Center for Tropical & Emerging Global Diseases
UNIVERSITY OF GEORGIA

35th Molecular Parasitology & Vector Biology Symposium

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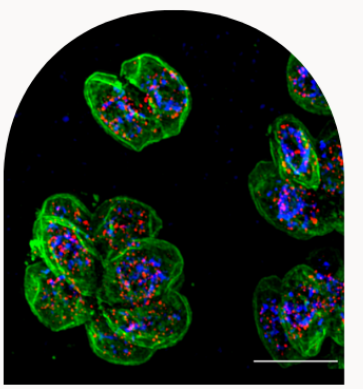


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Program

8:30 AM Registration and Poster Set-up

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

SESSION 1 — Kaelynn Parker & Mackenzie Sievert

9:10 AM **Kenna Berg**, Dept of Infectious Diseases, UGA
A suite of eight *Toxoplasma gondii* effectors cooperates to activate the non-canonical NF-κB pathway

9:25 AM **Watcharatip (Am) Dedkhad**, CTEGD & Dept. of Cellular Biology, UGA
Spatiotemporal dynamics of signal dependent exocytosis and parasitophorous vacuolar membrane rupture during *Plasmodium falciparum* egress

9:40 AM **Lindsay Berardi**, Dept. of Microbiology, UGA
Wolbachia peptidoglycan-associated lipoproteins inhibit ESCRT activity when expressed in yeast

9:55 AM **BREAK — POSTER VIEWING (even posters)**

SESSION 2 — Gonzalo Mondejar & Lena Argomaniz

10:50 AM **Rafeed Rahman Turjya**, Institute of Bioinformatics, UGA
The emergence of redundant yet non-identical multi-circular mitochondrial genomes in tissue coccidia

11:05 AM **INTRODUCTION OF THE EARLY CAREER SCHOLAR —Belen Cassera**

11:10 AM **Robyn Kent, Ph.D.**, Dept. of Microbiology and Immunology, College of Medicine, The University of Oklahoma Health Sciences Center
Uncovering replication during chronic *Toxoplasma infection*

12:10 PM **LUNCH — POSTER VIEWING**

SESSION 3 —Aylla von Ermland & Clyde Schmidt

1:30 PM **Taylor Pearson**, SCWDS & Dept. of Population Health, UGA
Diversity of ticks and SFG *Rickettsia* spp. in ticks collected from American black bears (*Ursus americanus*) in South Georgia, USA

1:45 PM **Jose Saenz-Garcia**, Center for Tropical and Emerging Global Diseases, UGA
The molecular basis of *Trypanosoma cruzi* persistence in muscle

2:00 PM **Javier Rosero**, Dept. of Microbiology and Cell Science, University of Florida
A non-canonical activation of the host's ESCRT machinery is required for the scission of parasitophorous vacuoles and the replication of *Leishmania donovani*

2:15 PM **Melissa Rogers**, Dept. of Cellular Biology, UGA
Investigating the role of a putative membrane contact site protein in *Toxoplasma gondii*

2:30 PM **BREAK — POSTER VIEWING (odd posters)**

SESSION 4 —Justine Shiau & Joe Dainis

3:30 PM **Benjamin Phipps**, Dept. of Genetics, UGA
Environmental microbial isolates support growth of *Anopheles stephensi* larvae but are decolonized during metamorphosis

3:45 PM **Colm Roster**, Dept. of Genetics and Biochemistry, Clemson University
A novel method for isolation of intact extrachromosomal rDNA reveals dynamic copy number regulation in *Naegleria fowleri*

4:00 PM **INTRODUCTION OF THE KENOTE SPEAKER —Rick Tarleton**

4:05 PM **David Horn, FRSE, FMedSci,** Parasite Molecular Biology, Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee
Sensory and druggable RNA-binding proteins in trypanosomes

5:00 PM Concluding Remarks: **Dennis Kyle**

Poster Presentations

P1 **Wayne Cheng,** CVI, CTEGD, & Dept, of Infectious Diseases, University of Georgia
Plasmodium vivax hypnozoite formation is intrinsic to the parasite

P2 **Ana C. Mengarda**, CTEGD & Biochemistry and Molecular Biology, UGA and Parasitology, USP
Unlocking the therapeutic potential of the *Trypanosoma cruzi* proline pathway

P3 **Kaci McCoy**, Pediatrics, Emory University
Refining vector-human exposure estimates by integrating routinely collected data: A case example in central Malawi

P4 **Kiara Darden**, Dept. of Ecology, Evolution, and Organismal Biology, Kennesaw State University
Intraspecific trait variation of trematodes infecting tree shrew (*Tupaia* spp.) from Borneo

P5 **Mya Covington**, Center for Tropical and Emerging Global Diseases, UGA
How does *Plasmodium falciparum* use N-linked glycosylation for blood-stage development?

P6 **Ibukunoluwa Tella**, Institute of Bioinformatics, UGA
A comparative transcriptomics approach to elucidate differences in Chagas disease vector capacity among *Rhodnius* species

P7 **Kathleen Weimer**, Pediatrics, Emory University
From transmission to taxonomy: Delineating *Triatoma* sp. nov., a novel species of Chagas Disease vector from northern Belize

P8 **Courtney Grant, Jr.**, Dept. of Bioinformatics, UGA
Lineage-specific gene family expansions drives sensory evolution in *Ixodiphagus hookeri*

P9 **John Amaka**, Dept. of Infectious Diseases, UGA
Defining the role of sugar feeding and metabolism in shaping *Anopheles* physiology and malaria transmission

P10 **Amy Bergmann**, Eukaryotic Pathogen Innovation Center, Clemson University
Exploring the cathelicidin antimicrobial peptide, LL-37, and its synthetic derivative, SAAP 148, against *Acanthamoeba* keratitis

P11 **Nupur Kittur**, Center for Tropical and Emerging Global Diseases, UGA
VEuPathDB: Tools for genomic-scale data exploration, analysis, integration and discovery

