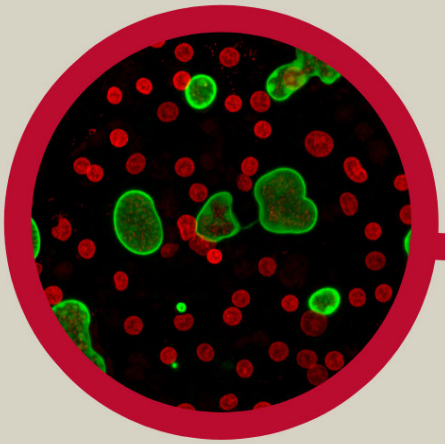

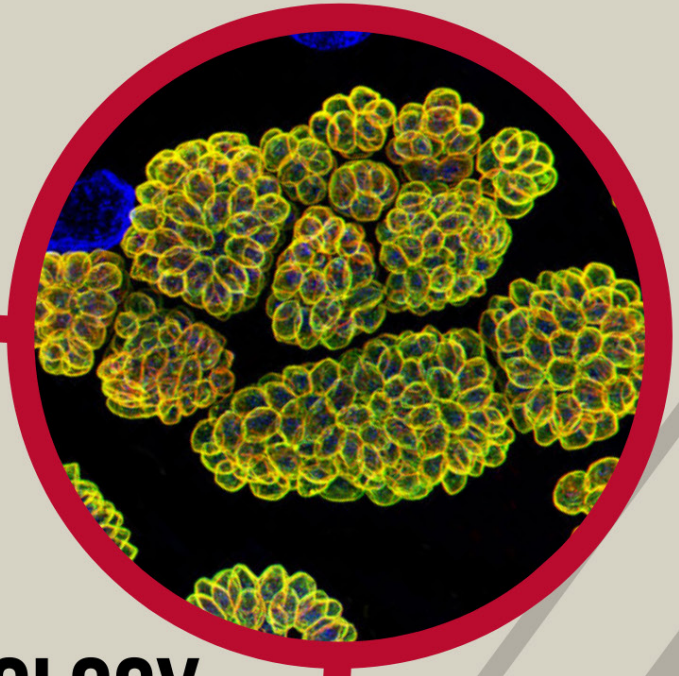

32ND MOLECULAR PARASITOLOGY & VECTOR BIOLOGY

SYMPOSIUM

GEORGIA CENTER FOR CONTINUING EDUCATION

ATHENS, GA

MAY 2, 2023

Center for Tropical & Emerging Global Diseases

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Program

8:30 AM	Registration and Poster Set-up
9:00 AM	Opening Remarks: **Dennis Kyle**, Director of CTEGD
	SESSION 1 — Moderators: Anna Gioseffi, Reagan Haney, Ruby Harrison
9:10 AM	**Justin Wiedeman**, Center for Tropical and Emerging Global Diseases, UGA A novel conditional knockdown system in *Trypanosoma cruzi* reveals essential roles for the Aurora and Polo-like kinases in parasite cell division
9:30 AM	**Watcharatip Dedkhad**, Center for Tropical and Emerging Global Diseases, UGA Exocytosis of egress-specific vesicles in *Plasmodium falciparum*
9:50 AM	**Benjamin Liffner**, Dept of Pharamcology & Toxicology, Indiana University School of Medicine Investigating sporozoite development through the mosquito
10:10 AM	**BREAK — POSTER VIEWING (even posters)**
	SESSION 2 — Moderators: Katherine Moen, Benjamin Phipps, Cassie Russell
10:50 AM	**Mayara S. Bertolini**, CTEGD and Dept. of Cellular Biology, UGA Vacuolar Transporter Chaperone 4 (TcVtc4) is essential for the infective stages of *Trypanosoma cruzi*
11:10 AM	**INTRODUCTION OF EARLY CAREER SCHOLAR**
11:15 AM	**Sabrina Absalon**, Dept. of Pharmacology & Toxicology, Indiana University School of Medicine Decoding new players in *Plasmodium* nuclear biology using expansion microscopy
12:10 PM	**LUNCH — POSTER VIEWING**
	SESSION 3 — Moderators: Saniya Sabnis, Justine Shiau, Grace Woods
1:10 PM	**Donovan Cantrell**, Dept. of Biochemistry & Molecular Biology, UGA The Skp1 α-Galactosyltransferase Gat1 regulates SCF assembly in *Toxoplasma gondii*
1:30 PM	**Charles-Chess**, CTEGD and Dept. of Cellular Biology, UGA Role of memory T regulatory cells in recurrent malaria
1:50 PM	**Baihetiya Baierna**, CTEGD and Dept. of Cellular Biology, UGA Characterization of the mitochondrial ubiquinone synthesis pathway in *Toxoplasma gondii*
2:10 PM	**Madelaine Usey**, CTEGD and Dept. of Cellular Biology, UGA *Toxoplasma gondii* ATP synthase subunits containing coiled-coil-helix-coiled-coil-helix (CHCH) domains are essential for mitochondrial function
2:30 PM	**BREAK — POSTER VIEWING (odd posters)**
	SESSION 4 — Moderators: Ruby Harrison, Anna Gioseffi, Benjamin Phipps
3:10 PM	**Brooke White**, Center for Tropical and Emerging Global Diseases, UGA Deep PCR sampling of blood allows consistent detection of *Trypanosoma cruzi* infection in chronically infected primates with widely different parasite loads
3:30 PM	**A. Cassiopeia Russell**, CTEGD and Dept. of Infectious Diseases, UGA Small RNAs within the extracellular vesicles of the brain eating amoeba, *Naegleria fowleri*, and their potential as diagnostic biomarkers
3:50 PM	**INTRODUCTION OF THE KEYNOTE SPEAKER**
4:00 PM	**Robert Seder**, Cellular Immunology Section, National Institutes of Health Scientific and clinical development of monoclonal antibodies to prevent and eliminate malaria
5:00 PM	Concluding Remarks: **Dennis Kyle**

Poster Presentations

P1 **Lindsey Baker,** Dept. of Natural Sciences, University of South Carolina Beaufort
Overexpression, purification, and biochemical evaluation of a *Trypanosoma cruzi* glucokinase bump mutation for drug-target validation

P2 **Juan Camilo Arenas-Garcia**, CTEGD and Dept. of Cellular Biology, UGA
The role of polyphosphates in *Toxoplasma gondii*

P3 **Emily Bremers**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Understanding *Plasmodium falciparum*'s multidrug resistance protein through the characterization of a novel antimalarial class

P4 **Alex Rosenberg**, CTEGD and Dept. of Infectious Diseases, UGA
Deciphering the mechanisms of host manipulation by *Toxoplasma gondii*

P5 **Leticia Do Amaral**, PREP, CTEGD, and Dept. of Biochemistry & Molecular Biology, UGA
Characterizing the mechanism of resistance of PRC1590 using known antimalarials

P6 **Erica Tovey**, Brigham Young University
Glucose levels signal glycosomal acidification in bloodstream-form *Trypanosoma brucei*

P7 **James Blauwkamp**, Dept. of Pharmacology & Toxicology, Indiana University School of Medicine
Landscape of nuclear pore complexes during the erythrocytic proliferation of the rodent malaria parasites using expansion microscopy

P8 **Janice Teal-Urquides**, CTEGD, CCRC, and Dept. of Biochemistry & Molecular Biology, UGA
Dependence of the expression and localization of a *Toxoplasma* GPN-GTPase on its O-fucosylation in *Toxoplasma gondii*

P9 **Nupur Kittur**, Center for Tropical & Emerging Global Diseases, UGA
Integration, exploration & reuse of clinical & epidemiological datasets: A case study using ICEMR data on the ClinEpiDB.org platform

P10 **David Anaguano**, CTEGD and Dept. of Cellular Biology, UGA
Plasmodium falciparum requires two rhoptries to invade host red blood cells

P11 **Amadis Vivas**, CTEGD and Center for Vaccine & Immunology, UGA
Development of a qPCR assay to detect *P. cynomolgi* gametocytes

P12 **Fernando Sanchez-Valdez**, Center for Tropical & Emerging Global Diseases, UGA
Dormant amastigotes of *Trypanosoma cruzi* display low levels of protein synthesis and mitochondrial activity

P13 **Reagan S. Haney**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
Identifying the mechanism of action of a novel antimalarial PRC1584 with collateral drug sensitivity associated with PfK13 C580Y mutation

P14 **Gonzalo Seminario Mondejar**, Dept. of Cellular Biology, UGA
Protein palmitoylation in *Trypanosoma cruzi*

P15 **Melissa A. Sleda**, CTEGD and Dept. of Cellular Biology, UGA
Discovery of new mitochondrial ubiquinone pathway inhibitors that are effective against the acute and chronic stages of *Toxoplasma gondii*

P16 **Clyde Schmidt-Silva**, CTEGD and Dept. of Cellular Biology, UGA
Targeting early liver-stage antigens confer better immunity to malaria

P17 **Susanne Warrenfeltz**, Center for Tropical & Emerging Global Diseases, UGA
The Eukaryotic Pathogen, Vector and Host Informatics Resources (VEuPathDB.org)

P18 **Raíssa Nogueira de Brito**, Dept. of Anthropology, UGA
Insect chitin synthase 1 (CHS1): A comparative analysis across species of fruitflies and blood-feeding disease vectors with contrasting climatic-ecological preferences

P19 **Elise Nanista**, CTEGD and Dept. of Genetics, UGA
Understanding the role of ebony in tapeworm reproductive development

P20 **Magdalena Argomaniz**, CTEGD and Center for Vaccines & Immunology, UGA
Adaptation of the *Plasmodium cynomolgi* Berok DC line to continuous in vitro culture

P21 **Rui Xiao**, Center for Tropical & Emerging Global Diseases, UGA
Long-read RNA Iso-seq and ATAC-seq data reveal and help explain *Cryptosporidium parvum*'s transcriptional landscape

P22 **Ruby Harrison**, CTEGD and Dept. of Cellular Biology, UGA
Mutual antagonism between the parasite *Trypanosoma cruzi* and the gut microbiota of its triatomine insect vector

P23 **V.G. Dellinger**, EPIC, Dept. of Genetics & Biochemistry and Dept. of Biological Sciences, Clemson University
Understanding fatty acid uptake in *Trypanosoma brucei* through gene knockouts

P24 **Anna Gioseffi**, Center for Tropical & Emerging Global Diseases, UGA
Evaluating the regulatory role of polyphosphorylation in *T. cruzi*

P25 **Anissa Waller Del Valle**, EPIC and Dept. of Biological Sciences, Clemson University
Targeting translational control of stress as a potential drug target for the treatment of *Entamoeba histolytica*

P26 **Benjamin L. Phipps**, CTEGD and Dept. of Genetics, UGA
Multiple blood meals support non-competitive egg production and *Plasmodium berghei* infection in the Indian malaria vector *Anopheles stephensi*

P27 **Saanvikha Saravanan**, Center for Tropical & Emerging Global Diseases, UGA
Analysis of varying drug treatment regimens on the activity of *Trypanosoma cruzi* amastigotes in-vitro

P28 **Grace W. Vick**, Center for Tropical & Emerging Global Diseases, UGA
A *Plasmodium* rhoptry neck protein is essential for parasitophorous vacuole formation

P29 **Lindsay Berardi**, Dept. of Infectious Diseases, UGA
Wolbachia protein, Wbm0152, disrupts eukaryotic endolysosomal membrane dynamics

P30 **Kaelynn Parker**, CTEGD, Dept. of Cellular Biology, UGA
Using proximity biotinylation to probe the mitochondrion-ER interaction in *Toxoplasma gondii*

P31 **James Oristian**, CTEGD and Dept. of Infectious Diseases, UGA
Induced in vitro sexual commitment of *Plasmodium cynomolgi*

P32 **J. Ryan Simmons**, Dept. of Biochemistry & Cellular and Molecular Biology, University of Tennessee Knoxville
Evolution of Programmed DNA Elimination in parasitic nematodes

P33 **Triet M. Pham**, University of South Carolina Beaufort
Rapid, novel, and sensitive tetrazolium-based colorimetric assay for helicase nsp13 in SARS-CoV-2

P34 **Steven P. Maher**, Center for Tropical & Emerging Global Diseases, UGA
Using chemobiology to study the liver stage of *Plasmodium vivax*

P35 **Trevor Haskins**, Center for Tropical & Emerging Global Diseases, UGA
An investigation of A-P polarized genes in *Hymenolepis diminuta*

P36 **Nathan Chasen**, CTEGD and Dept. of Cellular Biology, UGA
I have no mouth and that's okay: Viable endocytic null mutants of *Trypanosoma cruzi*

P37 **Saniya S. Sabnis**, Dept. of Infectious Diseases, UGA
Extrafollicular B cells are associated with persistent anemia during chronic *Plasmodium* infections

P38 **Erin Jones**, Clemson University
Inhibition of fructose 1,6-bisphosphatase in *Naegleria fowleri*

P39 **Sabrina S. Pizarro**, Clemson University
Use of peroxisomal targeting sequences in drug delivery

P40 **Megna Tiwari-Crowe**, CTEGD, CCRC, and Dept. of Biochemistry & Molecular Biology, UGA
The close cousins of O-GlcNAc-transferases: the O-fucose-transferases

P41 **Noah Travis Smith**, CTEGD and Dept. of Cellular Biology, UGA
Visualizing the cytostome-cytopharynx complex of *Trypanosoma cruzi*

P42 **Melanie Key**, Dept. of Biological Sciences, Clemson University
Host glycolytic metabolites serve as a vital fuel source for energy metabolism and intracellular survival of *Toxoplasma gondii*

P43 **Juliana Hoyos**, Odum School of Ecology, UGA
Detection of *Trypanosoma cruzi* and *T. rangeli* infections from *Rhodnius pallescen*s across different land use types in Panama

P44 **Camila Marques-da-Silva**, CTEGD and Dept. of Cellular Biology, UGA
Interferon-induced cell-autonomous immunity to *Plasmodium* in hepatocytes

P45 **Katherine Moen**, CTEGD and Dept. of Cellular Biology, UGA
The role of protein disulfide isomerase in the endoplasmic reticulum of *Toxoplasma gondii*

P46 **Luiz Gustavo Vasconcelos Machado**, Center for Tropical & Emerging Global Diseases, UGA
Live attenuated Centrin4 knockout *T. cruzi* as a new vaccine candidate

P47 **Brandon Estrem**, Dept, of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville
DNA break and end resection in *Ascaris* programmed DNA elimination

P48 **Anthony Ruberto**, Center for Tropical & Emerging Global Diseases, UGA
Lead optimization and target identification of drugs targeting hypnozoites

P49 **Vikky FNU**, Dept. of Biological Sciences, Clemson University
Is internalization of the transferrin receptor by *Toxoplasma gondii* a strategy for iron acquisition?

