reports of this disease in wildlife. We performed a retrospective search of elk necropsies at the University of Tennessee College of Veterinary Medicine (UTCVM) from 2002 to 2017 to identify elk with lesions consistent with echinococcosis. Archived paraffin blocks were sectioned, stained with H&E and examined microscopically to identify characteristic lesions. Subsequent PCR and sequencing targeting the parasite mitochondrial COX1 gene were performed on sections of paraffin blocks with corresponding lesions. In addition, from January to March 2018 we necropsied and performed complete helminth exams on 11 coyotes killed in areas surrounding the elk relocation zone. In total, 4 elk were PCR positive for *E. canadensis*. Each sequence had at least 98% coverage and identity to multiple *E. canadensis* genotypes in Genbank. Adult *E. granulosus* were not detected in any of the coyotes examined in this study. Continued surveillance of this disease in susceptible species in this area is warranted and these data further underscore the risk of pathogen introduction due to wildlife translocation.

P49. Pathogenesis of a Novel Avian Influenza Virus, A/New York/108/2016 (H7N2)

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Low pathogenicity avian influenza viruses (AIVs) are ubiquitous in waterfowl, generally causing little to no clinical disease. The H5 and H7 subtypes are known to mutate from low to high pathogenicity and therefore are a public health concern. The novel isolate was transmitted to a cat in a shelter in New York to a veterinarian and has been the first H7N2 subtype to be transmitted to a human in North America since 2003. In this study, we investigated the pathogenicity and pathogenesis of A/New York/108/2016 (hNY/16) (H7N2) in the murine model. Two strains of mice (DBA/2J and BALB/c) were intranasally inoculated with 1 x 106 PFU hNY/16 and clinical disease, virus titer, tropism, and serology were assessed. Infection of mice with hNY/16 caused morbidity in both BALB/c and DBA/2J mice, and caused significant mortality of DBA/2J mice. Weight loss, a measure a disease, was significant in both mouse strains. hNY/16 demonstrated a tropism for the upper and lower respiratory tract as it efficiently replicated in lung tissue, and was also capable of replicating in the nasal tract. Infection with hNY/16 induced protective HAI serum antibodies. Antigenic analysis revealed cross-protective HAI antibody response to a more closely related H7N2 avian isolate (A/chicken/NJ/150383-5/2002), but little to no cross reactivity against two more distantly related H7N2 isolates (A/chicken/ PA/13552-1/1998 and A/chicken/NY/13142-5/1994).

P50. Phosphoinositide Phospholipase C and Calcium Signaling in *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite that infects approximately one third of the world population. The infection may result in congenital disease and become clinically relevant in immunocompromised patients. *T. gondii* pathogenesis is directly linked to its lytic cycle, which starts when the parasite invades a host cell, replicates, and exits to find another host cell. Calcium signaling has been shown to have a role in gliding motility, host cell invasion, microneme secretion and host cell egress, all features of the *Toxoplasma gondii* lytic cycle. The genome of *T. gondii* reveals genes involved in Ca^{2+} signaling. Major Ca^{2+} stores have been characterized, such as the endoplasmic reticulum (ER), the acidocalcisome, and the plant-like vacuole. There is evidence for presence of an active IP3 signaling pathway. Phosphoinositide phospholipase C (PI-PLC), the IP3 producing enzyme, has been localized to the plasma membrane of the parasite and shown to be essential for parasite growth, and microneme secretion, and as a precursor of other signaling molecules such as phosphatidic acid. We characterized calcium entry in *T. gondii* and found that the parasites allow influx of calcium via two permeation pathways with different affinities. One of these mechanisms is regulated by intracellular signaling involving cyclic GMP, protein kinase G and a TRP channel. Our goal is to identify the participation of a PI-PLC in this pathway and study its effect in calcium signaling and influence in the parasite lytic cycle. Conditional down regulation of the PI-PLC gene led to reduced mRNA and protein levels after 48h incubation with anhydrotetracyclin. We studied intracellular calcium fluctuations in these parasites, loading their cytosol with Fura2AM calcium indicator. We found that PI-PLC is involved in calcium entry, specifically in the pathway activated by high concentrations of extracellular Ca^{2+} and sensitive to TRP channel inhibitors.