P12 **Abdul Malik Hussein**, CTEGD & Dept. of Cellular Biology, UGA
*Tg*GRP75 modulates calcium thresholds for microneme secretion and parasite egress in *Toxoplasma gondii*

P13 **Kalisa Bogati**, Dept. of Biochemistry and Molecular Biology, UGA
A yeast-based system to study horizontal transposon transfer in *Saccharomyces*i

P14 **Rachel Williams**, Dept. of Genetics and Biochemistry, Clemson University
Optimizing excystation in *Entamoeba histolytica*

P15 **Juan Camilo Arenas Garcia**, Dept. of Cellular Biology, UGA
The role of a vacuolar H+-ATPase assembly factor in *Toxoplasma gondii*

P16 **Sakshi Pandita**, Dept. of Infectious Diseases, UGA
Does RON11 control rhoptry number: A cross-species question in *Toxoplasma gondii*

P17 **Disha Bangalore Renuka Prasad**, Dept. of Genetics, UGA
Early establishment of virtual memory T cells in the neonatal lung

P18 **Amadis Vivas**, Dept. of Infectious Diseases, UGA
Vaccination with a *Plasmodium falciparum* MIF protein nanoparticle reduces inflammation and promotes recovery from anemia during malaria

P19 **Anotnia Blank**, Dept. of Genetics and Biochemistry, Clemson University
Characterizing EXP2's assembly into the parasitophorous vacuole in *Plasmodium falciparum*

P20 **Emma Varn**, Dept. of Genetics and Biochemistry, Clemson University
Assessing the role of the PEXEL motifinnon-exported proteins in *P. falciparum*

P21 **Jake Eaton**, Dept. of Cellular Biology, UGA
Harnessing intracellular ROS production to improve the efficacy of liver-stage malaria vaccine*s*

P22 **Gonzalo Seminario-Mondejar**, Dept, of Cellular Biology, UGA
Dissecting the molecular machinery of the *Trypanosoma cruzi* cytostome-cytopharynx complex

P23 **Abagail Goff**, Eukaryotic Pathogens Innovation Center, Clemson University
Development and Implementation of an in vitro adhesion assay for *Acanthamoeba castellanii*

P24 **Trent Beeland**, Center for Vaccines and Immunology, UGA
Dose-optimization of an anti-CD20 antibody to reduce B cells in owl monkeys

P25 **Annabella Castiglione**, Dept. of Infectious Diseases, UGA
Yeasty Boys: An in vitro system to assess the role of a *Plasmodium* protein in membrane fusion

P26 **Ibrahim Idris**, School of Veterinary Medicine, Texas Tech University
Epidemiology and temporal trends of gastrointestinal parasites and vector-borne pathogens in golden retrievers: An eight-year cohort study

P27 **Justine Shiau**, Dept. of Infectious Diseases, UGA
Mycobacterial ATP synthase inhibitor, Bedaquiline, limits *P. falciparum* parasite replication in blood and mosquito stages

P28 **Laasya Koganti**, CTEGD & Dept. of Cellular Biology, UGA
Characterization of two hypothetical subunits of the vacuolar transporter chaperone complex in *Toxoplasma gondii*

P29 **Samuel Nyarko**, Dept. of Cellular Biology, UGA
Diverging from the norm: Subunit k and its unique architecture in *Toxoplasma* ATP synthase

P30 **Aidan May**, Center for Tropical and Emerging Global Diseases, UGA
Characterization of the *Toxoplasma gondi*i steroidogenic acute regulatory protein-related lipid transfer domain-containing protein TgSTART2

P31 **Siddharth Yamujala**, Dept. of Cellular Biology, UGA
Functional characterization of novel SAM complex subunits Sam35 and Sam37 in *Toxoplasma gondii*

P32 **Solomon Ngwira**, Dept. of Biochemistry and Molecular Biology, Clemson University
Establishing a protein export reporter system in *P. falciparum*

P33 **Alka Nokhwal**, Department of Cellular Biology, UGA
Impact of exposure to malaria on susceptibility to respiratory viral infections

P34 **Elise Nanista**, Dept. of Genetics, UGA
Investigating nrps as a putative germ cell niche regulator in tapeworms

P35 **Mastura K Ruma**, Dept. of Infectious Diseases, UGA
PfDHODH is essential for mosquito-stage development of *Plasmodium falciparum:* A novel transmission blocking target

P36 **Kensley Parker & Somto Okoye**, Dept. of Biochemistry and Genetics, Clemson University
Characterizing divergent ER homeostasis mechanisms in Amicomplexa

P37 **Caroline Palmentiero Stephan**, Dept. of Genetics and Biochemistry, Clemson University
Establishment of a transfection system in *Naegleria fowler*i using expression vectors

P38 **Chandler Lowe**, Dept. of Genetics, UGA
Investigating the role of notch signaling on *Hymenolepis diminuta* segmentation

P39 **Kaelynn Parker**, Dept. of Cellular Biology, UGA
Communication is key: Investigating interorganellar communication in *Toxoplasma gondii*

P40 **Ross Low**, CVI & CTEGD, UGA
Genetic basis for hypnozoite formation in *Plasmodium vivax*

P41 **Suryanarayana Ganjikunta**, Dept. of Cellular Biology, UGA
Evaluating differential responses to *Naegleria fowleri* challenge

P42 **Saniya Sabnis**, Dept. of Infectious Diseases, UGA
Humoral immunity leads to control of chronic *Plasmodium* infections

P43 **Fernando Sanchez**, Center for Tropical and Emerging Global Diseases, UGA
Immune mechanisms regulating *Trypanosoma cruzi* control and persistence in muscle

P44 **Elisabet Gas-Pascual**, Dept. of Biochemistry and Molecular Biology, UGA
Protein glycosylation in pathogenic protist parasites - a few pathologically relevant examples

P45 **Mac Sievert**, Center for Tropical and Emerging Global Diseases, UGA
Collaborative cross mice yield genetic modifiers for sensitivity to infection with the brain eating amoeba *Naegleria fowleri*