Oral Presentations

A novel conditional knockdown system in *Trypanosoma cruzi* reveals essential roles for the Aurora and Polo-like kinases in parasite cell division

Justin Wiedeman, Drew Etheridge
Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, 30601

The protozoan parasite *Trypanosoma cruzi* is responsible for Chagas disease, the most severe parasitic illness of the Americas. Despite the medical importance of *T. cruzi*, our knowledge of the identity and function of essential genes is severely limited by the lack of a conditional knockdown system. Unlike its more genetically tractable cousin *Trypanosoma brucei*, *T. cruzi* lacks the necessary enzymatic machinery to facilitate RNAi based functional studies. Additionally, the diploid nature of the *T. cruzi* genome and arrangement of genes into polycistronic "cassettes" which lack clearly defined promoters, precludes the use of standard molecular approaches to analyze essential gene function. Here we describe the development and use of a functional conditional knockdown system based on tetracycline- and theophylline-responsive hammerhead ribozymes (HHRs) inserted into the 3' un-translated (UTR) regions of genes of interest. Using these new tools, we demonstrated the essential nature of multiple protein-coding genes, including the genes for the Aurora and Polo-like kinases. We discovered roles for these kinases in mitosis and cytokinesis, respectively, with these studies mirroring their known roles in *T. brucei*. Importantly, we demonstrated the effectiveness of the HHR knockdown system in intracellular amastigotes, the medically relevant mammalian stage of the parasite. This conditional knockdown system allows, for the first time, the straightforward functional characterization of essential genes in *T. cruzi*.

Exocytosis of egress-specific vesicles in *Plasmodium falciparum*

Watcharatip Dedkhad[1], Manuel A. Fierro[2], Vasant Muralidharan[1]
[1]University of Georgia, Athens, GA 30602 [2]Iowa State University, Ames, Iowa 50011

After completing the parasite's asexual proliferation via schizogony, daughter parasites (known as merozoites) egress from the host RBC by breaking down two membranes. Egress of merozoites requires a poorly understood signaling pathway that results in the exocytosis of specialized secretory vesicles, including micronemes and exonemes. Moreover, the proteins required for this signal-dependent exocytosis remain mostly unknown. We fused a pH-sensitive GFP, which fluoresces only in neutral pH, known as a super ecliptic pHlourin or SEP with the exoneme-localized protease, Plasmepsin X (PMX). Observation of live mature schizonts suggests that exoneme exocytosis occurs for several hours before egress, and as expected, exocytosis is blocked by the Protein Kinase G inhibitor, compound 1 or 2. We have previously shown that an ER-resident calcium-binding protein (PfERC) is essential for egress. Using PMXSEP/PfERC-glmS and PMXSEP/PfERC-M9 double mutants, we show that PfERC is required for exoneme exocytosis. We identified proteins interacting with PfERC (PIEs), one of which is a putative transmembrane protein Pf3D7_1308000 or PIE4 expressed primarily during schizogony. We generate conditional knockout mutants of PIE4 using rapamycin (RAP)-inducible DiCre recombinases knockout system (PIE4KO). Treating PIE4KO with RAP for 4 hours inhibited parasite growth, suggesting that PIE4 is essential for the blood stage life cycle. Giemsa-stained thin blood smears show all intraerythrocytic stages are morphologically normal. However, schizonts are unable to egress upon PIE4 knockout. These data show knockout of PIE4 inhibits *P. falciparum* egress. Immunofluorescence microscopy showed that PIE4 colocalized with the exoneme protein, PMX. Ongoing work will determine whether PIE4 is required for PVM or RBCM membrane breakdown and will elucidate whether PIE4 function in signal-dependent exocytosis.

Investigating sporozoite development through the mosquito

Benjamin Liffner[1], Elizabeth Glennon[2], Veronica Primavera[2], Cecilia Kalthoff[2], Elaine Hilton[1], Alexis Kaushansky[2,3], Sabrina Absalon[1]

[1]Department of Pharamcology and Toxicology, Indiana University School of Medicine, Indianapolis, IN, USA [2]Seattle Children's Research Institute, Center for Global Infectious Disease Research, Seattle, WA, USA [3]Department of Pediatrics, University of Washington, Seattle, WA, USA

Human malaria parasites have complicated lifecycles that involve both human and mosquito hosts. In just a few days, a single parasite transmitted from human to mosquito can form an oocyst in the mosquito midgut that gives rise to thousands of daughter sporozoites. These sporozoites will then egress from the oocyst and undergo a complex series of translocation and invasion events, eventually residing in the mosquito salivary gland duct ready to transmit to their new host. Sporozoite formation and salivary gland invasion are both incredibly important and interesting processes from a cell biology perspective but are relatively poorly understood. The small size of these parasites and the difficulty of imaging in their anatomical context has made it challenging to unravel much of their cell biology. To better understand cellularisation of sporozoites and their invasion of salivary glands in situ, we used ultrastructural expansion microscopy (U-ExM), a sample prep method that physically expands the sample ~4.5x. We performed U-ExM on whole mosquito midguts and salivary glands, which allowed us to visualise sporozoites in 3D from whole tissue architecture down to parasite ultrastructural detail. In this study, we have defined when and where sporozoites establish the polarity that will define their apical end and described a series of events leading to fission of their apicoplast. Further, we found that the rhoptries of mature oocyst-sporozoites vary drastically in size, shape, and number compared to salivary gland sporozoites. These findings begin to uncover the fascinating cell biology of the least well understood stages of the parasite lifecycle and pave the way to development of treatments or prevention strategies targeting these stages.

Vacuolar Transporter Chaperone 4 (TcVtc4) is essential for the infective stages of *Trypanosoma cruzi*

Mayara S. Bertolini, Miguel A. Chiurillo, and Roberto Docampo

Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, UGA, GA, US

Polyphosphate (polyP) is a polymer of inorganic phosphate, and it typically accumulates in acidic, calcium-rich organelles known as acidocalcisomes. PolyP synthesis in eukaryotes was unclear until it was demonstrated that the protein named Vtc4 (vacuolar transporter chaperone 4) is a polyP polymerase that localizes to the yeast vacuole. The human pathogen *Trypanosoma cruzi*, who causes Chagas disease, possesses a Vtc4 homolog (TcVtc4). It was demonstrated that TcVtc4 catalyzes polyP synthesis and localizes to acidocalcisomes in this parasite. Here, we report the use of a CRISPR/Cas9-based strategy to generate knockout mutants of TcVtc4. After several attempts, we confirmed by PCR and Southern blot analyses that only one TcVtc4 allele was replaced by the DNA donor cassette at the specific locus. This result suggests that null alleles can have lethal effects in epimastigotes. TcVtc4 single-gene knockout (TcVtc4-SKO) epimastigotes have a lower proliferation rate and they are defective in short chain polyP when compared with control cells. Moreover, TcVtc4-SKO trypomastigotes have a reduced capacity to invade host cells and to replicate within them as amastigotes. In conclusion, our data show that TcVtc4 is important in epimastigotes, and critical for the infective stages. Given that Vtc genes are not present in the genomes of higher eukaryotes, drugs that target Vtc4 function are promising candidates for the treatment of Chagas disease.

The Skp1 α-Galactosyltransferase Gat1 regulates SCF assembly in *Toxoplasma gondii*

Donovan A. Cantrell[1], Msano Mandalasi[1,3], Elisabet Gas-Pascual[1,3], Hyun W. Kim[1], Hanke van der Wel[1], and Christopher M. West[1,2,3]

[1]Department of Biochemistry and Molecular Biology, UGA, Athens, GA, USA; [2]Complex Carbohydrate Research Center, UGA, Athens, GA, USA; [3]Center for Tropical and Emerging Diseases, UGA, Athens, GA, USA

The SCF (Skp1/Cullin-1/F-Box protein (FBP)) complex is involved in proteomic regulation within all eukaryotes. Protein targets are specifically recognized by a variety of FBPs which bind the Skp1 adaptor protein linking the substrate recognizing FBPs to the SCF complex. In the agent for human toxoplasmosis, *Toxoplasma gondii*, the oxygen-dependent prolyl hydroxylase PhyA hydroxylates a conserved proline on Skp1. This primes it for the addition of 5 sugars, the last of which is mediated by the glycosyltransferase Gat1. Substantial evidence Indicates that glycosylation promotes both disassembly of the strong Skp1 homodimer and association with FBPs. Deletion of Gat1 results in a modest growth defect that is strongly exacerbated in a PhyA-KO background. This was surprising since Gat1's only known substrate is the Skp1 glycan, whose generation depends on the prior action of PhyA. Co-immunoprecipitation data indicate that, unlike the other glycosyltransferases, Gat1 stably associates with Skp1 in vivo suggesting that Gat1 plays an additional role in associating with non-substrate glycoforms. Gel filtration and analytical ultracentrifugation studies indicate that Gat1, a stable homodimer, directly and stably interacts with monomeric Skp1 with a sub-micromolar affinity. Evidence suggests that glycosylation promotes the formation of a 2:2 rather than a 2:1 Gat1:Skp1 complex, which may be related to the dissociative effect of glycosylation on Skp1 homodimerization. AlphaFold modeling suggests that the Gat1 binding interface of Skp1 overlaps with its homodimerization and FBP interfaces. This model is supported by evidence that Gat1 inhibits Skp1 hydroxylation and competes with FBP binding to Skp1 in vitro. These findings offer a biochemical mechanism for the genetic data by suggesting a second role for Gat1 in titrating the availability of Skp1 for the SCF complex. A related effect was previously proposed for the unrelated terminal glycosyltransferase of *Dictyostelium* Skp1, suggesting that this mechanism was a driving force for convergent evolution.

Role of memory T regulatory cells in recurrent malaria

Charles Chess[1,2], Sam Kurup[1,2]

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Regulatory T cells (Tregs) are a subset of CD4 'helper' T cells that allow the host to maintain immune tolerance and homeostasis. Defined by the expression of transcription factor Foxp3, Tregs are known to expand and restrain pro-inflammatory immune responses in primary blood-stage malaria, while also impeding the effective control of the infection. Considering that people residing in malaria-endemic regions get repeated infections with *Plasmodium*, it is important to understand how memory Tregs influence immunity to such recurrent infections. To our surprise, individuals living in malaria-endemic regions with high pre-existing frequencies of memory Tregs exhibited lower parasitemia levels when naturally reinfected with *Plasmodium* in the subsequent malaria season. In agreement with this observation, in mice, selective ablation of memory Tregs resulted in loss of protection from reinfection and transfer of memory Tregs conferred better immunity to fresh *Plasmodium* infections in the recipients. These findings are in stark contrast to the expectations based on how Tregs function in primary malaria infection. We show that, upon *Plasmodium* re-infection, memory Tregs get transcriptionally reprogrammed into a new subsets of helper cells – T follicular helper (Tfh) cells and localize to the germinal centers in the secondary lymphoid organs, where they facilitate the generation of robust antibody responses. Our findings show that memory Tregs protect against recurrent blood-stage malaria by undergoing transcriptional and phenotypic reprogramming. These observations will have a significant impact on the design of anti-malaria strategies in the endemic areas, where reinfection makes up the majority of malaria cases.

Characterization of the mitochondrial ubiquinone synthesis pathway in *Toxoplasma gondii*

Baihetiya Baierna, Juan Camilo Arenas-Garcia, Silvia NJ Moreno
Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, UGA, GA, US

Toxoplasma gondii is a protozoan parasite of the Apicomplexan phylum that infects approximately one third of the human world population. The disease caused by *T. gondii*, toxoplasmosis, represents one of five neglected parasitic infections in the United States and could be fatal in immunocompromised patients. Current medicines used to treat toxoplasmosis have toxic side effects and require long term treatments, especially for immunocompromised patients. Therefore, medicines with enhanced efficacy that target unique metabolic steps of *T. gondii* is of great interest to improve toxoplasmosis therapy. The mitochondrion is a valid target for *T. gondii* chemotherapy. The ubiquinone pathway synthesizes Coenzyme Q (UQ), which functions in the mitochondrial electron transport chain shuttling electrons from complex I or II to complex III. The UQ synthesis appears to diverge from human, and several enzymatic steps could represent novel therapeutic targets. In *Saccharomyces cerevisiae*, several Coq enzymes form a complex called ubiquinone synthome, which has not been characterized in *T. gondii*. Moreover, very little is known about this pathway in *T. gondii* and other apicomplexan parasites. We used conditional gene disruption and selected for clonal populations along with various functional analysis assays to investigate the role of two methylation activities, TgCoq3 and TgCoq5, in mitochondrial membrane potential and mitochondrial oxygen consumption. We validated that these two proteins are involved in the UQ synthesis pathway. By performing 2-dimentional BlueNative PAGE, we were able to demonstrate that the synthome is also present in *T. gondii*. We created C-terminal tagged cell lines with TurboID biotin ligase and demonstrated that TurboID was able to biotinylate mitochondrial proteins. By using subcellular fractionation and mass spectrometry analysis, we were able to find some novel proteins that could be involved in this pathway. We will further characterize these enzymes as potential drug targets for the pathway.