P51. The Impact of STING Pathway Activation During *Trypanosoma cruzi* Infection

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Host cell invasion by the protozoan parasite *Trypanosoma cruzi* is a markedly silent process that induces a modest, yet reproducible type I interferon (IFN-I) response. Previous studies have demonstrated the requirement of the signaling molecules TBK1 and IRF3 in the induction of IFN-I during *T. cruzi* infection, but the upstream sensors involved remain unknown. In this study, we report that the *T. cruzi*-mediated IFNβ induction occurs through the STING pathway with the involvement of the cytosolic DNA sensor cGAS. The IFNβ transcript induction in wild-type (WT) primary murine macrophages following infection was almost entirely abolished in macrophages lacking cGAS or STING. Moreover, infected cGAS-/-, STING-/- and IFNAR-/-macrophages had significantly higher amastigotes, indicating that activation of the STING pathway during infection limited intracellular parasite growth. To further assess the impact of STING activation in vivo, mice were infected in the footpads with tdTomato-expressing parasites and the fluorescence intensity at the site was quantified to monitor parasite growth kinetics. Despite an initial increase in parasite growth, we unexpectedly found that the absence of STING and IFNAR resulted in less parasite growth at the site than WT mice. Additionally, immunophenotypic analysis revealed that this decreased parasite growth was correlated with a limited proportion of inflammatory monocytes recruited to the site. Administration of recombinant IFNβ to STING-/-- mice at the site of infection subsequently increased parasite growth compared to mock-treated controls. Therefore, STING signaling promotes parasite growth at the site of infection through an IFN-I-mediated recruitment of monocytes that favor parasite growth. However, the absence of STING did not confer significant protection in preventing the establishment of chronic infection in mice. Future studies will assess the impact of exogenous STING activation on parasite control, while exploring potential parasite modulation of the STING pathway to address the modest nature of the endogenous response to infection.

P52. Regulation of Calcium Entry by Calcium-binding Proteins

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Fluctuations of the cytosolic calcium ion (Ca^{2+}) concentration regulate a variety of cellular functions in all eukaryotes. Ca^{2+}signals derive from either intracellular stores or from the external medium. Plasma membrane channels control Ca^{2+} entry from the external medium in response to stimuli like membrane depolarization, extracellular agonists and also depletion of intracellular stores. We studied Ca^{2+} entry in *Toxoplasma* and its downstream impact in gliding motility, microneme secretion, conoid extrusion, and invasion. We found two mechanisms of calcium influx with different affinity for calcium and different downstream roles. We characterized five calmodulin-like proteins for their potential role in calcium influx and a molecule annotated as Transient Receptor Potential (TRP) channels. Phenotypic analyses of mutants for two calcium-binding proteins (ELC1 and ELC2) showed calcium entry defects, which are recovered in the complemented lines. We propose that these calmodulin-like proteins regulate calcium entry in addition to their role in activation of the glideosome. CaM2, another Ca^{2+}-binding protein, localizes to the conoid and mutants for this gene showed a defect in the regulation of calcium entry, and calcium release from intracellular stores, suggesting a role for this Ca^{2+}-binding protein in Ca^{2+} homeostasis. In addition, we characterize a new Ca^{2+} binding protein, TgGT1_255660 (EF60), that localizes to the Golgi. Mutants for this gene show a defect in the release of calcium from intracellular stores, suggesting a role in the regulation of the mechanisms of release or uptake of intracellular stores. Our hypothesis is that similarly to neuronal synapses and cilia, plasma membrane channels will create functional domains of Ca^{2+} signaling molecules and calcium-binding proteins will be part

of these domains.

P53. Extracellular Vesicles Produced by African Trypanosomes a Potential Tool for Diagnostics

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African trypanosomes are the causative agents of human sleeping sickness and Nagana, a disease of cattle. Infections are initiated by the bite of tsetse flies with the parasites initially restricted to the blood and lymphatics. During late-stage infections trypanosome invade the central nervous system causing neurological symptoms. We recently discovered that African trypanosomes produce membranous nanotubes, from the flagellar membrane, that breakdown to form small (~100nm) extracellular vesicles (EVs) and contribute to virulence, host immune evasion, and erythrocyte clearance (anemia) corresponding with disease. Trypanosome EVs deliver proteins and lipids to other trypanosomes and host cells by fusion with the plasma membrane of the recipient cell. There is currently a lack of diagnostic methods able to detect and differentiate the different subspecies of African trypanosome. We postulate that EVs can serve as a sensitive biomarker to allow detection of trypanosome infection in mammals. Our goal is also to identified EV proteins that will serve to distinguish trypanosome subspecies in samples collected in the field. The proposed studies use a combination of biochemical approaches to identify EV proteins for use in the diagnostic assays and animal studies to assess the sensitivity of the assays in trypanosome-infected mice.