P46 **Eden O'Connell**, Dept. of Infectious Diseases, UGA
The role of putative ATP synthase subunits during sexual conversion of malaria parasites

P47 **Juan David Valencia-Hernandez**, Dept. of Cellular Biology, UGA
Characterization of the protein phosphatase calcineurin (CN) network in *Toxoplasma gondii*

P48 **Magdalena Alba Argomaniz**, Dept. of Infectious Diseases, UGA
Developing transgenic systems for studying relapsing malaria parasite biology

P49 **Max Williams**, Dept. of Genetics and Biochemistry, Clemson University
RNA-seq analysis of gene regulation during early encystation in *Entamoeba histolytica*

P50 **Celia Saney**, Center for Vaccines and Immunology, UGA
Automated detection and quantification of *Plasmodium cynomolgi* with the Sysmex XN-30 hematology analyzer

P51 **Leticia Do Amaral**, Dept. of Infectious Diseases, UGA
Chemoproteomics-driven target discovery for the novel antimalarial PRC1910

P52 **Anissa Waller Del Valle**, Dept. of Cellular Biology, UGA
Optimizing treatment for primary amoebic meningoencephalitis caused by *Naegleria fowleri*

P53 **Sharaf Aroni**, Dept. of Genetics and Biochemistry, Clemson University
Investigating the role of CDC42 in early encystation and stress response in *Entamoeba histolytic*a

P54 **Melissa Sleda**, Center for Tropical and Emerging Global Diseases, UGA
Targeting tissue cysts in chronic *Toxoplasma gondii*: Mitochondrial inhibition with 4(1H)-Quinolones

P55 **John Joseph**, Dept. of Infectious Diseases, UGA
Investigating the role of Adenyl Cyclase beta (ACβ) and Armadillo Interacting Protein (AIP) in biogenesis of *Plasmodium* rhoptries

P56 **Clyde Schmidt-Silva**, Dept. of Cellular Biology, UGA
Determining the mechanism of antigen-presenting cell recruitment during liver-stage malaria

P57 **Godspower Okeke**, Dept. of Cellular and Molecular Biology, University of Southern Mississippi
Harnessing anti-α-Gal immunity: A cross-species virus-like particle vaccine platform against cutaneous leishmaniasis

Oral Presentations

A suite of eight *Toxoplasma gondii* effectors cooperates to activate the non-canonical NF-κB pathway

Kenna Berg [1,3,*], Michael Panas [4,*], Samarchith P Kurup [2,3], John C Boothroyd [4], Alex Rosenberg [1,3]
[1]Department of Infectious Diseases, University of Georgia, Athens, GA, USA, [2]Department of Cellular Biology, University of Georgia, Athens, GA, USA, [3]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, USA, [4]Department of Microbiology and Immunology, Stanford University School of Medicine

As a master of host-cell reprogramming, *Toxoplasma gondii* (*T. gondii*) tachyzoites manipulate diverse signaling networks to establish a niche permissive for long-term infection. While the parasite's subversion of canonical NF-κB signaling (p65/p50) is well established, how infection impacts the non canonical NF-κB pathway has been largely unexplored. Here, we report that *T. gondii* infection induces robust nuclear accumulation of the non-canonical NF-κB subunits RelB and p52 in both human and murine cells. This activation follows a gradual kinetic profile and is conserved across both Type I and Type II parasite genetic backgrounds. We demonstrate that this reprogramming is strictly dependent on the MYR1-dependent export of dense granule effectors. Mechanistically, *T. gondii* infection drives the depletion of the negative regulator TRAF3, leading to the stabilization of NF-κB-inducing kinase (NIK), phosphorylation of p100, and its subsequent processing into p52. Utilizing a panel of combinatorial knockout parasites, we reveal that no single effector is responsible for this phenotype. Instead, a suite of eight MYR1-dependent effectors, IST, NSM, HCE1/TEEGR, GRA16, GRA18, GRA24, GRA28, and GRA84, functions through a collaborative, additive network to trigger the non-canonical response. These findings highlight a distributed regulatory strategy used by the parasite to overcome host transcriptional robustness and shape host signaling.

Spatiotemporal dynamics of signal dependent exocytosis and parasitophorous vacuolar membrane rupture during *Plasmodium falciparum* egress

Watcharatip Dedkhad[1,2], Tia Tran[1,2,§], Manuel A. Fierro[1,2,#], Carrie Brooks[1], and Vasant Muralidharan[1,2,*]
[1]Center for Tropical and Emerging Global Diseases [2]Department of Cellular Biology University of Georgia, Athens, GA 30602. [§]Current address: Saint Louis University School of Medicine, Saint Louis, MO. [#]Current address: Department of Genetics and Biochemistry, Eukaryotic Pathogen Innovation Center, Clemson University, Clemson, SC

Malaria remains a major global health concern. It is caused by intracellular *Plasmodium falciparum* parasites, residing and replicating within a vacuole in host red blood cells. Egress of daughter parasites out of the membranes is tightly regulated via a complex mechanism. Prior studies have suggested that a cyclic-GMP driven calcium signaling pathway leads to the signal-dependent exocytosis of egress-specific vesicles that discharge several proteases into the parasitophorous vacuole. However, signal-dependent exocytosis during egress has not yet been observed in live parasitized RBCs. We targeted the exocytosis reporter, superecliptic pHlourin or SEP, to these egress-specific vesicles and utilized live imaging to observe exocytosis. The spatiotemporal relationship between exocytosis and the breakdown of the parasitophorous vacuolar membrane (PVM) as well as parasite egress was also determined using a fluorescent reporter fused to EXP2. Our data showed that exocytosis is triggered as early as 3 hours prior to merozoite egress. These data suggest that the PVM rupture occurs at a single site and rapidly expands from that initial site of rupture, releasing the merozoites into the RBC. Using conditional mutants of *Plasmodium* endoplasmic reticulum calcium-binding protein (PfERC), we demonstrate that loss of PfERC inhibits signal-dependent exocytosis of egress-specific vesicles. Together, these data demonstrate that signal-dependent exocytosis of egress-specific vesicles starts well before merozoites are formed via cytokinesis, PVM ruptures at a single site, and that PfERC is required for exocytosis of egress-specific vesicles.