Toxoplasma gondii ATP synthase subunits containing coiled-coil-helix-coiled-coil-helix (CHCH) domains are essential for mitochondrial function

Madelaine Usey[1,3] and Diego Huet[2,3]
[1]Department of Cellular Biology, UGA, Athens, GA, USA [2]Department of Pharmaceutical and Biomedical Sciences, UGA, Athens, GA, USA [3]Center for Tropical and Emerging Global Diseases, UGA, Athens, GA, USA

Apicomplexan parasites include the causative agents of several debilitating global diseases, including malaria and toxoplasmosis. Recent work in *Toxoplasma gondii* has revealed that their ATP synthase, an enzyme critical for mitochondrial energy production, is comprised mostly of subunits with no known homologs outside the apicomplexan phylum. Three of these subunits contain coiled-coil-helix coiled-coil-helix (CHCH) domains, which are known to mediate the function of electron transport chain complexes and maintain mitochondrial morphology in eukaryotes. These domains consist of specifically spaced cysteine residue pairs in separate α-helices that are oxidized to form disulfide bonds after mitochondrial import. However, because CHCH domain proteins have not been identified as part of the ATP synthase in organisms outside the phylum, their role in apicomplexans is unclear. The ATP synthase is essential for the life cycles of *T. gondii* and *Plasmodium* spp., making it critical that we advance knowledge of ways it diverges from the mammalian host. To investigate two *T. gondii* ATP synthase subunits containing CHCH domains, we utilized a conditional gene knockdown system and demonstrated that both subunits are essential for the parasite lytic cycle. Using native gel electrophoresis, metabolic assays, three-dimensional mitochondrial volume analysis, and transmission electron microscopy, we have also shown that these subunits are critical for ATP synthase structure, energy production, mitochondrial volume, and cristae density. Mutation of key cysteine residues in the CHCH domains resulted in mis-localization of the proteins, illustrating the importance of the CHCH domains for protein function. This investigation into divergent apicomplexan ATP synthase subunits reveals vulnerabilities that could be targeted by novel drugs against these opportunistic parasites.

Deep PCR sampling of blood allows consistent detection of *Trypanosoma cruzi* infection in chronically infected primates with widely different parasite loads

Brooke E. White[1], Carolyn L. Hodo[2] and Rick L. Tarleton[1]
[1]Center for Tropical and Emerging Global Diseases, UGA, Athens; [2]Keeling Center for Comparative Medicine and Research, University of Texas, Bastrop

Trypanosoma cruzi, the causative agent of Chagas disease, persists chronically in most infected subjects, eventually leading to muscle-focused pathology. Detection of *T. cruzi* via PCR of blood is a common way to definitively establish infection status, but this standard protocol is undependable due to the frequently low parasite numbers in circulation. Likewise, assessing cure following therapeutic treatment is a major challenge. The commonly used method for PCR-based assessment of treatment outcomes in human clinical trials is to perform serial PCR on 2-9 aliquots of DNA from blood, with a single positive aliquot considered evidence of treatment failure but 9 negative reactions does not dependably signify treatment success. Our ultimate goal in this project is to determine if PCR of deep-sampled DNA from blood – up to a maximum of ~200 aliquots per blood sample - could provide dependable evidence of parasitological cure. Toward this end, we have screened DNA from duplicate blood samples obtained monthly from naturally infected, *T. cruzi* seropositive *Macaca mulatta*. Initially, we PCR-screened 5 aliquots of each blood DNA sample and if found negative, additional sets of aliquots were screened, up to a total of 194 qPCR reactions/sample. We also cultured blood samples to check for outgrowth of parasites. We found a wide range of positivity across the 20 infected macaques, ranging from strongly positive (100%; 10 out of 10 aliquots positive) to negative or barely detectable positive (<1%; 1-2 out of 194 aliquots). The percent PCR positive aliquots as expected, correlated with the quantitative PCR score (Cq value) for each positive aliquot and occasionally with parasite growth in hemocultures and with few exceptions, was highly consistent over the nearly 1-year sampling period. These data suggest that higher quality diagnostic and perhaps treatment outcome data can be obtained by deeper sampling of blood DNA, vastly beyond that currently used in human clinical trials.

Small RNAs within the extracellular vesicles of the brain eating amoeba, *Naegleria fowleri*, and their potential as diagnostic biomarkers

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The pathogenic free-living amoeba, *Naegleria fowleri* is the causative agent for primary amoebic meningoencephalitis (PAM), an acute brain disease with a case mortality rate of >97%. Several factors contribute to this rate of mortality including delayed/incorrect diagnosis, ineffective therapeutics and lack of understanding of the amoebic pathogenesis. Recently, there have been advances in the study of the parasite biology, but the understanding of the molecular basis for parasite-host interactions is lacking. Apparatuses termed extracellular vesicles (EVs) have been implicated in the field of intercellular communication. EVs are secreted from cells and house various signaling molecules that elicit a response in recipient cells. One class of small secretory molecules found in EVs known as small RNAs can be reliably detected in many bodily fluids. These small RNAs have been implicated as regulators of gene expression, can modulate host immune responses, and are used as biomarkers for the diagnosis and prognosis of various diseases. Thus, we hypothesized that *N. fowleri* also produces small RNAs and packages them into EVs to be secreted. Our previous work shows that EVs are secreted by *N. fowleri* (Nf-EVs) via multiple routes, contain thousands of proteins, and readily fuse with host-cells and other amoebae. Deep-sequencing of the RNA contents of Nf-EVs followed by computational processing uncovered multiple classes of small RNAs, including a highly prevalent small RNA that we have validated with RT-qPCR in EV RNA from eight *N. fowleri* clinical isolates, in clinical PAM-infected cerebrospinal fluid samples (the standard fluid used to diagnose PAM by the CDC), and in various PAM-infected mouse biofluids at timepoints as early as 24h post infection. This work implicates this small RNA as a less invasive early-stage diagnostic biomarker for PAM infections and expands the knowledge of intracellular interactions among these pathogenic amoebae.

Poster Presentations

P1. Overexpression, purification, and biochemical evaluation of a *Trypanosoma cruzi* glucokinase bump mutation for drug-target validation

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Trypanosoma cruzi is a pathogenic protozoan parasite that causes Chagas' disease owing to chronic stage symptoms including endocarditis and cardiomyopathy, which decrease life expectancy. Therapeutics including nifurtimox and benznidazole have been in clinical use since the 1970's but have adverse side effects that have led to poor patient compliance; therefore, the development of new therapeutics is in high demand. *T. cruzi* parasites have several interconnected and important biochemical pathways for its survival such as glycolysis, the pentose phosphate pathway, and gluconeogenesis. These three pathways involve the metabolite glucose-6-phosphate (G6P). Moreover, the enzyme *T. cruzi* glucokinase (TcGlcK) catalyzes a reaction to produce G6P from D-glucose and ATP, and without TcGlcK there appears to be the inhibition of these pathways necessary for *T. cruzi* cell survival. Carboxybenzyl glucosamine (CBZ-GlcN) is one of the strongest known competitive inhibitors of any glucose kinase and we have conceived of a plan to use this competitive inhibitor in a bump-and-hole approach to validate the enzyme as a "confirmed" drug-target. The current state of understanding is that TcGlcK is a "potential" drug-target because experimentation for genetic essentiality is still lacking in this field. We have envisioned a site-directed mutation within the active site cavity of TcGlcK, particularly TcGlcK(N105F), so that CBZ-GlcN would not have the ability to bind firmly or not bind at all. The inhibitory constant (Ki) for wt-TcGlcK and CBZ-GlcN was previously determined to be 0.71 ± 0.05 µM. Herein, we present the bump mutation TcGlcK(N105F) in the presence of 20 µM CBZ-GlcN and the compound does not exhibit any inhibitory effect with the mutant enzyme.

P2. The role of polyphosphates in *Toxoplasma gondii*

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Polyphosphate (PolyP) is a linear polymer of orthophosphate units linked by high energy phosphoanhydride bonds. PolyP chains are variable in size, ranging from 3 to thousands of units. PolyP play roles in bacterial virulence, infection control, blood coagulation, protein folding, and regulation of cell signaling pathways. In eukaryotes, PolyP concentrate in the cytosol, nucleus, lysosomes, and mitochondria, but is specially stored in acidic vacuoles like the yeast vacuole and acidocalcisomes. A critical role of PolyP was described recently as a molecular chaperone in bacteria and was shown that PolyP binds covalently to lysines of PASK (polyacidic serine and lysine (K)) domains-containing proteins and modify their activity in a process that is named polyphosphorylation. In *Saccharomyces cerevisiae*, a vacuolar transporter chaperone complex (VTC complex) was identified that synthesizes PolyP, in expense of Pi and ATP, and translocates PolyP to the yeast vacuole and acidocalcisomes. The yeast VTC complex is composed of 5 subunits: VTC1, VTC2, VTC3, VTC5 and the catalytic subunit VTC4. The genome annotation of *Toxoplasma gondii* predicts two genes with homology to the yeast VTC proteins ScVTC2p (TgVTC2) and ScVTC4p (TgVTC4). In this work we generated conditional mutants with an N-terminal 3HA-tag for the annotated TgVTC2 (iΔTgVTC2-3HA) and TgVTC4 (iΔTgVTC4-3HA) using the CRISPR/Cas9 system. Analysis of the mutants showed that TgVTC2 is essential for the *T. gondii* lytic cycle. We also demonstrated that TgVTC2 and TgVTC4 co-localize with the plant-like vacuole (PLV) markers VP1 and CPL, and with the Zinc transporter previously localized to the PLV and acidocalcisomes. Both mutants (iΔTgVTC2 and iΔTgVTC4) showed a significant decrease in PolyP content. An in silico screening of PASK motifs in *T. gondii* GT1 proteome found 326 proteins with a negative phenotype CRISPR score. The future goal of this project is to identify proteins that are polyphosphorylated and the role of the PolyP modification.

P3. Understanding *Plasmodium falciparum*'s multidrug resistance protein through the characterization of a novel antimalarial class

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Malaria is a deadly parasitic disease that affects over half the world's population. Currently, resistance has been identified to all available antimalarials, highlighting an urgent need to develop novel compounds and better understand common mechanisms of resistance. A common form of resistance occurs in the parasite's multidrug resistance protein (PfMDR1), which is a protein situated in the membrane of the parasite's digestive vacuole (DV). Interestingly, different mutations of pfmdr1 can result in collateral drug sensitivity, where specific mutations can aid in resistance to some antimalarials while simultaneously increasing sensitivity to other compounds. While PfMDR1 is a major driver of resistance, we do not understand how different mutations of pfmdr1 modulate resistance in the malaria parasite. We hypothesize mutation type of PfMDR1 is mediated by the drug's molecular target and is explanatory of collateral drug sensitivity. Our lab group has discovered a novel β-carboline compound, PRC1590, that recapitulates this collateral drug sensitivity. Through in vitro selection of resistance to PRC1590, we have identified that a single nucleotide polymorphism (SNP) on pfmdr1 is the driver of PRC1590-resistance. To characterize this SNP, we used cross-resistance screenings with other compounds and identified that the PRC1590 resistant line is more sensitive to drugs with mechanisms of resistance that result in gene amplification of PfMDR1. We have identified through fluorescence microscopy that PRC1590 localizes to the DV of a drug-sensitive strain of malaria. We are expanding these microscopy experiments to resistant strains of malaria to characterize PRC1590-localization. We will then use chemoproteomics to determine PRC1590's mechanism of action. Understanding how compounds like PRC1590 modulate drug response through PfMDR1 will be informative for purposeful drug design and partner drug pairings that exploit its collateral drug sensitivity.

P4. Deciphering the mechanisms of host manipulation by *Toxoplasma gondii*

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Microbial virulence often relies on secreted effectors that modulate host signal transduction. The revelation of the functional mechanisms of pathogen effectors not only leads to a better understanding of microbial virulence strategies but also has the potential to impact human health through novel treatment strategies. Over the past decades, extensive studies of viral and bacterial virulence have immensely expanded our knowledge and understanding of host-pathogen interaction. However, viruses and bacteria are only two classes of microbes that can cause disease, and surprisingly little is known about how eukaryotic pathogens manipulate their hosts. In our lab, we use *Toxoplasma gondii* as a model organism to study the sophisticated mechanisms this pervasive human eukaryotic pathogen uses to modulate the host immune system. We are using multidisciplinary approaches, including genome-wide genetic screens, proteomics, cell biology, microscopy, and biochemistry, to uncover novel *T. gondii* secreted effectors and understand their mechanism of action. We are especially interested in understanding the crosstalk between the host's circadian clock and *T. gondii* infection. We are also applying proteomic tools developed for the discovery of *T. gondii* secreted effectors to the study of the *Plasmodium* liver stages secretome.

P5. Characterizing the mechanism of resistance of PRC1590 using known antimalarials

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Malaria is a disease caused by the *Plasmodium* parasite, with the deadliest species being *Plasmodium falciparum*. In 2021 there were 247 million cases of malaria reported worldwide. Resistance to all available antimalarial drugs has been identified in regions endemic to this disease. As a result, it is imperative to find novel antimalarials with new molecular targets, and to better understand common mechanisms of resistance to the known antimalarials. Mutations in the multidrug resistance protein (PfMDR1) can lead to collateral drug sensitivity, which is when the parasite becomes resistant to certain compounds, but more sensitive to others. This is important to study because it can inform partner drug pairing and lessen failed attempts for treatment due to antimalarial resistance. PRC 1590 is a potential antimalarial compound discovered by our lab. Through early characterization of this compound, we identified that the mechanism of resistance is due to a single nonsynonymous mutation on the pfmdr1 gene. One of the first steps in antimalarial discovery is to generate in vivo resistance to compounds. Previously, the lab was able to select for a PRC1590-resistant strain of parasites using the drug-sensitive 3D7 strain. Single nucleotide polymorphisms (SNPs) and gene amplification mutations such as copy number variants (CNVs) lead to resistance to antimalarials. In order to understand the mechanism of resistance to PRC1590, we conducted cross-resistance screenings using compounds that have known mechanisms of resistance. Currently, we are conducting assays to continue analyzing the characteristics of three different strains when exposed to known antimalarials such as amodiaquine, quinine, chloroquine, and mefloquine. Our results are setting the basis for future studies to understand how antimalarials may select for mutations in PfMDR1.