P54. Curative Effect of Modified Benznidazole Dosing Regimens in Chronic *Trypanosoma cruzi* Infection

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Developing effective chemotherapies for *Trypanosoma cruzi* infection remain an urgent need. Currently, treatment relies on two compounds: benznidazole (BNZ) and nifurtimox (NFX). Both exhibit potential side effects, variable efficacy and are administered in an intensive daily regimen (~ 60 days) that leads to cessation of treatment in ~20% of individuals. Previously, we showed that reducing BNZ treatment to only once every 5 days for 60 days was as effective as a 40-dose daily BNZ protocol to achieve cure. Here, we asked whether less frequent (weekly) but continuous BNZ therapy for months might be more effective than the daily protocols. We established three treatments in mice infected 100 days previously: a) 500mg/kg weekly, b) 100 mg/kg weekly, or c) 10 daily doses of 100mg/kg of BNZ followed by weekly 100 mg/kg doses. To assess treatment efficacy, we determined the memory status of *T. cruzi*-specific CD8+ T cells that indicates whether or not mice had recently encountered parasite antigen, and thus remained infected. We also monitored antibody levels over time and determined the presence of parasite DNA in tissues in a subset of mice. Both the mice receiving a weekly dose of BNZ at 100 mg/kg and those receiving 500mg/kg, showed an increased frequency of CD127+ cells among the *T. cruzi*-specific CD8+ T cells. However, mice in the high dose group had a higher frequency of CD127+ cells as well as a decline in the levels of antibodies over the treatment course. A subset of mice with the highest frequency of CD127+ terminated at 37 weeks of treatment showed undetectable levels of parasite DNA in tissue or parasites in hemocultures, suggesting parasitological cure. Our results suggest that less frequent BNZ dosing over an extended period of time might be as, or more effective in curing *T. cruzi* infection than the currently used intensive (daily), shorter-term treatment.

P55. Developmental Changes in Extracellular Vesicles from African Trypanosomes (*Trypanosoma brucei brucei*)

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*Trypanosoma brucei bruce*i, a subspecies of African trypanosomes, produces extracellular vesicles (EVs) that are released from the plasma membrane. Two developmental stages of this parasite, the procyclic form found in the midgut of infected tsetse flies and the mammalian bloodstream form, produce nanotubes that extend from the flagellar pocket and break down into EVs. The procyclic trypanosomes also release EVs from multivesicular bodies in a form of exocytosis. Extracellular vesicles are suggested to have a variety of functions including the transfer of virulence factors between bloodstream form cells and the regulation of social motility in the procyclic form. Such differences in function make it likely that the EVs of both forms have significantly different protein contents and likely differ from the total protein samples of whole cells of their respective developmental forms. Analysis of the EVs of both forms using nanoparticle tracking analysis and the preliminary use of SDS-PAGE will be used to compare procyclic and bloodstream EVs. This will be followed by mass spectrometry of the EVs of both developmental forms to determine specific protein contents compared to the known contents of the overall organism. Extracellular vesicles from procyclic and bloodstream *T. b. brucei* will also be analyzed for the ability to fuse and deliver proteins, RNAs and lipids to mammalian and insect cells. This research will provide a better understanding of the difference in the proteomes of the developmental forms of *T. b. brucei* and their EVs and how these differences impact the various functions of the EVs.

P56. Role of a Secreted Effector of *Toxoplasma gondii* in Modulating the Host Cell Cycle

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The obligate intracellular protozoan parasite, *Toxoplasma gondii*, infects nearly a third of the global human population and has the remarkable capacity to infect any nucleated cell of warm blooded animals. As a consequence of infection, host cells undergo a rapid and dramatic reprogramming of transcriptional pathways relating to metabolism, the immune response and cell cycle. How *T. gondii* is able to manipulate such a broad range of host cell processes is the current focus of this work. After invasion, *Toxoplasma* forms and resides within a membrane bound compartment known as the parasitophorous vacuole (PV) that protects it from many host cell defense mechanisms. It is within the PV that the parasite replicates and exports secreted proteins into the host cell to modulate host cellular machinery. The source of many PV targeted proteins is a unique secretory organelle known as the dense granules (DG) which have recently been shown to be an important compartment harboring host modulating protein effectors. In this study, we have identified a dense granule protein that translocates across the PV membrane and traffics to the host cell nucleus. Parasites lacking this protein do not demonstrate altered growth or virulence yet produce distinct changes in their host cell cytoskeleton. RNAseq analysis has demonstrated that the primary pathways affected by loss of this protein center on changes in the host cell cycle. As a result, we have since named this protein ICC1 for Inducer of the Cell Cycle protein 1. Our data suggests that ICC1 is responsible for driving the infected host cell into the S-phase of the cell cycle as parasites lacking this protein fail to effectively transition out of G0/1. We have therefore revealed an important role of this effector in altering host cell proliferation.

In our characterization of ICC1, we aim to investigate its host targets and ultimately the mechanistic basis for its activity.