Wolbachia peptidoglycan-associated lipoproteins inhibit ESCRT activity when expressed in yeast

Lindsay Berardi[1] Emma Bethel[1] and Dr. Vincent J. Starai[1]
[1]Department of Microbiology, University of Georgia, Athens, GA

Brugia malayi is a mosquito-borne filarial nematode that causes debilitating and disfiguring lymphatic disease. Affecting millions of individuals worldwide, current anthelmintic treatments are ineffective at completely eradicating all life stages of the worms, resulting in prolonged treatment times and potential disease recrudescence. *Wolbachia pipientis*, the essential endosymbiont of several filarial nematodes—including *Brugia malayi* and *Dirofilaria immitis*—has therefore emerged as a promising target for drug discovery, as its presence is required for the growth and reproduction of these parasites. My research seeks to understand the molecular basis of the *Wolbachia*–nematode relationship through the expression of select *Wolbachia* proteins in the eukaryotic model organism *Saccharomyces cerevisiae*. One such protein, Wbm0152 from the *Wolbachia* endosymbiont of *Brugia malayi,* is a predicted peptidoglycan-associated lipoprotein (PAL) and has been shown to disrupt endosomal cargo trafficking pathways through inhibition of the ESCRT complexes when expressed in yeast. The Endosomal Sorting Complexes Required for Transport (ESCRT) are highly conserved multiprotein complexes that play essential roles in endosomal intraluminal vesicle formation, cytokinesis, and viral budding in mammalian cells. Recently, we have also found that a homolog of *w*Bm0152 from the *Wolbachia* endosymbiont of *Dirofilaria immitis*, *w*DimmPAL, exhibits similar ESCRT-inhibitory activity when expressed in yeast. Using BLAST alignments and AlphaFold2 modeling, we have identified key, conserved residues that may facilitate these PAL–ESCRT interactions. Together, these findings suggest that *Wolbachia* PAL proteins play critical roles in maintaining *Wolbachia:*nematode endosymbiosis by altering host endolysosomal membrane dynamics, thereby addressing an important gap in our understanding of the molecular mechanisms that enable *Wolbachia* persistence within nematode hosts.

The emergence of redundant yet non-identical multi-circular mitochondrial genomes in tissue coccidia

Rafeed R. Turjya[1], George L. Kitundu[1,2], Sriveny Dangoudoubiyam[3], Daniel K. Howe[4], and Jessica C. Kissinger[1,5,6]
[1]Institute of Bioinformatics, UGA, [2]Dept. of Biochemistry and Biotechnology, Pwani University, Kenya, [3]Dept. of Comparative Pathobiology, Purdue University, West Lafayette, IN, [4]Dept. of Veterinary Science, University of Kentucky, Lexington, KY, [5]CTEGD & [6]Dept. of Genetics, UGA, Athens, GA

Among apicomplexans, *Toxoplasma gondii* and other closely related tissue coccidia exhibit a fragmented mitochondrial genome (mtDNA) consisting of sequence blocks (SBs), recognizable stretches of sequence which are redundantly present in specific non-random orders and orientations (PMID:33906963, 38363119). Evolutionarily, the genus *Sarcocystis* represents tissue coccidia branching in between *T. gondii* with fragmented mtDNA and Eimeriidae, which exhibit unfragmented mtDNA. We investigated *S. neurona,* the causative agent of equine protozoal myeloencephalitis, to resolve its mtDNA structure. Mitochondrial reads were identified from Oxford Nanopore data generated from *S. neurona* merozoites. We discovered 18 unique SBs with a total length of 5959 bp occurring in 24 non-random SB orders. In contrast, *T. gondii* has 23 SBs with a total length of 5859 bp and 31 SB orders. Interpreting the mtDNA organization as a genome graph revealed connected graphs of multiple circles. Mapping longer mtDNA reads to this graph supports the hypothesis of homologous recombination between redundant regions on circular DNA molecules, generating significant sequence and read length diversity. Comparison of *S. neurona* and *T. gondii* mtDNAs reveals two evolutionarily conserved SB boundaries – designating this fragmentation as a defining trait of the tissue coccidia. Divergent fragmentation paths in the tissue coccidia raise interesting questions regarding the generation and conservation of small RNAs that are known to be incorporated into apicomplexan mitoribosomes (PMID: 39690155, 39827269), as well as unexplored molecular processes for mtDNA replication and maintenance.

Diversity of ticks and SFG *Rickettsia* spp. in ticks collected from American black bears (*Ursus americanus*) in South Georgia, USA

Taylor Pearson[1], Haley-Marie Jones [2,3], Alex Coombs [2,3], Bri Neily [2,3], Bailey Ward [1,] Jacob Lyons [1], Christopher Cleveland [1], and Michael J. Yabsley [1,2]

[1]Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia, Athens, GA, [2]Warnell College of Forestry and Natural Resources, University of Georgia, Athens, GA [3]Georgia Department of Natural Resources (GDNR), Social Circle, GA

Rickettsial tick-borne pathogens are the causative agents of severe and potentially fatal spotted fever group (SFG) diseases in domestic animals and humans. Despite the public-health importance of SFG *Rickettsia* spp., there are few data on the current prevalence and diversity of rickettsial pathogens in ticks found across the lower coastal plains of Georgia, USA. The aim of this study was to characterize SFG *Rickettsia* spp. prevalence and diversity in ixodid ticks collected from hunter-harvested and live-captured black bears from 6 counties expanding the Okefenokee Swamp, Georgia, USA. Ticks were removed from black bears from February 2023 - August 2025. Ticks were identified using dichotomous keys and were individually screened for SFG *Rickettsia* spp. using a nested PCR targeting the *17-kDa* gene followed by a PCR targeting the *ompA* gene. Rickettsial species identification was determined using bidirectional Sanger sequencing of the *17kDa* and/or *ompA* gene targets. To date, 238 ticks have been collected and identified from 86 black bears. Tick species included *Dermacentor variabilis* (186; 78%), *Amblyomma maculatum* (41; 17%), and *Amblyomma americanum* (11; 5%). Out of 150 screened ticks, 51 ticks had SFG *Rickettsia* spp. detections (34%), including *R. rhipicephahli, R. parkeri*, *R. amblyommatis*, and *R. bellii.* These data indicate that black bears are infested with a high diversity of ixodid ticks suitable for maintenance of SFG *Rickettsia* spp. of public health importance.