P6. Glucose levels signal glycosomal acidification in bloodstream-form *Trypanosoma brucei*

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Several eukaryotic parasites, including *Trypanosoma brucei* and *Leishmania* spp. cause significant morbidity and mortality globally. Currently available therapeutic agents have significantly improved the outcome for infected individuals during the past decade; however, continuing the trajectory of developing better-targeted therapeutics remains a critical need. One apparent target is parasite metabolism, due to their reliance on glycolysis in the host and their unique localization of glycolytic enzymes to a peroxisome-like organelle called the glycosome. Previously, the Christensen Lab reported that glycosomal pH could serve as a regulator for metabolism using a small molecule pH reporter in the insect form of *T. brucei*. We have now utilized a fluorescent protein pH sensor, pHLuorin2, which localizes to the glycosome to make pH measurements in the glycosome of the parasite's more relevant host bloodstream form. Here we report the validation of this pH reporter in glycosomes and show that the bloodstream form of *T. brucei* appears to also use pH as a regulator of glycolysis under nutrient-deprived conditions. Furthermore, our recent findings indicate that sodium-proton exchangers (NHEs) within the bloodstream form are responsible for this glycosomal acidification. This differs from the parasite’s procyclic form, which requires inhibition of both V-ATPases and NHEs in order to prevent cell signaling via pH.

P7. Landscape of nuclear pore complexes during the erythrocytic proliferation of the rodent malaria parasites using expansion microscopy

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During blood-stage infection, *Plasmodium* parasites undergo an atypical cell division called schizogony in which multiple rounds of closed mitosis occur, followed by a single event of cellularization. While in recent years parasite replication has been increasingly studied, very little is known about Nuclear Pore Complexes (NPCs) biogenesis, their functions in selective bi-directional nucleocytoplasmic transport, and genome organization during parasite replication. NPCs consist of over 30 proteins labeled nucleoporins (Nups) highly conserved within eukaryotes. However, *Plasmodium* Nups are highly divergent, with only 11 confirmed Nups in the rodent malaria model, *Plasmodium berghei*. While recent studies highlight new tools for identifying novel Nups, new ways of characterization are needed. Here, we utilize ultrastructure expansion microscopy to study *P. berghei* Nup138, Nup221, and Nup313 at the single nucleus level. We confirmed these are Nups using a previously described NPC antibody against yeast NPCs. We found all these Nups evenly distributed around the nuclei and in an organized rosette structure previously described around the microtubule organizing center (MTOC). We first used the recombination-induced tag exchange (RITE) system in *Plasmodium* to show novel Nup221 synthesis at the later stages of the erythrocytic life cycle, signifying Nup turnover during the life cycle. Our results map the *P. berghei* NPCs and examine their homeostasis during closed mitosis. Further studies into these NPCs can reveal more information about their roles during the atypical cell division of malaria parasites.

P8. Dependence of the expression and localization of a *Toxoplasma* GPN-GTPase on its O-fucosylation in *Toxoplasma gondii*

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Toxoplasma gondii (*T. gondii*) is an obligate intracellular parasite of the phyla Apicomplexa. *T. gondii* has a wide range of warm-blooded hosts including humans. Throughout its life, *T. gondii* has to adapt to its different hosts and environments, making regulation of protein expression, protein activity, and protein stability important factors for its survival. Recent work has identified a sugar modification in *T. gondii* in which an O-fucose molecule is linked to a target protein by an O-fucosyltransferase (OFT). The OFT is encoded by the SPY locus, which modifies serine and threonine residues of at least 33 different proteins. Previous studies showed that fucosylated proteins are concentrated in assemblies near the nuclear envelope. More recently, ectopically overexpressed OFT substrate models suggested that O-fucosylation is required for protein abundance and stability. However, our current data based on endogenously tagged GPN GTPase, a protein possessing a serine-rich domain (SRD), exhibits only a very modest decrease in abundance when the OFT is not present despite confirmation that it is normally abundantly fucosylated. Furthermore localization appeared to not be affected. To further assess the role of its SRD, we fused it with a yellow fluorescent protein (YFP) and stably expressed the fusion protein at the expendable UPRT locus in the presence or absence of SPY. This fusion protein was also stably expressed irrespective of its fucosylation based on Western blotting and exhibited a molecular weight shift in the absence of SPY suggesting the domain is O-fucosylated by the OFT. Currently, we are examining the localization of SRD-YFP to determine if the SRD domain is sufficient to concentrate the protein in nuclear envelope assemblies. Beyond a hypothesized modest role in protein stability, the role of O-fucosylation remains enigmatic.

P9. Integration, exploration & reuse of clinical & epidemiological datasets: A case study using ICEMR data on the ClinEpiDB.org platform

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Open access to data from epidemiological studies has tremendous potential to increase secondary data use and accelerate discovery and translational impact. Data sharing is increasingly a requirement for funding and publication of epidemiological research, but it comes with technical and ethical challenges. We present a case study of successful data sharing from the clinical epidemiological database, ClinEpiDB.org, built in 2018 to facilitate access to de-identified data from large, high-quality global health studies. ClinEpiDB currently hosts data from >1.2 million participants representing 37 global studies in several domains including infectious diseases. The PRISM2 team, an International Center of Excellence for Malaria Research (ICEMR), conducted a cohort study of malaria in Uganda and collected socioeconomic, demographic, clinical, entomological, and other data, and was interested in data sharing to maximize use and impact of their research and meet funder and journal requirements. De-identified data was securely transferred to ClinEpiDB along with codebooks and other contextual metadata. PRISM2 variables were ontologically harmonized for increased interoperability, and after extensive data and quality checks, data was released on the free, open-access, online data platform ClinEpiDB.org. In their publication, the PRISM2 team included a link to recreate key findings of PRISM2 analyses on ClinEpiDB. Readers can follow the link to learn about PRISM2 cohort study methodologies, discover additional variables collected but not included in the published analysis, download data with no restrictions, and modify their copy of the published analysis to explore their own hypotheses in a point and click interface. Metrics reveal that PRISM2 data is being accessed regularly even three years after publication. The PRISM2 team gained visibility while retaining ownership of data and making all data access decisions. ClinEpiDB will expand in 2023 with integration of new datasets as well as enhanced visualization tools and the ability to derive variables.

P10. *Plasmodium falciparum* requires two rhoptries to invade host red blood cells

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Malaria is a global and deadly human disease caused by the apicomplexan parasites of the genus *Plasmodium*. Parasite proliferation within human red blood cells (RBC) is associated with the clinical manifestations of the disease. Establishment of infection within the human host begins with the invasion of RBCs by *P. falciparum*, which is mediated by the secretion of effectors from specialized secretory organelles in merozoite-stage parasites known as rhoptries. We investigated the function of one of these effectors, the Rhoptry Neck Protein 11 (RON11). RON11 contains 7 transmembrane domains and a putative calcium-binding EF-hand domain. Using RON11 conditional mutants, we show that knockdown of RON11 inhibits parasite growth by preventing merozoite invasion. Using ultrastructure expansion microscopy (U-ExM), we observed a unique phenotype in the absence of RON11, fully developed merozoites with single rhoptries. More interestingly, RON11 depletion does not inhibit merozoite attachment, nor does it hinder the secretion of rhoptry effectors into the RBC during invasion, but it blocks internalization of merozoites into the RBC. Together, these data suggest that RON11 is required for generating two rhoptries as well as the final step of merozoite invasion, internalization into the RBC. We are currently investigating the exact function of RON11 in merozoite invasion into the RBC.

P11. Development of a qPCR assay to detect *P. cynomolgi* gametocytes

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Understanding malaria transmission is essential for control and elimination, but there is little information available on gametocyte biology for parasites other than *P. falciparum*. This is due to the lack of culture systems that support gametocytogenesis for malaria parasites other than *P. falciparum*. Recently, we adapted the *P. cynomolgi* Berok strain, termed the DC line, to continuous in vitro culture, and we intend to study gametocytogenesis using this parasite as a model for *P. vivax*. The objective of this project is to develop quantitative PCR assays to detect *P. cynomolgi* gametocytes from in vitro and ex vivo samples. The pcs25 gene, an orthologue of the *P. vivax* gametocyte gene pvs25, was selected as a gametocyte marker, and rps18 was selected as a housekeeping gene. Primers and probes for each gene were designed, optimized, and their efficiencies evaluated. The assay then was validated using ex vivo samples from P. *cynomolgi* infections. Interestingly, there was an enrichment in the bone marrow for *P. cynomolgi* gametocytes, indicating that *P. cynomolgi* gametocytes are also enriched in the bone marrow like *P. vivax* in humans. Overall, this assay establishes a needed tool for evaluating gametocyte levels from in vitro and ex vivo samples, which is an essential first step to testing factors that influence gametocytogenesis of relapsing malaria parasites using our in vitro culture system.

P12. Dormant amastigotes of *Trypanosoma cruzi* display low levels of protein synthesis and mitochondrial activity

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Dormant amastigotes of *Trypanosoma cruzi*, the parasite causing Chagas disease, are less susceptible to current trypanocidal drugs and also express lower levels of reporter fluorescent proteins, suggesting a lower general metabolism compared to drug-susceptible, actively replicating amastigotes. Our understanding of the full metabolic activity of dormant *T. cruzi* amastigotes as well as methods to track the dynamics and the mechanisms involved in entering and exiting the dormancy state, are still very limited. In this study, we monitored the global synthesis of proteins in *T. cruzi* and the mitochondrial activity of dormant parasites using high-resolution expansion microscopy protocols. We assessed protein synthesis in vitro and in mice infections by detecting the incorporation of the clickable amino acid L-azidohomoalanine (AHA) into amastigote nascent proteins. In vitro, dormant amastigotes (detected by retention of CellTrace violet), presented a substantially lower AHA signal compared with replicating intracellular amastigotes. Intracellular amastigotes infecting skeletal muscle fibers of mice also showed a heterogenous pattern of protein synthesis, consistent with a range of metabolic activity in amastigotes in vivo. To assess the mitochondrial activity of dormant parasites we studied infected cell cultures incubated with the membrane potential-dependent probe Mitotracker. Dormant amastigotes displayed lower mitochondrial membrane potential, suggesting a reduced mitochondrial activity compared to replicating parasites in the same cell. These results demonstrate a decreased metabolic activity in *T. cruzi* dormant forms, which could consequently limit the efficacy of drugs whose activity depends on active parasite metabolism. Future studies will use these and additional tools to identify the cause and time course of the entry into and exit from dormancy, as well as the factors which might trigger the resumption of a more active metabolic profile.

P13. Identifying the mechanism of action of a novel antimalarial PRC1584 with collateral drug sensitivity associated with PfK13 C580Y mutation

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Malaria is a devastating disease that caused approximately 619,000 deaths in 2021 worldwide. Cases of malaria have increased from previous years in part by the quick development of resistance to current antimalarials. Due to the rising resistance to current antimalarial drugs such as chloroquine and artemisinin, there is an urgent need to discover and develop new chemotherapeutic agents that engage new targets in the malaria parasite. This research focuses on a novel antimalarial PRC1584. We investigated how one of known mechanisms of resistance to dihydroartemisinin (DHA) modulates sensitivity to PRC1584. DHA resistance can be conferred by a single nucleotide polymorphism (SNP) in the PfK13 gene known as K13 and a few SNPs have been reported, with C580Y and R539T being the most relevant mutations. Preliminary data indicate that parasites carrying a K13 C580Y mutation are more susceptible to PRC1584 treatment. Therefore, we hypothesize that K13 or its interactors may be potential molecular targets of PRC1584. We selected a set of *P. falciparum* DHA-resistant strains with K13 C580Y mutations and assessed PRC1584 EC50 values, measured the amount of K13 protein present, and performed localization experiments to evaluate if PRC1584 colocalizes with K13. Altogether, these studies will help reveal how the K13 mutations cause enhanced potency to parasites treated with PRC1584 and if K13 or its interactors play a role in the mechanism of action of PRC1584.

P14. Protein palmitoylation in *Trypanosoma cruzi*

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Palmitoylation is a reversible post-translational modification that involves the addition of palmitate to cysteine residues on proteins. This lipidation often leads to protein recruitment to membranes, making it essential for various biological responses such as signal transduction. Recent studies have shown that palmitoylation is also critical for the life cycle progression and virulence of many eukaryotic pathogens. However, in the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas disease, we still know very little about the palmitoylome, as only a few palmitoylated proteins have been characterized to date. Therefore, further research is necessary to better understand the role of this lipid modification in the parasite's life cycle. To identify the palmitoylated proteins in *T. cruzi*, we used a click-chemistry based methodology to label and capture them for identification via mass spectrometry. Through this process, we discovered an inducible palmitoylation pattern in insect stage epimastigotes, which occurred only when parasites were grown in fresh medium. After a series of nutrient screens, we narrowed down the inducing factor to the essential amino acid arginine and demonstrated that its addition alone is enough to induce the distinct palmitoylation response. Using this methodology, we isolated and identified several proteins being palmitoylated in response to arginine, including the palmitoyl acyltransferase 1 (PAT1), phosphoinositide-specific phospholipase C (PI-PLC), and a PDZ-domain-containing protein (PDZ-PLC) enzymes. We are currently using our in-house conditional knockdown system to further characterize the role of these proteins in the palmitoylation response and the parasite's life cycle.