P57. Effects of *Plasmodium falciparum* on Placental Expression of Inflammatory and Coagulation Factors

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Plasmodium falciparum infection during pregnancy can develop into placental malaria (PM), the cause of over 200,000 infant deaths annually. PM involves sequestration of infected red blood cells (iRBCs) and deposition of hemozoin (HZ) in the maternal blood space of the placenta. HZ is a toxic byproduct of the parasite's digestion of hemoglobin. The role of the syncytiotrophoblast, the outermost cell layer of the placenta, during pathogenesis remains unclear. It is hypothesized that the syncytiotrophoblast responds to infection but leads to negative outcomes including low birthweight and growth restriction. HZ is hypothesized to induce expression of tissue factor, the initiator of the extrinsic coagulation pathway, which results in fibrin deposition. Coagulation factors activate protease activated receptors (PARs) expressed on the syncytiotrophoblast. Activated PARs increase expression of tumor necrosis factor (TNF), which leads to more tissue factor expression. In vivo syncytiotrophoblast-parasite interactions are simulated using a human choriocarcinoma cell line, BeWo, and primary human cells, trophoblasts. BeWos and trophoblasts are unstimulated or stimulated with lipopolysaccharide (LPS; positive control), HZ, TNF, or HZ/TNF. RNA isolation, cDNA generation, and qPCR for tissue factor, PAR-1, and PAR-2 is conducted. Results to date show TNF stimulation results in 2-fold increase in tissue factor mRNA expression at 2 hours. LPS stimulation results in 2-fold increase in both tissue factor and PAR-1 expressions between 2 to 4 hours. HZ stimulation results in a 2-fold increase in tissue factor expression. Identifying the extent to which the syncytiotrophoblast is involved in PM pathogenesis will advance the understanding of how this syndrome impacts fetal development and specify potential therapeutic targets.

P58. Using Forward Genetics to Identify Calcium Channels in *Toxoplasma gondii*

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Toxoplasma gondii, the causative agent of toxoplasmosis, is an apicomplexan parasite currently infecting one third of the world's population. Among humans, *T. gondii* becomes clinically evident in immunocompromised individuals and fetuses through congenital transmission. Our lab is interested in understanding the mechanics of intracellular calcium (Ca^{2+}) signaling in *T. gondii*. Calcium signaling is a conserved evolutionary process in all branches of life, yet remains poorly understood in apicomplexans. To remain viable, the tachyzoite life stage must successfully invade host cells, replicate inside a parasitophorous vacuole and egress to seek another host cell to invade. Calcium has been shown to stimulate each major step of the lytic cycle, strongly supporting its importance for the overall physiology of the parasite. Our lab has biochemical evidence showing Ca^{2+} influx into the cytosol of *T. gondii* tachyzoites in response to the addition of extracellular calcium. Furthermore, we have detected inhibition of calcium entry in the presence of calcium channel blockers. These data suggest that *T. gondii* has developed a network to regulate intracellular calcium levels. However, plasma membrane Ca^{2+} channels or molecules homologous to characterized proteins involved in Ca^{2+} entry have not been discovered in *T. gondii*. We hypothesize that *T. gondii* expresses specific Ca^{2+} channels at the cell membrane essential for fundamental signaling pathways. My goal is to identify these calcium channels using a forward genetics approach.

P59. Identification of Macrophage Cell Surface Receptors with Binding Affinity for the Helminth Glycan, LNFP3

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Parasitic helminths are capable of modulating host immune responses to survive. Lacto-N-fucopentaose III (LNFP3) is a helminth immunomodulatory glycan found on the surface of schistosome eggs and schistosomula. Helminth infections often result in a CD4+ type 2 (Th2) immune response along with anti-inflammatory activation of antigen presenting cells (APCs). Studies have demonstrated that helminth glycans are required to bias the host to Th2 type. Unraveling the mechanism(s) by which helminth glycans such as LNFP3 bias APCs to an anti-inflammatory phenotype is critical to discovering anti-inflammatory therapeutics to treat type 1 mediated autoimmune diseases. Alternative activation of APCs and Th2 immune responses occur after LNFP3 is internalized by APCs via a receptor mediated and clathrin dependent process. The aim of this study is to identify APC receptors that bind LNFPIII neoglycoconjugates (NGC), subsequently driving anti-inflammatory activation. Membrane proteins were extracted from RAW 264.7 macrophages. LNFP3 NGC was biotinylated with EZ-Link Sulfo-NHS-Biotin. A pull down assay was performed to confirm the existence of a physical interaction between the macrophage receptors and LNFP3 NGC. The samples were run on an acrylamide protein gel. Bands unique to LNFP3-macrophage interactions will be identified by mass spectrometry.