The molecular basis of *Trypanosoma cruzi* persistence in muscle

Jose Saenz-Garcia, Fernando Sanchez-Valdez, Jihyun Lim, Caleb Hawkings, Brooke White, and Rick Tarleton

CTEGD, University of Georgia, Athens, GA

Chagas disease is a result of infection by *Trypanosoma cruzi,* that is controlled but not eradicated, with parasite persistence and damage primarily in muscle tissues. Our previous studies have indicated that in chronically infected hosts, amastigotes inside muscle cells appear to replicate robustly even when surrounded by intense inflammation. IFN-γ is absolutely required for immune control but how it exerts this control, particularly in chronic *T. cruzi* infection, has not been previously determined. To address this question, we generated mice with a muscle-specific inducible knockout of the IFN-γ receptor (IFNγR). Knockout of IFNγR in skeletal muscle before infection, or during the post-acute or chronic stages of infection resulted in uncontrolled parasite burden, indicating that muscle-intrinsic IFN-γ signaling contributes to infection control. In vitro studies determined that while IFN-γ–stimulated fibroblasts, endothelial cells, and macrophages restricted intracellular parasite development, primary myoblast-derived myotubes failed to do so. To further investigate the differential response of these different cell types to IFN-γ, we employed RNA sequencing and found that myotubes mount a core IFN-γ transcriptional program similar to that of fibroblasts, including the induction of genes involved in cell-intrinsic innate immunity (including guanylate-binding proteins), antigen presentation, and chemokine and cytokine production. However, myotubes failed to induce key inflammatory and metabolic programs, including genes such as *Casp1* and immune–metabolic regulators, revealing a cell-type–specific metabolic component of the response. Importantly, this same pattern of transcriptional response to IFN-γ was evident in human primary cardiomyocytes. Consistent with these findings, myotubes failed to produce nitric oxide or reactive oxygen species following IFN-γ stimulation or infection. Collectively, this study shows that myocytes are differentially responsive to IFN-γ and as a result, allow for the persistence of *T. cruzi*, which accompanying tissue damage, nearly exclusively in muscle. However, our additional studies (see poster by F. Sanchez) that myocytes are not helpless in the parasite control process as their ability to recruit immune effectors and to present antigens are essential and to controlling *T. cruzi* in chronically infected hosts.

A non-canonical activation of the host's ESCRT machinery is required for the scission of parasitophorous vacuoles and the replication of *Leishmania donovani*

Javier Rosero[1] and Peter E. Kima[1]
University of Florida, Department of Microbiology and Cell Science[1]

Leishmania donovani (Ld) are the causative agents of visceral leishmaniasis. In mammalian cells, Ld live in vacuolar compartments called parasitophorous vacuoles (LdLPVs) that enigmatically divide following parasite replication to accommodate one parasite per vacuole. We evaluated the role of the host cell's endosomal sorting complex required for transport (ESCRT) machinery in the scission of LdLPVs. We found that ESCRT components are constitutively recruited to LdLPVs. The knockdown (KD) of upstream components of the ESCRT machinery in macrophages revealed that KD of ESCRT accessory ALIX but not ESCRT-I TSG101 or VPS28 led to a significant reduction in the parasite burden in infected cultures. Interestingly, LdLPVs in ALIX-KDs were more distended and harbored more than 2 parasites. Incorporation of BrdU into Leishmania in THP-1 macrophages revealed that parasite replication was inhibited in ALIX-KD due to defective LdLPV scission. These findings establish that non-canonical activation of the ESCRT machinery is required for *Leishmania* to replicate within host macrophages.

Investigating the role of a putative membrane contact site protein in *Toxoplasma gondii*

Melissa Rogers[1]; Cyrille Botté[2]; Diego Huet[1]
[1]University Of Georgia, Athens - USA; [2]Universite´ Grenoble Alpes, Grenoble - France

Membrane contact sites (MCSs) are regions of the cell in which two organelles are close enough together to be connected by tethering proteins without their membranes fusing. MCSs in yeast and mammals facilitate inter-organellar lipid and calcium transport, alongside other crucial functions for maintaining homeostasis. START domain-containing proteins have been implicated in MCS-mediated lipid transport in yeast and mammals. In the apicomplexan parasite *Toxoplasma gondii*, little is known about the proteins involved in MCS formation or what roles MCSs play in the parasite. In this study, we investigate the role of a START domain-containing protein called TgSTART in MCS function in *T. gondii*. A blast search to the human proteome reveals sequence homology between the START domains of TgSTART and the phosphatidylcholine transfer protein STARD7. Using CRISPR/Cas-9 to generate tagged parasite strains, we determined the localization of the protein in the apicoplast and used a conditional knockdown system to show the protein is essential for the lytic cycle of the parasite. TgSTART-deficient parasites exhibited aberrant apicoplast morphology via immunofluorescence microscopy and disrupted apicoplast gene expression via qPCR. Staining lipid stores in TgSTART-deficient parasites revealed increased lipid droplet accumulation during TgSTART knockdown in both the parasites and the host cells. Altogether, our results suggest that TgSTART is essential for apicoplast maintenance in the parasite via the transfer of phosphatidylcholine from lipid droplets to the apicoplast. Further studies will assess the role of TgSTART in parasite lipid composition and lipid transfer between organelles, as well as the role of the protein in apicoplast biogenesis and MCS architecture in the parasite.