P15. Discovery of new mitochondrial ubiquinone pathway inhibitors that are effective against the acute and chronic stages of *Toxoplasma gondii*

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The current treatments against toxoplasmosis are ineffective because they are only effective against the tachyzoite stage and have little effect on the slow growing bradyzoite, found in tissue cysts. The mitochondrion of *T. gondii* is essential for its survival and is a validated target. Several antiparasitic drugs, such as atovaquone and endochin-like quinolones, act through inhibition of the mitochondrial electron transport chain at the coenzyme Q:cytochrome c oxidoreductase. The ubiquinone (UQ) molecule consists of a water soluble quinone head and a lipophilic isoprenoid tail that anchors UQ to membranes. Previous work from our lab showed that the synthesis of the isoprenoid tail and of UQ is essential for cell growth. In this work we test inhibitors of the isoprenoid and ubiquinone pathways against the acute and chronic stages of *Toxoplasma gondii*. We found a bisphosphonate that is able to protect mice against a lethal acute infection of *T. gondii*. Several bisphosphonates (BPH-1218, BPH-1236, and BPH-1238) were also able to inhibit the replication of ME49 (a type II cystogenic strain), change the morphology and reduced the viability of in vitro bradyzoites. We also found that some bisphosphonates decrease the viability and growth of ex vivo derived bradyzoites. Most interestingly, BPH-1218 and BPH-1236 were able to reduce the number and size of tissue cysts in the brains of chronically infected mice. We also monitored the activity of the infected mice throughout the entire chronic infection and found reduced activity during the acute infection and increased activity during the chronic infection. Treatment with atovaquone or BPH-1218 modulated the increased activity during the chronic infection. Altogether we found that the UQ pathway is a viable target for acute and chronic stages of Toxoplasmosis.

P16. Targeting early liver-stage antigens confer better immunity to malaria

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Intracellular eukaryotic pathogens, such as protozoans, undergo extensive development, remodeling, and maturation within host cells, which can result in varying timelines of diverse antigen expression. The extent to which host cells present these antigens to the immune system is a key determinant of immune-mediated protection. Hepatocytes infected by *Plasmodium*, the causative agent of malaria, can presents *Plasmodium* antigens on MHC-I molecules for detection and targeted cytolysis by CD8 T cells, resulting in vaccine or infection-induced protection. However, it is unclear how the timing of *Plasmodium* antigen expression within the infected hepatocytes contributes to such protection. We hypothesize that *Plasmodium* antigens expressed early in the parasite's life-cycle within the hepatocytes would serve as superior targets for CD8 T cells, by offering more time for the CD8 cells to seek and destroy the infected hepatocytes before the parasites progress to the blood-stage. Our study shows that *Plasmodium berghei* expressing the model antigen ovalbumin early in its life-cycle in hepatocytes is cleared more efficiently compared to when expressed later. These findings suggest that immunizations targeting the early liver-stage antigens would provide better protection from malaria and is an essential consideration when designing malaria vaccines.

P17. The Eukaryotic Pathogen, Vector and Host Informatics Resources (VEuPathDB.org)

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VEuPathDB provides free, online access to genomic-scale data-mining resources for >500 species of eukaryotic pathogens and related taxa, including protozoan parasites, fungi & oomycetes, arthropod vectors of disease, and selected host species. VEuPathDB resources empower end-users to leverage diverse multi-Omics datasets, without requiring specialized analytical or computational skills. Advanced search capabilities, data visualizations and custom analysis tools are applied to genomic-scale datasets, enabling in silico hypothesis development and testing, and facilitating the discovery of meaningful biological relationships. Available data types include genome sequence and population-level variation data; manually-curated and automatically generated annotation; epigenetic, transcriptomic and proteomic data; pathway information, including metabolomic datasets; genome-wide phenotypic analyses, information on host-pathogen interactions and selected clinical results. VEuPathDB provides a phylogenetic framework to facilitate cross-species functional inference via orthology. These resources merge evidence from diverse data and across species, making the power of bioinformatic analysis accessible to research scientists worldwide. An active user support team offers an email help desk, social media, video tutorials, webinars, and a worldwide program of workshops.

P18. Insect chitin synthase 1 (CHS1): a comparative analysis across species of fruitflies and blood-feeding disease vectors with contrasting climatic-ecological preferences

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The insect cuticle is a highly effective barrier to water loss, particularly in dry habitat adapted species. Chitin, a sugar polymer assembled by chitin synthases (CHS), is the main component of the cuticle. Here, we asked whether and how amino acid (aa) sequences of CHS1 (isoform a) differ between insect species adapted to dry habitats and closely-related species adapted to moister habitats, with an emphasis on blood-feeding disease vectors. We compared CHS1a sequences from 21 species of *Drosophila* fruitflies, *Anopheles* malaria vectors, *Glossina* sleeping-sickness vectors, and *Rhodnius* Chagas disease vectors. CHS1a is highly conserved over functionally critical stretches. However, we identified private polymorphisms in the four species that differ most in climatic-ecological adaptations from their close relatives: five substitutions (two of them radical) in the desert-adapted *D. mojavensis*; five substitutions (three radical) in the moist-adapted *D. erecta*; one coiled-coil radical substitution and a 13-aa insertion in the arid-adapted *An. arabiensis*; and a catalytic-domain (conservative) plus a coiled-coil (radical) substitution in the drier-adapted *R. prolixus*. Further, the moist-adapted *G. palpalis* differed by one radical replacement from its drier-adapted closest relatives; the more distantly related *G. brevipalpis*, which breeds in high-moisture habitat patches, shares this aa with *G. palpalis*. Although most mutations in CHS1a functionally critical domains are likely deleterious, we identified and characterized a set of polymorphisms that may be involved in adaptation to drier vs. moister habitats. Private polymorphisms with larger potential impacts on enzyme function were more common in blood-feeding vectors than in fruitflies. Regulation of CHS1 expression and chitin post-processing (e.g., fibril organization or chitin-protein interactions) likely play crucial roles inmodulating dehydration resistance; our results widen the scope of research on candidate molecular mechanisms possibly underpinning climatic-ecological adaptations in insects.

P19. Understanding the role of ebony in tapeworm reproductive development

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Tapeworms are parasitic flatworms, competent to regenerate new tissue. Their anatomy is simple: head, neck, and a body made up of thousands of proglottids (segments). Each proglottid buds from the neck and contains all the necessary components to fertilize, incubate, and disseminate embryos. The constant production of proglottids means tapeworms continuously produce germ cells throughout their life, however, the mechanism of germ cell specification and differentiation is still unknown. The gene ebony was found to be necessary for schistosomes, the flatworm cousin of tapeworms, to be able to produce differentiated germ cells and lay eggs (Chen et al. 2022). Ebony conjugates β-alanine with tryptamine, as well as other monoamines, creating the dipeptide β-alanyl-tryptamine (BATT). This substance, BATT, is what is secreted by the male schistosomes while in physical contact with the female worm, resulting in the female taking up BATT and then being able to lay eggs. While schistosomes are diecious, we are curious if ebony performs similar functions in the hermaphroditic tapeworm. Using the rat intestinal tapeworm, *Hymenolepis diminuta*, as a model we observe ebony expression in the most anterior of the neck in a portion of osmoregulatory canals. This region of the canals flank undifferentiation germ cells. Thus ebony+ cells are poised to act as a germ cell niche. We find ebony expression influences germ cell development, as knockdown of the gene via RNA interference (RNAi) results in a decrease of the number of germ cells in the tapeworm neck. Using mass spectrophotometry, the presence of BATT was also identified in tapeworm tissue. Consistent with our model, BATT supplementation has a positive correlation with the production of undifferentiated germ cells. In conclusion, we identify ebony and BATT as important factors within the early germ cell niche. Ongoing experiments will resolve whether ebony plays sex-specific roles in later development.

P20. Adaptation of the *Plasmodium cynomolgi* Berok DC line to continuous in vitro culture

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Plasmodium vivax research has lagged behind *P. falciparum* because in vitro culture has been unsuccessful due to the need of a continuous source of reticulocytes. Luckily, the closely-related monkey malaria parasite, *Plasmodium cynomolgi*, does not require reticulocytes for growth, and recently, the K4 line of the *P. cynomolgi* Berok strain was adapted to continuous culture. Unfortunately, laboratories have struggled to continuously culture the K4 line because the parasite became exquisitely adapted to the specific nonhuman primate serum and erythrocytes utilized during its adaptation. This has limited the utility of the K4 line to a few labs able to identify nonhuman primates suitable for its growth. To address this limitation, we adapted another *P. cynomolgi* Berok strain parasite to continuous in vitro culture and designated it the DC line. Similar to K4, DC is able to infect monkeys with similar growth kinetics as the non-adapted strain, and it remains infectious to mosquitoes. However, the DC line has not developed a predilection for a specific source of NHP serum or erythrocytes, and it is currently being adapted to grow in human serum. Importantly, we have shown this parasite can be genetically manipulated efficiently in vitro. Overall, the DC line overcomes the prior limitations of K4 and should be able to be widely used to study asexual stages of vivax-type malaria parasites as well as new therapies and treatments for *P. vivax*.

P21. Long-read RNA Iso-seq and ATAC-seq data reveal and help explain C*ryptosporidium parvum*'s transcriptional landscape

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Cryptosporidium parvum is an apicomplexan protist parasite. Cryptosporidiosis, which is caused by this parasite is one of the leading causes of diarrhea in infants globally. Despite its compact genome size of 9.1 Mb, with 80% being coding sequence, *C. parvum* has lost several pathways including de novo nucleotide synthesis and RNA interference pathways. Previous work from our lab identified substantial, developmentally regulated long noncoding RNA (lncRNA) in vitro in *C. parvum*. We hypothesize that lncRNA may play important roles in regulating gene expression during the parasite's lifecycle. In this study, we utilized PacBio Iso-seq to characterize the RNA landscape of *C. parvum*. Additionally, we have conducted the first chromatin accessibility assay in *C. parvum* using ATAC-seq to investigate the relationship between chromatin state and the transcriptome. Our results revealed that the majority of *C. parvum*'s lncRNA are anti-sense and occupy the 3-prime ends of protein coding genes. Iso-seq also allowed us to annotate 5-prime and 3-prime untranslated regions (UTRs) of protein-coding genes and identified alternative splicing events despite the small number of genes with introns. Moreover, the combination of ATAC-seq and Iso-seq identified alternative transcription start site (TSS) usage. Overall, our study provides a comprehensive understanding of the *C. parvum* transcriptome and highlights a potential role for lncRNA in regulating gene expression during the in vitro parasite lifecycle.

P22. Mutual antagonism between the parasite *Trypanosoma cruzi* and the gut microbiota of its triatomine insect vector

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The single-celled parasite *Trypanosoma cruzi* alternates between mammalian and insect hosts to complete its lifecycle. Humans infected with *T. cruzi* develop Chagas disease, a debilitating infection potentially resulting in megaorgan syndromes or cardiomyopathy estimated to affect 7-8 million people in Latin America. While promiscuous in its ability to infect a variety of mammalian hosts, *T. cruzi* is exclusively acquired and transmitted by blood-feeding insects of the subfamily Triatominae, also known as kissing bugs. Extracellular life stages of *T. cruzi* (epimastigotes) colonize the kissing bug gastrointestinal tract, from which they are fecally transmitted back to vertebrate hosts. Because *T. cruzi* colonization of the kissing bug intestine is chronic, this parasite has likely evolved a variety of adaptations allowing it to contend with other gut-dwelling microorganisms – i.e. the gut microbiota – that it encounters in this unique environment. Due to the long evolutionary history of trypanosomatids as luminal residents of arthropod intestines, we hypothesized that *T. cruzi* and the microbiota compete for resources and space within the bug gut. To test this, we examined the dynamics of bacterial populations after introduction of *T. cruzi* in in vivo and in vitro settings. We observed that infection of the kissing bug, *Rhodnius prolixus*, with *T. cruzi* Y strain parasites suppresses the proliferation of the gut microbial populations. Intriguingly, we found that *T. cruzi* is also able to directly inhibit the growth of the major kissing bug bacterial symbiont, *R. rhodnii*, when these organisms are co-cultured in vitro. Finally, we compared *T. cruzi* colonization dynamics when infecting either axenic (microbe-free) or conventionally reared insects, and found that parasitemia was significantly higher when insect gut microbes were absent. Taken together, our results provide strong evidence that *T. cruzi* and the kissing bug microbiota are mutually antagonistic.

P23. Understanding fatty acid uptake in *Trypanosoma brucei* through gene knockouts

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Trypanosoma brucei is a parasite that causes African Sleeping Sickness, a disease that impacts humans and livestock in sub-Saharan Africa. This disease can be deadly. Though effective treatments are available, options are limited and epidemic re-emergence remains a threat. We aim to find new ways to treat this infection by better understanding the parasite's metabolism. My project aims to understand the function of genes related to the uptake of fatty acids in *T. brucei*. Little is known about the proteins involved in fatty acid uptake. We focus on two genes, a homolog to Fatty Acid Transfer Protein (FATP) and Long-chain Acyl-CoA Synthetase 5 (LACS5), that may play a role in the uptake process based on these genes' related functions in other organisms. To do this, we are creating knock-out *T. brucei* cell lines for FATP and LACS5 to test the impact of their deletion on fatty acid uptake. We are using Gibson Assembly to generate constructs that will direct the replacement of each allele with Blasticidin (BLA) and Puromycin (PUR) resistance genes. Once the constructs are completed and validated, they will be transfected into *T. brucei* to generate the knock-out cell lines, followed by validation and phenotypic characterization. By identifying genes essential for fatty acid uptake, we can understand how the parasite acquires essential nutrients from its host. This work may enable us to create better treatments for African Sleeping Sickness and related veterinary diseases in livestock.