Environmental microbial isolates support growth of *Anopheles stephensi* larvae but are decolonized during metamorphosis

Benjamin L. Phipps[1] and Michael R. Strand[2]
[1]Department of Genetics and [2]Department of Entomology, University of Georgia, Athens, GA

Like many animals, mosquitoes are colonized by environmental microbes that shape their fitness. Changes in mosquito behavior, diet, and physiology during the transition from larva to adult profoundly alter the gut microbiota. However, the different microbial taxa found in larvae and adults can fill similar functional roles, provisioning essential nutrients deficient in the mosquito's diet. The gut microbiota of *Anopheles* mosquitoes has been implicated in malaria transmission, but the specific taxa and functions that affect vector competence are poorly understood due to lack of techniques for culturing anophelines to adulthood without a microbiota. To overcome this challenge, we monocolonized aseptically hatched *A. stephensi* larvae with each of seven microbial isolates from our laboratory mosquito colony and assessed their ability to support maturation to adulthood. We then tested whether isolates that could promote larval growth persisted in adults. We observed that most isolates were expelled in the feces during metamorphosis and could not be detected in most adults. These results demonstrate that diverse microbes can provide the necessary factors for larval development on standard diet, but many cannot persist throughout metamorphosis. This approach could also be leveraged for future studies dissecting the role of the microbiota in vector competence because it can be used to obtain germ-free adults.

A novel method for isolation of intact extrachromosomal rDNA reveals dynamic copy number regulation in *Naegleria fowleri*

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Naegleria fowleri, a pathogenic free-living amoeba, is the etiological agent of primary amebic meningoencephalitis (PAM), a rapidly progressing central nervous system infection that is nearly universally fatal. A unique feature of this amoeba is the presence of a nucleolar-localized closed circular extrachromosomal ribosomal DNA element (CERE) that encodes the entirety of the cell's ribosomal RNA template. Despite potential biological significance, CERE has remained poorly characterized largely due to technical challenges in maintaining CERE integrity during extraction. Here, we describe a novel, expedited, and scalable methodology for extracting intact CERE from cell lysates, enabling biological analysis. Using this approach, we've shown that CERE copy number is dynamic, being responsive to environmental conditions. Additionally, higher copy numbers may be required for virulence. Drug pressure - including amphotericin B, a cornerstone of PAM treatment - seems to be a strong driver of increased CERE copy number, suggesting CERE may play an adaptive role in response to stress. To further investigate CERE as a therapeutic target, we screened a collection of inhibitors predicted to have CERE-related activity and identified 12 lead compounds with potent amoebicidal activity, favorable blood-brain barrier permeability, and promising pharmacokinetics. We plan to investigate the biological effects of these compounds on CERE to enhance our limited understanding of this nucleic acid's biology and assess their potential as lead therapeutic candidates.

Poster Presentations

P1. *Plasmodium vivax* hypnozoite formation is intrinsic to the parasite

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Plasmodium vivax (*Pv*) causes a significant portion of malaria globally and remains a public health problem because it forms dormant liver-stages termed hypnozoites (HZs). Hypnozoites exit dormancy after an initial infection and cause recurrent infections, known as relapses, that sustain transmission and disease. Universal treatments that kill HZs are not available and developing new treatments is difficult due to poor understanding of HZ biology. This study aims to improve our understanding of HZ biology by determining if HZ formation is intrinsic to the parasite or influenced by other factors. To address this question, the *Pv* Chesson and North Korean isolates were propagated in monkeys and fed to mosquitoes to obtain sporozoites. The sporozoites of these isolates are expected to form a mixture of HZs and schizonts (SZs) and nearly all HZs, respectively, and thereby, provide a unique opportunity to study HZ formation. Despite adaptation to grow in monkeys, sporozoites form these strains remain infectious to human hepatocytes and retain similar morphology and drug susceptibility profiles as field isolates. As expected, the Chesson isolate formed a mixture of HZs to SZs whereas as the North Korean isolate formed nearly all HZs. Importantly, these proportions are consistent across multiple NHP infections and hepatocyte donors. Together, these data suggest that HZ formation is intrinsic to the parasite and minimally influenced by other factors. Comparative analysis of these isolates will be useful for elucidating the mechanisms controlling HZ formation.

P2. Unlocking the therapeutic potential of the *Trypanosoma cruzi* proline pathway

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Chagas disease affects 6-7 million people and only two drugs (nifurtimox and benznidazole) are available for the treatment of the disease. However, both drugs present limitations regarding efficacy and tolerance. In this context, there is an urgency to find new molecules with anti-*T. cruzi* potential. It is known that proline (L-Pro) is important for several biological processes in *T. cruzi*. Given the crucial function of the L-Pro pathway in *T. cruzi*, it is possible that identifying small molecules that inhibit enzymes involved in the pathway of L-Pro could result in new treatments. The main goal of this work is to screen a proprietary library of novel β-carbolines as a starting point for the development of novel therapies for Chagas' disease and produce knockdown strains for the enzymes as a drug target validation strategy. We screened the library composed of chemical scaffolds and PRC1901 demonstrated a promising trypanocidal effect showing that the trypomastigote invasion was inhibited with an EC_{50} of 0.08 µM, while the release of trypomastigotes was inhibited at an EC_{50} of 0.13 µM. This resulted in SI >40 and >20, respectively. PRC1901 inhibited the replication of intracellular amastigotes as well reduced the total number of infected cells by 96.8 and 88.3% respectively. At present, we are broadening the screening process to encompass additional scaffolds and knockdown strains are producing.