P24. Evaluating the regulatory role of polyphosphorylation in *T. cruzi*

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Trypanosoma cruzi is a kinetoplastid parasite which causes Chagas disease and infects up to 8 million people across the Americas. While this disease has significant impacts on public health, including potentially fatal cardiac manifestations, the complex network of host-pathogen interactions and how they are regulated remains unclear. To address this knowledge gap, we are particularly interested in the post-translational modifications of parasite-derived virulence factors. Lysine polyphosphorylation is the covalent, non-enzymatic attachment of the ubiquitous molecule polyphosphate (polyP) to lysine residues within polyacidic serine and lysine (PASK)-rich clusters. This post-translational modification of proteins is common yet relatively newly described and remains to be evaluated in kinetoplastid parasites such as *T. cruzi*. In yeast, polyphosphorylation has previously been demonstrated to modulate both localization and enzymatic activity of proteins, suggesting it plays a significant role in controlling the function of proteins. In this work, we first performed a bioinformatic screen of the *T. cruzi* genome and identified 341 proteins which contain predicted PASK domains, and we hypothesize are polyphosphorylated. Next, we selected candidate molecules from this bioinformatic screen to evaluate using mobility shift assays to determine their ability to undergo polyphosphorylation. Using this approach, we have so far identified two *T. cruzi* proteins, the nuclear bromodomain factor 2 (TcBDF2) and the surface-localized phosphoinositide phospholipase C (TcPI-PLC), which demonstrated electrophoretic shifts consistent with polyphosphorylation when incubated with polyP130 and polyP700 respectively. Additionally, using fluorescence microscopy and enzymatic activity assays we are studying the ability of polyphosphorylation to modulate the localization and activity of these PASK domain containing proteins. Together, we show that essential parasite proteins are able to undergo lysine polyphosphorylation in vitro, potentially regulating their activity and contributing to the fine-tuning of pathogenic processes.

P25.Targeting translational control of stress as a potential drug target for the treatment of *Entamoeba histolytica*

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Entamoeba histolytica is an enteric parasite responsible for amebic dysentery and amoebic liver abscesses. It exists in two stages: the cyst, which causes infection in the host once ingested, and the trophozoite, which underlies invasive disease. Once present in the large intestine, *E. histolytica* must overcome stress brought on by nutrient deprivation and host immune pressure. One well-defined stress response includes the phosphorylation of eukaryotic initiation factor-2 alpha (eIF2α), which halts eiF2α activity and results in the down-regulation of general protein translation. *E. histolytica* possesses two putative eIF2α kinases: EHI_035950 and EHI_109700. Our previous studies have demonstrated that the phosphorylation of eIF2α in *Entamoeba* species is a key aspect of this pathogen's translation control system, response to stress, and stage conversion. Although a canonical eIF2α phosphatase has not yet been found, we believe that eIF2α phosphorylation and dephosphorylation may serve as valuable anti-amoebic drug targets. To explore this, we investigated the effects of guanabenz acetate (GBZ), an FDA-approved drug shown to inhibit eIF2α phosphatase in *Toxoplasma gondii*, and GSK2656157, an eIF2α kinase inhibitor. We treated trophozoites with a range of concentrations of each compound and measured the viability. GSK2656157 did not induce a loss of viability of trophozoites at the treatment concentrations and times tested. However, GBZ did reduce the viability of trophozoites. We identified the 50% inhibitory concentration (IC50) of GBZ in vitro to be between 8 µM and 10 µM. We also determined that sublethal concentrations of GBZ inhibited the conversion of trophozoites to cysts. Taken together, these findings suggest that GBZ can be repurposed to treat disease caused by *E. histolytica*.

P26. Multiple blood meals support non-competitive egg production and *Plasmodium berghei* infection in the Indian malaria vector *Anopheles stephensi*

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Mosquitoes feed on vertebrate blood to acquire nutrients for egg production and can acquire malaria parasites (*Plasmodium* spp.) when they feed on an infected individual. *Plasmodium* development within the mosquito host is intimately associated with egg production because parasites use nutrients mobilized during vitellogenesis for their own growth. Between ingesting malaria parasites and becoming infectious to the next vertebrate host, mosquitoes can take several more blood meals, each supporting an additional clutch of eggs. Whether malaria infection affects mosquito fecundity and longevity remains unclear. How multiple blood meals influence Plasmodium development is also poorly understood. To address these questions, we assessed the number of eggs per clutch, *P. berghei* sporozoite prevalence and intensity, and mosquito survival over the course of four additional blood meals after the infectious feed in *Anopheles stephensi*. Egg clutch sizes remained constant after several blood meals and were not influenced by infection status. Multiple blood meals accelerated sporozoite development in mosquitoes fed multiple blood meals relative to those that only received the infectious feed. Mosquito survival was also unaffected by the parasite. These results suggest multiple blood meals provide sufficient nutrition for both egg production, parasite development, and survival.

P27. Analysis of varying drug treatment regimens on the activity of *Trypanosoma cruzi* amastigotes in-vitro

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Trypanosoma cruzi is the causative agent of Chagas disease, the highest impact parasitic disease in Latin America. Intracellular amastigotes of *T. cruzi* are the replicative stage in mammals, but spontaneous dormancy in a fraction of these amastigotes allow them to survive drug treatment. Using currently available drugs, the rate of killing of *T. cruzi* amastigotes is slow and there are gaps in understanding drug kinetics. This research focuses on understanding how two drugs, benznidazole, in use for *T. cruzi* infection treatment for more than 50 years, and AN15368, a new compound that has yet to be tested in humans, control both actively replicating and dormant *T. cruzi* in-vitro. We used time lapse videos to track in-vitro intracellular amastigote activity (proliferation, stasis, and death), in the presence and absence of drug for up to 11 days. CellTrace violet pre-staining of infective trypomastigotes allows for the identification of dormant (non-replicating) amastigotes. In a four-day treatment period at 10X IC50, the dose required for ½ the maximal killing, of AN15368 and benznidazole, followed by washing out the drugs, AN15368 shows earlier, and more rapid killing. Killing of *T. cruzi* amastigotes continues after removal of the drugs, mainly in AN15368-treated cultures. Likewise, parasite rebound after drug removal is more extensive and happens sooner in benznidazole treatment. Fewer parasites rebound post AN15368 treatment. Our results provide a better understanding on the killing kinetics of these two drugs, which will be useful for modifying treatment regimens to effectively kill the parasites as they come out of dormancy.

P28. A *Plasmodium* rhoptry neck protein is essential for parasitophorous vacuole formation

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Malaria is a serious public health issue that affects millions around the world and is especially detrimental to children under the age of 5. During infection, *Plasmodium falciparum* parasites invade red blood cells (RBCs), creating and residing within a membrane-bound compartment called a parasitophorous vacuole (PV). PV formation is critical for intracellular *Plasmodium* survival as it is required for nutrient uptake, protein trafficking, and waste export. After replicating within RBCs, *Plasmodium* will egress from the host cell in the form of infectious daughter merozoites which reinvade surrounding RBCs. This cyclical intraerythrocytic development cycle causes the manifestation of clinical disease. Recent work has identified that an ER-resident calcium binding protein (PfERC) is an essential regulator of *Plasmodium* egress. Using quantitative proteomics, we identified proteins interacting with PfERC (PIEs) as candidates for further study based on essentiality and peak expression during schizogony. Our work is focused on discovering the function of two conserved membrane proteins, Pf3D7_1117400 (PIE1) and Pf3D7_0522600 (PIE2). Using CRISPR/Cas9 genetic engineering to incorporate the PfDOZI-TetR-based knockdown system or the Cre recombinase knockout system, we have manipulated parasites to generate conditional mutants to study how these two proteins function. We have determined that PIE2 is not essential for the asexual life cycle, despite its predicted essentiality. Using immunofluorescence microscopy, we determined that PIE1 localizes to the rhoptry neck. Rhoptries are unique secretory organelles whose contents are secreted into the RBC and rhoptry neck proteins play a critical role in merozoite invasion. Surprisingly, knockdown of PIE1 did not inhibit invasion of *P. falciparum* merozoites. Instead, knockdown of PIE1 results in merozoites that invade RBCs but lack a PV. These data suggest that PIE1 has a role in PV formation. Ongoing studies will determine if PIE1 is required for PV formation during invasion or PV maintenance post-invasion.

P29. *Wolbachia* protein, Wbm0152, disrupts eukaryotic endolysosomal membrane dynamics

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Wolbachia is a Gram negative, intracellular bacterium capable of parasitizing an extremely broad range of arthropod and nematode hosts. Although the *Wolbachia*:insect relationship is usually considered parasitic, *Wolbachia* has become an essential endosymbiont in human pathogenic filarial nematodes such as *Brugia malayi*, as nematodes lacking *Wolbachia* are completely unable to colonize the human host. Upon expression of *Wolbachia* protein, Wbm0152 in the model system *Saccharomyces cerevisiae*, disrupted delivery of endosomal cargo to the degradative vacuole was observed. Based on the phenotypes observed in yeast, we hypothesized that Wbm0152 expression inhibited the conserved eukaryotic Endosomal Sorting Complex Required for Transport (ESCRT) protein complexes. To further illuminate the potential interactions of Wbm0152 with ESCRT, colocalization analysis of GFP-tagged Wbm0152 and mRuby-tagged ESCRT protein subunits showed colocalization with subunits from complexes ESCRT-I and -II, moderate localization with subunits from ESCRT-III, and no localization with the ESCRT-affiliated protein Bro-1. This may suggest Wbm0152 plays a preventative role in the assembly of ESCRT-III subunits on pre-existing ESCRT-II complexes or the interaction of downstream regulatory proteins (like Bro1). To determine specific binding partners of Wbm0152 in the ESCRT complexes, a biomolecular complementation assay was performed which showed direct binding of Wbm0152 with ESCRT proteins Vps2p, Vps20p, and Vps25p. We therefore hypothesize that this protein is likely important in maintaining the *Wolbachia*:nematode endosymbiosis by altering host endolysosomal membrane dynamics. Further characterization of the activities of this protein is likely to fill an important knowledge gap regarding the molecular mechanisms by which *Wolbachia* can persist within the nematode host.

P30. Using proximity biotinylation to probe the mitochondrion-ER interaction in *Toxoplasma gondii*

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Apicomplexan parasites are responsible for several debilitating diseases illnesses afflicting both humans and animals. *Toxoplasma gondii* is a member of this medically significant phylum and the causative agent of toxoplasmosis, a significant threat to pregnant or immunocompromised individuals. Moreover, *T. gondii* is also emerging as a model to study cell biology in divergent eukaryotes. In other model organisms, it has been shown that mitochondrion-ER interactions are essential for calcium and lipid exchange, ER morphology, mitochondrial dynamics, and cell fate. These interactions are supported by areas of close proximity between the two organelles, maintained by protein tethers, and referred to as membrane contact sites (MCSs). Currently, the majority of our knowledge on mitochondrion-ER MCSs is restricted to yeast and mammals, which are part of the opisthokont clade. To improve our knowledge on MCSs across the evolutionary tree, we will study the mitochondrion-ER association in *T. gondii*. Here, I have adapted an ER handle to *T. gondii*, which can be used to anchor proteins to the cytoplasmic side of the ER membrane. I used this new handle to generate a strain expressing TurboID, a biotin ligase, on the ER membrane. We also generated strains expressing TurboID both on the outer mitochondrial membrane, as well as a cytoplasmic-localized version. Together, they will be used to uncover MCSs in *Toxoplasma gondii* through an unbiased proximity biotinylation-based approach. This methodology will not only allow us to identify canonical MCSs between the two organelles, but also identify proteins that are apicomplexan-specific, expanding our knowledge of how these contacts are conserved throughout evolution.

P31. Induced in vitro sexual commitment of *Plasmodium cynomolgi*

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Plasmodium vivax is the most geographically widespread malaria species, yet our understanding of its unique biology has been hindered by a lack of in-vitro culture systems and access to human clinical samples. Phylogenetically related species such as *P. cynomolgi* have been historically utilized in non-human primate models to further our understanding of *P. vivax* biology. Recently, *P. cynomolgi* was successfully adapted to long-term in vitro culture, expanding the utility of this model species. Gametocytes, sexually committed parasites capable of mosquito infection, are relatively uncharacterized in *P. cynomolgi* and are essential for mosquito infection, making them an ideal target for in vitro study. Previous reports indicate overexpression of gametocyte associated genes Api-AP2G (AP2-G) and Gametocyte Development protein 1 (GDV1) in *P. falciparum* and *P. berghei* leads to massive in vitro sexual commitment, yet this has not been accomplished in *P. cynomolgi*. However, transcriptional and genomic studies indicate the process of sexual commitment is likely conserved within the *Plasmodium* genus. To gain greater insight into the mechanism of sexual commitment in *P. vivax*-like parasites, we are currently generating molecular tools to overexpress *P. cynomolgi*-specific homologues to AP2-G and GDV1 within in vitro cultured *P. cynomolgi*. We aim to utilize both the centromere-containing overexpression plasmid, pCyCEN, as well as CRISPR/Cas9 to achieve our goals. *P. cynomolgi* mutants capable of in vitro sexual commitment will be tested for mosquito infectivity via standard membrane feeding assay. This work will increase our understanding of sexual commitment in *P. vivax*- like parasites and create a valuable tool for future studies.