P3. Refining vector-human exposure estimates by integrating routinely collected data: A case example in central Malawi

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Quantifying human exposure to malaria vectors requires an understanding of both mosquito and human nighttime activity patterns. While entomological surveillance routinely captures vector biting rates, comparable data on human location and behavior have historically been less available. Here, we present a case example integrating large-scale human behavioral survey data with entomological surveillance data to generate behavior-adjusted estimates of vector exposure in central Malawi. Entomological data consisted of hourly biting rates collected via human landing catches (HLCs) in June 2021. The Malawi Malaria Behavior Survey, conducted between May and July 2021, provided hourly nighttime estimates of human location, sleeping status, and insecticide-treated net (ITN) use. We integrated these data using established frameworks to produce hourly, weighted estimates of exposure to mosquito bites that accounted for human location and ITN use. Our analyses indicated that most exposure to vector bites occurred indoors (96%), during sleeping hours (87%). Although ITNs would be expected to offer protection during these times, only 35% of all exposure to bites was estimated to be prevented by ITNs; this gap in protection was likely driven by limited household ITN access rather than a behavioral gap. Other key contextual factors for interpretation included ITN durability, insecticide resistance, and activity outside of the peri-domestic space. This case example suggests that integrating routinely collected entomological and human behavioral data can refine estimates of malaria vector exposure. Such approaches can strengthen interpretation of vector biting patterns in relation to human behavior, improving exposure metrics.

P4. Intraspecific trait variation of trematodes infecting tree shrew (*Tupaia* spp.) from Borneo

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Parasitism is one of the most successful life history strategies on Earth. The success of an individual parasite depends on its strategies for dispersal, establishment, and persistence. We can use functional ecology to understand how individuals are interacting with other organisms and their environment and how these interactions affect their success. The first step is to quantify the inter- and intraspecific trait variations to determine what traits are present. Using digenean trematodes of tree shrews (*Tupaia* spp.) from Borneo, we sought to determine 1) how much trait variation existed within and between trematode species within a host population and 2) what traits were the most successful, using abundance and prevalence within the parasite community as proxies for success. We will measure six functional traits: body size, ventral sucker size, oral sucker size, egg size, vitelline fields, and testis size. The data will be analyzed in R, and preliminary results will be discussed. With this work, we aim to advance our understanding of host–parasite interactions and the functional ecology of parasites.

P5. How does *Plasmodium falciparum* use N-linked glycosylation for blood-stage development?

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The malarial parasite *Plasmodium falciparum* requires the trafficking of proteins throughout its vacuole and the red blood cell for successful blood-stage development. Post-translational glycan modifications are one way to ensure proteins reach their proper subcellular location after synthesis in the endoplasmic reticulum. Glycans attached to asparagine residues, N-linked glycans, serve as a mechanism for quality control and a signal for protein trafficking within the secretory pathway. Although *P. falciparum* has lost most proteins in this conserved pathway, it retains three Asparagine-linked glycosylation (ALG) proteins and the oligosaccharyltransferase (OST) complex. The loss of the conserved N-glycosylation machinery in *P. falciparum* has fueled a debate on whether the parasite uses this glycan modification for blood-stage development as well as its importance during intraerythrocytic growth. To investigate the essentiality of N-glycosylation machinery during the intraerythrocytic developmental cycle (IDC), we generated conditional knockdowns of the catalytic unit of the OST complex, STT3, and upstream glycotransferase ALG14. In the absence of these proteins, parasites experience a delayed death following arrested growth as trophozoites in the 3rd IDC. Preliminary data from blood smears and parasites with a fluorescently labeled hemoglobinase show ALG14 and STT3 knockdowns impact the digestive vacuole as early as the 2nd IDC. Using crosslinking proteomics, we aim to identify candidate N-glycosylated proteins in *P. falciparum*. Armed with these tools, we will elucidate the biological role of N-glycosylation in the secretory pathway of *P. falciparum*.

P6. A comparative transcriptomics approach to elucidate differences in Chagas disease vector capacity among *Rhodnius* species

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Chagas disease is caused by the parasite *Trypanosoma cruzi*, which is primarily transmitted by blood-feeding insect vectors known as kissing bugs. Within the kissing bug genus *Rhodnius*, two sister species (*R. prolixus* and *R. robustus)* exhibit clear differences in ecological behavior and vector capacity despite being separated by less than 1.5 million year of divergence. *R. prolixus* readily colonizes human dwellings due to its adaptation to drier, warm microclimates, making it a major vector of Chagas disease. In contrast, *R. robustus* sporadically colonizes human dwellings, and prefers cooler, moist microclimates, and is not considered a major Chagas disease vector. We hypothesize that physiological differences associated with vector capacity are encoded in the genomes of the two species that can be detected at the transcriptomic level. To test this hypothesis, we generated and analyzed a whole organism RNA-seq dataset (5 replicates per condition) comprised of mild and moderate dehydration stress treatments for both species. Genome-wide analysis of gene expression profilers under these conditions revealed hundreds of genes that differ in expression between these two species. Genes upregulated in vector *R. prolixus* relative to the non-vector *R. robustus* are enriched in adaptive immune response, response to bacteria, and histamine binding. In contrast, genes upregulated in the non-vector *R. robustus* relative to the vector *R. prolixus* are enriched in cuticle structure, chitin structure and binding, extracellular matrix, and reproductive processes. These results provide important insights into the molecular processes involved in kissing bug vector capacity and can potentially inform future mitigation strategies.

P7. From transmission to taxonomy: Delineating *Triatoma sp. nov.,* a novel species of Chagas Disease vector from northern Belize

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Chagas disease is the leading cause of non-ischemic cardiomyopathy in the Americas, where >6 million people are estimated to be infected. Caused by the parasite *Trypanosoma cruzi*, transmission most often occurs through contact with the feces of triatomine vectors deposited near the bite site. *Triatoma dimidiata* sensu lato is considered the prevailing vector taxon of human health importance in Belize, where vectorial transmission of Chagas disease was reportedly interrupted in 2015. However, following identification of an acute case of Chagas disease in March 2020, molecular analysis of *Triatoma sp.* collected around the case-patient's home detected *T. cruzi* and Discreet Typing Units corresponding to that of the infected patient, implicating vector-borne transmission. Notably, molecular identification revealed only 95% identity to the predominating *T. dimidiata* - indicating emergence of a taxon not previously described. Here, we describe this newly identified taxon, *Triatoma* sp. nov**.** Morphological description was carried out with 6 collected specimens (four males and two females) from the Corozal District of Belize and compared to *T. dimidiata* from Central America and specimens from Belize featured in the Florida State Collection of Arthropods. Genetically, *T.* **sp. nov.** is most closely related to *T. huehuetenanguensis,* Lima-Cordón & Justi 2018. The new species boasts several distinguishing characteristics including a dark ventral abdomen, larger overall size, extended head length, and greater width of the pronotum. Further defining this species, scanning electron microscopy of eggs revealed a distinct symmetric ellipsoidal shape lacking a neck or collar, with a narrow ring-shape band forming a "chorial edge" adjacent to the plane of the operculum and the shell. Given the changing vector landscape within Belize and the region, it is essential to reevaluate the risk of Chagas disease to humans: identification of this novel species is an important step in understanding the current potential for vector-borne *T. cruzi* transmission.