P32. Evolution of Programmed DNA Elimination in parasitic nematodes

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The human/pig parasite *Ascaris* is a member of the Ascarididae family of nematodes (ascarids) that infect a wide variety of animals and includes the horse parasite *Parascaris univalens*. *Ascaris* and *Parascaris* undergo Programmed DNA Elimination (PDE), an exception to the genome constancy rule. During ascarid PDE, large germline chromosomes are broken into smaller somatic chromosomes with loss of selected sequences. The eliminated DNA is enriched in highly repetitive sequences and genes involved in germline function. The resulting DNA ends are repaired through addition of new telomeres, forming stable somatic chromosomes. The functions and molecular mechanisms of PDE in nematodes are under intensive investigation. However, the evolutionary trajectory of chromosomes in nematodes with PDE remains largely unknown. Here, we generated complete germline and somatic genomes for *P. univalens* using PacBio and Hi-C and compared them to the *Ascaris* genomes to delineate the genome dynamics between the two closely related parasites. The single germline chromosome of *P. univalens* is broken into 36 smaller somatic chromosomes, with 90% of the germline genomic sequence being eliminated. Interestingly, we found that each of these 36 somatic chromosomes in *Parascaris* is mapped uniquely to one of the 36 somatic chromosomes in *Ascaris*, suggesting that the content and organization of the somatic chromosomes are the same in these two parasites. Our data indicates that PDE may function to restore the karyotypes of somatic chromosomes from drastically different germline chromosomes in *Ascaris* and *Parascaris*. These germline chromosomes may have arisen from fusion events during evolution. As ascarids are important parasites, our completed genome, comprehensive transcriptomes, and annotations also provide rich genomic resources for future molecular studies that may identify potential new drug targets or mechanisms of drug resistance.

P33. Rapid, novel, and sensitive tetrazolium-based colorimetric assay for helicase nsp13 in SARS-CoV-2

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a human pathogenic virus that has essential proteins ranging from cell surface attachment, RNA and viral genomic replication, and translation of viral structural proteins. Once expressed in the host cell, 16 non-structural proteins are produced, which represent a drug discovery targeting opportunity. One target that has received attention in early-stage drug discovery is Coronavirus non-structural protein 13 (nsp13) that is also known as SARS-CoV-2 helicase (SC2Hel). The enzyme serves the function of unravelling double-stranded RNA (dsRNA) or double-stranded DNA (dsDNA) in an NTP-dependent method from the 5' to 3' directionality. The standard nucleic acid unwinding assay involving helicase from studies of SARS-CoV and SARS-CoV-2 encompasses the concept of fluorescence resonance energy transfer (FRET). Although effective in detection of nucleic acid unwinding activities, there is the drawback of relatively expensive fluorophore and dark quencher components for the nucleic acid. Herein, we have developed a rapid, novel, sensitive, tetrazolium-based colorimetric assay for the detection of SC2Hel activities (nucleic acid unwinding activity and ATPase activity) using a standard unmodified duplex nucleic acid substrate. This SC2Hel assay combines three enzyme-coupled steps involving the ADP-dependent *Thermococcus litoralis* glucokinase (TlGlcK), *Leuconostoc mesenteroides* glucose-6-phosphate dehydrogenase (LmG6PDH), and *Clostridium kluyveri* diaphorase (CkDIA). In the final step of the assay, a colorimetric tetrazolium reagent was used, known as iodonitrotetrazolium chloride (INT), that converts into INT-formazan upon reduction and exhibits an intense colorimetric response at a wavelength maximum of 505 nm (in a pH 7.6 buffered solution). The assay is more cost effective than the canonical FRET-based assay since the duplex nucleic acid substrate lacks any modification.

P34. Using chemobiology to study the liver stage of *Plasmodium vivax*

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Treating the reservoir of hypnozoites residing in the liver is essential for controlling and eliminating malaria caused by *Plasmodium vivax*. The only drugs which kill hypnozoites and prevent relapse—primaquine and tafenoquine—are contraindicated or not approved for vulnerable groups, including pregnant women and children. Discovery and development of new antimalarials targeting hypnozoites are now possible in our robust 384-well plate phenotypic screening format. To date we have screened over 65,000 compounds against *P. vivax* hypnozoites, resulting in over 30 confirmed hit scaffolds. Included in this collection of hits are drug-like compounds, with properties amendable to medicinal chemistry, and probe-like compounds, which are less attractive for development but useful for interrogating hypnozoite biology. Herein we summarize our implementation of a make-test cycle to understand the structure-activity relationship of one hit, and how a second hit is being pursued as a repurposed drug. Meanwhile, using our collection of chemical probes, we can study some of the biological processes governing hypnozoite dormancy, revealing hypnozoites are active forms with dynamic structures and functions. Ongoing work aims to produce compounds suitable for confirmation of efficacy in vivo, as well as further elucidate the chemobiololgy of *P. vivax* hypnozoites.

P35. An investigation of A-P polarized genes in *Hymenolepis diminuta*

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The rat tapeworm, *Hymenolepis diminuta*, is a tractable model to study parasite stem cells, which underlie the development, regeneration, transmission, and potentially pathology of tapeworms including those that infect humans. In *H. diminuta*, the neck is the only regeneration-competent region with proglottids forming at the posterior end of the undifferentiated neck. *H. diminuta* can regenerate after serial rounds of amputation while the head remains intact, but if the head is removed or damaged, regeneration is finite. The neck will be lost, and the worm becomes fully segmented. Therefore, signals from the head are required to maintain regeneration of proglottids. We hypothesized that head-dependent signals create asymmetries in the neck along the anterior-posterior (A-P) axis that influence regeneration. Subdivision of the neck into anterior, middle, and posterior fragments revealed that the most anterior neck fragments regenerated more proglottids than the middle and posterior neck fragments over a 12-day growth period. RNA-sequencing of the subdivided neck fragments uncovered 461 anterior- and 241 posterior-enriched transcripts. This led us to investigate how A-P polarized gene expression patterns within the neck effect regeneration. We validated and described four general polarized expression patterns using whole mount in situ hybridization. Analysis of six anterior enriched transcripts by double fluorescent in situ hybridization identified at least two distinct cell populations within the anterior neck: β4gat1+ and β4gat1-. We also find that gene expression of β4gat1 is elevated and mis-expressed if the tapeworm head is removed. The β4gat1- population expresses many components of the Wnt signaling pathway, which has been implicated previously in A-P polarity, stem cell regulation, and regeneration competence in many species. This preliminary work has laid the foundation for a deeper understanding of the microenvironment signals that pattern the neck and enable tapeworm regeneration.

P36. I have no mouth and that's okay: Viable endocytic null mutants of *Trypanosoma cruzi*

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Trypanosoma cruzi is the sole pathogenic trypanosomatid of humans that retains the endocytic organelle known as the cytostome-cytopharynx complex (SPC). In free-living kinetoplastids such as *Bodo saltans*, this tubular feeding apparatus functions to capture and endocytose bacterial prey. How this endocytic organelle functions at a mechanistic level and why *T. cruzi* alone retains the SPC, remains largely unknown. This work builds upon our initial efforts identifying the first known SPC proteins of any protozoan and the identification of several SPC targeted myosins and an associated regulatory protein essential for endocytosis. As myosin motors canonically use actin as a substrate, we next focused our efforts on identifying actin-related components essential for SPC function. Accomplishing this aim, we identified a putative formin enzyme (TcFormin3) and an actin-related protein (TcARC), both of which generated endocytic null mutants when deleted, a phenotype which was rescued upon complementation. Using in-silico sequence and structure analysis, site-directed mutagenesis, and advanced microscopy techniques, we sought to characterize these proteins in greater detail, analyzing their domains, dynamic localization and functional role in the SPC. Notably, we found that one of our endocytic null mutants entirely lacked the cytostome entrance to the SPC, but remained viable in culture. This ability to identify the first functional SPC components, and the generation of several viable endocytic null mutants targeting different aspects of the SPC machinery and structure, provides us with the unique capacity to dissect the mechanism behind SPC-mediated endocytosis and ultimately, to uncover the functional role of this unusual organelle in the *T. cruzi* life cycle.

P37. Extrafollicular B cells are associated with persistent anemia during chronic *Plasmodium* infections

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While *Plasmodium falciparum* (Pf) malaria is known for acute febrile illness, chronic infections with minimal symptoms become the norm as individuals acquire immunity. These 'asymptomatic' infections were thought to be inconsequential, but new research indicates persistent anemia is present in 32.8% of individuals with chronic Pf infections. The mechanisms that contribute to anemia during chronic Plasmodium infections are not understood but are important to understand because persistent anemia places individuals at a higher risk of developing severe anemia and leads to cognitive deficits in children. Here, we used samples collected from rhesus macaques infected with *P. coatneyi*, a model for human Pf infections, to identify potential mechanisms involved in chronic malarial anemia. Using whole blood RNA sequencing, detailed clinical data, and weighted gene co-expression network analysis, we identified gene clusters that correlated with fluctuations in hematocrit and hemoglobin levels during acute and chronic phases of the infections. These clusters were overrepresented with genes related to B cells, and thus, we hypothesized that 'anti-self' antibodies directed against red blood cell (RBC) membrane proteins may contribute to persistent anemia. Indeed, anti-RBC antibodies significantly increase during the acute phase and persist during the chronic infection. The amount of anti-RBC antibodies correlated with the expansion of extrafollicular B cells (CD20+IgD-CD27-CD11c+CD21-T-bet+) indicating this population may be responsible for their production. These cells showed signs of proliferation during acute infection but in the chronic phase, suggesting this subset is generated during the acute phase and then maintained. In sum, these data suggest that extrafollicular B cells specific for RBC membrane proteins expand during acute infection and are maintained during chronic infections where they differentiate to produce anti-RBC antibodies resulting in chronic anemia. Better understanding of the differentiation of these cells may lead to new treatments for malarial anemia.

P38. Inhibition of fructose 1,6-bisphosphatase in *Naegleria fowleri*

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Naegleria fowleri infections, which have a very high mortality rate (~98%), leads to the disease primary amebic meningoencephalitis (PAM). Because of limited drug treatments and the high mortality rate even in the face of aggressive treatments, new approaches are needed. Here, we are exploring the potential for targeting a metabolic enzyme, fructose 1,6-bisphosphatase (FBPase), due to its involvement in gluconeogenesis and lack of human FBPase homology. The role of FBPase is to dephosphorylate fructose 1,6-bisphosphate. Inhibiting this enzyme while simultaneously inhibiting glycolysis is an effective drug combination for *N. fowleri* during nutrient deprivation. To date, we have successfully cloned the gene into a heterologous expression plasmid but have yet to generate recombinant FBPase using standard approaches. This potentially suggests that FBPase is incompatible with current expression conditions and further testing is required.

P39. Use of peroxisomal targeting sequences in drug delivery

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Trypanosoma brucei is the causative agent of African sleeping sickness in humans and nagana in cattle. The parasite organizes several essential metabolic pathways, including glycolysis, into specialized peroxisomes called glycosomes. Inhibitors of trypanosome glycolytic enzymes developed against recombinant enzymes have historically had poor activity against the parasites, likely due to target inaccessibility in the glycosome. To overcome this potential hurdle, inhibitors were modified with a type one peroxisomal targeting sequence (PTS1). This sequence is found on proteins destined for peroxisomes and glycosomes, and our previous work has demonstrated that addition of the PTS1 tripeptide is sufficient to traffic exogenous small molecule cargo, including inhibitors of glycolytic enzymes, to glycosomes. Notably, these PTS modified inhibitors were more toxic to trypanosomes than the unmodified compound. With the goal being optimized delivery of drugs by modification with a PTS1 sequence, we have begun to assess the impact of the spectrum of potential PTS1 sequences found on cellular proteins on rates of cellular uptake and glycosomal trafficking in *T. brucei*.

P40. The close cousins of O-GlcNAc-transferases: the O-fucose-transferases

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Toxoplasma gondii infects approximately 30% of the human population worldwide and is responsible for the disease toxoplasmosis. The parasite propagates between an asexual and sexual life cycle in which the parasite must adapt to its various environments. These adaptations require tight regulation of gene expression, protein levels, and protein activity. Of interest is the recent discovery of an O-fucosyltransferase (OFT) encoded by the SPY locus that modifies serine and threonine residues of at least 33 different nucleocytoplasmic proteins with a single fucose residue during the proliferative phase of the parasite. Spy-like sequences in amoebozoan protists that lie along a distant evolutionary branch, *Dictyostelium* and *Acanthamoeba*, and another apicomplexan parasite, *Cryptosporidium*, are also O-fucosyltransferases (OFTs). This process is related to the O-GlcNAcylation of nucleocytoplasmic proteins of animal and plant cells via O-GlcNAc transferases (OGTs), which have been implicated in mediating stress and nutritional responses. *Aleuria aurantia* lectin (AAL) staining of tachyzoite parasites indicate that O-fucosylated proteins accumulate in assemblies near the nuclear pore complex (NPC) of the parasites. Furthermore, AAL staining revealed complete loss of terminal fucose modifications in the nucleocytosol of cells lacking TgSPY, suggesting that the OFT is solely responsible for the PTMs. To facilitate further investigation of these differences in *Toxoplasma* and other protists containing the OFT, we present here the development of rabbit antibodies specific for fucose-O-Ser and fucose-O-Thr (anti-FOS/T). Unlike AAL that cross-reacts with terminal fucose on N- and O-linked glycans of ER, Golgi, secreted, and plasma membrane proteins, these anti-FOS/T antibodies only bind to nucleocytosolic proteins modified by the OFT. We show that anti-FOS/T antibody labels *Toxoplasma* nuclei without cross-reacting with host secreted proteins, and labeling is inhibited by fucose and lost in spyΔ tachyzoites. The anti-FOS/T antibodies promise a revolution in the identification and localization of nucleocytosolic proteins modified with O-fucose in protists, plants, and bacteria, which cannot be studied with AAL because of the presence of terminal fucose on secreted proteins.

P41. Visualizing the cytostome-cytopharynx complex of *Trypanosoma cruzi*

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Eating is fundamental for life, and from this basic principle living organisms have evolved an array of strategies to capture energy and nutrients from their environment. As part of the world's aquatic ecosystems, the expansive family of protozoan predators hunt and eat their prokaryotic prey using an ancient feeding apparatus known as the cyto**s**tome-cyto**p**harynx **c**omplex or SPC. This feeding structure begins as a plasma membrane surface opening (cytostome), descends into an internal tubular invagination (cytopharynx) and ends with prey being enveloped within budding vesicles destined for digestion. However, despite its ubiquitous presence in environmental protozoans, very little is known mechanistically about how this endocytic organelle is formed or functions. Intriguingly, a class of phagotrophic predators known as the kinetoplastids, gave rise to a lineage of parasitic protozoa that can infect a wide variety of organisms ranging from plants to humans. Curiously, one important human pathogen known as *Trypanosoma cruzi*, the etiological agent of Chagas disease, that infects an estimated 10 million people in the Americas, retained this ancestral organelle much like its free-living relatives (e.g. bodonids) and continues to use it as its primary route of endocytosis. Using currently available data we have sought to produce a 3D representation of the cytostome-cytopharynx complex of *T. cruzi* in insect-stage (epimastigote) parasites within the vector intestine. Ongoing research in the Etheridge lab seeks to elucidate the molecular components of the SPC in *T. cruzi* and assess their role in its the function. Overall, by using *T. cruzi* as a model for dissecting SPC function, we can begin to elucidate the mechanistic basis of this ancient protozoal feeding apparatus with the goal of providing important insights into processes ranging from microbial food webs to parasitic diseases.

P42. Host glycolytic metabolites serve as a vital fuel source for energy metabolism and intracellular survival of *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite that acquires nutrients from its host during infection. Previous literature demonstrated that host cells increase the production of glycolytic intermediates upon *Toxoplasma* infection, and lactate/pyruvate supplementation can partially rescue parasite growth when its glycolysis is blocked, suggesting that *Toxoplasma* can take up lactate/pyruvate as a fuel source. However, the molecular mechanism underlying this phenomenon remains unclear. Through our bioenergetic analysis, we show that *Toxoplasma* can ingest and deliver lactate and pyruvate into its mitochondrion for energy production. To further elucidate how *Toxoplasma* transports lactate/pyruvate, we individually deleted three reported lactate/pyruvate transporters (FNT1-3) in wildtype (WT) and glycolysis-deficient parasites Δhk, a mutant lacking hexokinase. Our bioenergetic study showed that deletion of individual FNTs did not completely prevent lactate/pyruvate acquisition. However, the removal of both FNT1 & FNT2 fully blocked lactate/pyruvate uptake. Through qPCR, we observe that TgFNT2 is highly upregulated in the absence of TgFNT1, suggesting a compensation mechanism between both TgFNTs. To acquire lactate/pyruvate from host cells, the parasites need to adopt a strategy to allow these metabolites across the parasitophorous vacuole membrane (PVM) during intracellular replication. One dense granule protein, TgGRA17, was reported to mediate transport of small solutes through the PVM. Here, we utilize a FRET-based lactate sensor to test lactate concentrations within the PVM in ΔhkΔgra17 via live-cell imaging. In addition, our murine infection model revealed that ΔhkΔfnt1Δfnt2 displayed significant growth and virulence defects, indicating the importance of lactate/pyruvate ingestion for parasite survival in vivo. Collectively, our work presents a novel host-parasite interaction strategy for nutrient acquisition in *Toxoplasma gondii*.

P43. Detection of *Trypanosoma cruzi* and *T. rangeli* infections from *Rhodnius pallescens* across different land use types in Panama

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Triatominae species are the only known vectors of *Trypanosoma cruzi*, the cause of Chagas disease in vertebrates. In addition, they can be infected with *Trypanosoma rangeli*, that also infects humans and animals in Central and South America but does not cause any significant pathology. The dynamics of both parasites seem to be affected by anthropogenic changes in the landscape, which should be considered as part of the whole ecological dynamics of Chagas disease. Studies suggest that co-infection may affect the ability of kissing bugs to transmit *T. cruzi*, thereby altering the prevalence of this disease in different habitats. The objective of this study is to evaluate ecological factors associated with Chagas disease vector *Rhodnius pallescens* across land use gradients. We tested single infection and co-infection rates in populations of *Rhodnius pallescens* (N=82) in a landscape gradient in central Panama where the species is considered the main vector of Chagas disease. We collected kissing bugs from 46 palms located in 12 communities across different habitat types, including secondary forest, grassland, and regenerating forest. Genomic DNA was extracted from whole bodies and real-time PCR (RT-PCR) assays were performed using probes targeting the 18S ribosomal RNA (rRNA) genes of both parasites. Thus far, we have collected 82 specimens of *R. pallescens*: Of those, 45% were Adults (N=37), 32% Nymphs between stages 5 and 3 (N=26), and 23% Nymphs between stages 2 and 1 (N=19). Preliminary data indicate that the prevalence of single infection with only *T. cruzi* is 39%, with *T. rangeli* is 13%, and co-infections were present in 39% of individuals in this landscape. Only 9% were not infected with any species. We will analyze and compare fine scale (palm-level), medium scale (community-level), and landscape scale (region) factors associated with *T. cruzi-T.rangeli* infection patterns and discuss results in relation to *T. cruzi* infection dynamics.

P44. Interferon-induced cell-autonomous immunity to *Plasmodium* in hepatocytes

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Malaria, caused by *Plasmodium* parasites remains an unresolved global health emergency that impacts over half of the world's population. The sporozoite stage of Plasmodium inoculated into the host by mosquitoes migrates to the liver and establishes infection in the hepatocytes, where they undergo replication and development. We have shown that type I interferon-driven responses in the infected hepatocytes are pivotal in controlling *Plasmodium* in the liver. Yet, the mechanism of such control remains unknown. Transcriptional analysis of primary murine hepatocytes infected with *Plasmodium berghei* (Pb) showed that several members of a specific class of interferon-stimulated immune effectors called Guanylate Binding Proteins (GBPs) are upregulated in such cells. Hepatocytes deficient in GBP-1 failed to optimally control *Plasmodium* in the liver. Type I IFNs drove the recruitment of GBP-1 to *Plasmodium* parasitophorous vacuole (PV), mediated by IFN-induced accessory cell-intrinsic immune effectors such as p62, LC3, and Ubiquitin in both murine and human hepatocytes, following which, GBP-1 induced disruption of the PV and lysis of the parasite within the hepatocytes. Taken together, we describe a new pathway of cell-autonomous immunity to liver-stage malaria.

P45. The role of protein disulfide isomerase in the endoplasmic reticulum of *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite that belongs to the phylum Apicomplexa. It can infect most warm-blooded animals as intermediate hosts. During the acute phase of toxoplasmosis, the fast-growing tachyzoite actively invades host cells, undergoes asexual replication, and egresses rupturing host cell membranes causing damage to host tissues. The host's immune response will slow down replication of the tachyzoite, which will differentiate into the slow-growing bradyzoites that encyst in the host's tissues. The endoplasmic reticulum (ER) is a dynamic organelle in eukaryotic cells. It is the site for post-translational processing and serves as the largest calcium store in the cell. Protein disulfide isomerases (PDIs) are resident ER enzymes that catalyze the breakage and formation of disulfide bonds between cysteine residues in proteins in order to chaperone protein folding and regulate ER caclium homeostasis. In mammals, the PDI family, which is a subgroup of the thioredoxin superfamily of proteins, is composed of 21 different proteins, which have not been characterized in *T. gondii*. Members of the PDI family are functionally diverse but all contain at least 1 thioredoxin-like domain. Those proteins, which carry out disulfide bond breakage and/or formation, have canonical CXXC motifs that function as the active site for oxidative protein folding. We characterize TGGT1_211680 (TgPDI1) which is essential for parasite growth and ablation of TgPDI1 causes a defect in parasite replication and host cell invasion. We aim to characterize the role of TgPDI1 (TGGT1_211680) in the *T. gondii* lytic cycle, specifically its potential roles in calcium signaling in the ER, protein folding, microneme secretion, and the ER stress response. We also intend to use subcellular fractionation and co- immunoprecipitation techniques to create a library of ER proteins in *T. gondii* through proximity biotinylation with TgPDI1-TurboID.

P46. Live attenuated Centrin4 knockout *T. cruzi* as a new vaccine candidate

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Chagas disease is a parasite disease caused by *Trypanosoma cruzi* that affects around 8 to 10 million people worldwide. The treatment for Chagas disease is the same since the seventies, using Nifurtimox and Benznidazole, those medicines are not highly efficacious, and they have higher side effects and cost. Due to the lack of treatments and prevention for this disease, the development of a new strategy such as a prophylactic vaccine using attenuated parasites has become a good option. Attenuated parasites with Centrin knockout it's been widely used for leishmaniosis vaccines because of the role of centrin during the proliferation. Overall, Cen-/- has been showing promise as a candidate vaccine against parasite diseases by providing long-term immunity. The ultimate goal of this project was to create an attenuated parasite by knockout the Centrin 4 gene using the CRISPR/Cas9 technique. We create sgRNA that recognize the beginning of the sequence for the Centrin 4 gene and were able to target all the copies. We transfected the *T. cruzi* Columbiana strain expressing TdTomato and rsLuciferase with the sgRNA and SaCas9, and also a repair template that was able to add the three stop codons and a BamHI restriction site into the sequence. Two days after the transfection we checked the population by DNA extraction, PCR, and BamHI digestion to check if the transfection worked and after that, we cloned by putting 1 parasite per well in a 96-well plate. After they grow we selected the parasites that were showing morphological differences and confirmed them by PCR and digestion. We were able to get null-/- and heterozygotes+/- clones that are going to be morphologically characterized for the first time in *T. cruzi*, as well as used for an in vitro and in vivo infection essay.

P47. DNA break and end resection in *Ascaris* programmed DNA elimination

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Organisms must maintain the integrity and full complement of their genome in all cells. However, in the human and pig parasitic nematode *Ascaris*, a process of DNA loss known as programmed DNA elimination occurs during early embryogenesis in all pre-somatic cells. In *Ascaris*, all 24 germline chromosomes are fragmented with 72 DNA double-strand breaks (DSBs) at specific genomic loci. The broken ends are healed with de novo telomere addition to generate new chromosome ends. Genomic analysis of the telomere addition sites in a population of *Ascaris* revealed a 3-6 kb chromosomal break region (CBR). We found no sequence or structural motifs, common histone marks, or small RNAs associated with the CBRs. Yet, CBRs exhibit more accessible chromatin during and after DNA elimination, suggesting that specific mechanisms are involved in this process. However, CBR recognition, DSB generation, and broken end processing are poorly understood. To further characterize DSBs introduced during PDE, we used END-seq to identify break sites and end resection. We found that the initial DNA break occurs before the onset of mitosis. We also revealed extensive end resection at the CBRs after DSB induction. Interestingly, telomere addition is biased towards the retained but not the eliminated DNA, suggesting a selective process governs the telomerase activity. We also identified alternative break sites where DSBs form within the eliminated DNA in both *Ascaris* and *Parascaris* (a horse parasite that also undergoes PDE). These alternative break sites may act as a "fail-safe mechanism" to ensure the elimination of sequences. Our results reveal the timing of DNA breaks, extensive resection of DSBs, and the presence of alternative break sites, thus providing insights into the DNA break and repair process during *Ascaris* DNA elimination.

P48. Lead optimization and target identification of drugs targeting hypnozoites

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Malaria caused by *Plasmodium vivax* is a neglected tropical disease that infects up to 15 million people each year, with ~2.2 billion people at risk. Hypnozoites of *P. vivax* are the dormant parasite reservoir in the liver that causes the majority of vivax malaria cases worldwide. Currently only two drugs—primaquine and tafenoquine— are approved to prevent relapse, but both drugs are 8-aminoquinolines that can't be administered to patients with specific genetic backgrounds. New, safe and effective therapies are needed. However very little is known about the biochemical pathways that are active in hypnozoites; and furthermore, there are no validated drug targets. Using a medium throughput assay for *P. vivax* liver stages in vitro, we identified a new non-8-aminoquinoline compound (MMV987) with hypnozonticidal activity. We have performed optimization of the MMV987 hit series for improved potency, stability, and bioavailability. Current efforts involve identifying the target(s) of our hit compound. As *P. vivax* is not amendable to continuous culture, we have used *P. falciparum* as a proxy for our initial studies. In-vitro evolution followed by whole genome sequencing, we reveal numerous mutations in parasites resistant to our lead compound compared to sensitive parasites. Furthermore, using a thermal shift assay and mass spectrometry we reveal compound–protein interactions. Work is currently underway to identify protein-compound interactors in hepatocytes infected with *P. vivax* hypnozoites. Together, our work highlights a novel pipeline, including compound optimization and various -omics approaches, to identification of drugs targeting hypnozoites.

P49. Is internalization of the transferrin receptor by *Toxoplasma gondii* a strategy for iron acquisition?

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Iron is a crucial micronutrient for all living organisms, including intracellular pathogens that must acquire iron from the host for various cellular processes, such as de novo heme biosynthesis. When infecting host cells, *Toxoplasma* parasites replicate within parasitophorous vacuoles that are enclosed by a membrane derived from the host's plasma membrane. Although this membrane structure can protect the parasites from the host's immune response, it also poses a challenge for the parasites in acquiring essential nutrients. To overcome this obstacle, the parasites can exploit the host's iron transport system, which primarily involves transmembrane proteins, such as the transferrin receptor (TFRC). Previous studies have shown that host cells increase the expression of TFRC upon *Toxoplasma* infection. Using the pronase protection and immunofluorescence assays, our study found that the parasites ingest the host's TFRC and transport it to their endolysosomal system. Interestingly, the internalized TFRC was detected in the endosome-like compartment (ELC) but not the plant-like vacuolar compartment (PLVAC). Currently, we are attempting to genetically ablate or knock down the TFRC gene in the host cells to elucidate its roles in *Toxoplasma* infection.