P8. Lineage-specific gene family expansions drives sensory evolution in *Ixodiphagus hookeri*

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Parasitoid wasps rely on specialized sensory systems to locate hosts, yet the evolutionary mechanisms behind this adaptation still remains unclear for some species such as *Ixodiphagus hookeri*. *I.hookeri* is a parasitoid of ticks, which are a major vector of pathogens such as Borrelia burgdorferi, making host detection a key trait with potential ecological relevance. We investigated the evolution of odorant-binding proteins (OBPs) and chemosensory proteins (CSPs) in I.hookeri using comparative analyses with *Nasonia vitripennis* (*N.vitripennis*) and *Copidosoma floridanum* (*C.floridanum*). Phylogenetic reconstruction identified lineage -specific expansions, and codon-based models (PAML) were used to test for positive selection in select gene family groups. Branch-model analyses were done to detect significance for lineage-specific positive selection, with dN/dS ratios. This study aims to clarify whether sensory adaptation in I.hookeri is primarily driven by gene family expansion and diversification or by positive selection acting on conserved loci.

P9. Defining the role of sugar feeding and metabolism in shaping *Anopheles* physiology and malaria transmission

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Malaria, a devastating parasitic disease disproportionately affecting sub-Saharan Africa, is transmitted exclusively by *Anopheles* mosquitoes. The rise of insecticide resistance as well as changes in vector behavior, such as daytime and outdoor biting, threaten current control strategies, creating an urgent need for innovative approaches that target novel aspects of vector biology. While mosquito blood feeding is well-studied, the equally essential behavior of sugar feeding, which fuels mosquito flight and long-term survival, is a validated but underexploited modality for mosquito control and malaria elimination, and how this specific nutritional input shapes Plasmodium-Anopheles interactions remain a critical knowledge gap in our understanding of malaria transmission. The central objective of this project is to define the role of sugar feeding and metabolism in shaping mosquito physiology and its capacity to transmit malaria. We hypothesize that sugar intake not only sustains mosquito survival but also remodels metabolic pathways that directly and indirectly influence *Plasmodium* development. Here we present initial findings showing that *Anopheles stephensi* mosquitoes reared on contrasting sugar diets (mono-, di-, and tri-saccharides), including both pentose and hexose sugars, display different long-term survival and reproductive outputs, shedding light on their energy metabolism and specific ecological niches. Future work will use isotopically labeled sugars and metabolomic profiling to track carbon flow through key metabolic pathways. By comparing fluxes between *Plasmodium*-infected and uninfected mosquitoes, we aim to determine how the parasite rewires host metabolism. This study is expected to identify metabolic pathways for new vector control targets, paving the way for next-generation interventions like attractive toxic sugar baits (ATSBs) that are optimized for metabolic disruption.

P10. Exploring the cathelicidin antimicrobial peptide, LL-37, and its synthetic derivative, SAAP 148, against *Acanthamoeba* keratitis

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Acanthamoeba castellanii (*A. castellanii*) is a free-living amoeba found abundantly in soil and water sources. It is the causative agent of *Acanthamoeba* keratitis (AK), a difficult-to-treat eye infection that can lead to irreversible scarring and vision loss. Current treatments for AK are intensive and can be cytotoxic to host tissue. Here, we examine LL-37, a naturally occurring antimicrobial peptide with microbicidal and immunomodulatory activity, and its synthetic derivative, SAAP 148. LL-37, which is present at low concentrations in human tears, has been shown to be effective against certain bacteria, yeasts, and fungi. SAAP 148 is a 28-mer synthetic derivative of LL-37 and has been shown to be more effective against pathogens. We aim to examine the effects of both LL-37 and SAAP 148 against *A. castellanii* in human corneal epithelial cells (HCE-S). Our initial data show that while both LL-37 and SAAP 148 have dose-dependent effects on *A. castellanii*, SAAP 148 appears to be more effective at lower doses while having minimal cytotoxic effects on HCE-S. These findings suggest that SAAP 148 may have therapeutic potential as a treatment for *Acanthamoeba* keratitis.

P11. VEuPathDB: Tools for genomic-scale data exploration, analysis, integration and discovery

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Biomedical research is increasingly driven by Big Data, from genome sequencing to population-level diversity and multi-omics datasets. Effectively collecting, storing, maintaining, and integrating these data is essential to ensure FAIR (Findable, Accessible, Interoperable, Reusable) access and to advance biological understanding across laboratory, field, and clinical settings. The Eukaryotic Pathogen & Vector Genomics Resource (VEuPathDB.org; including PlasmoDB, ToxoDB, CryptoDB, VectorBase, and related platforms) serves thousands of users daily and enables discovery in eukaryotic pathogen and vector research without requiring advanced computational expertise. The resource provides access to diverse datasets, including genomes, transcriptomes, proteomes, motifs, and pathways, alongside tools for comparative genomics, epigenetics, and orthology-based function prediction. It supports integration and in silico analysis of public and user-generated datasets, facilitates annotation improvement through community curation, and helps users comply with funder and publisher data policies. Recent developments include incorporation of AlphaFold structural predictions, long-read and single-cell RNA-seq datasets, improved orthology detection methods, AI-assisted transcriptomic summaries, and enhanced tools for differential expression analysis. Together, these features accelerate hypothesis generation and enable data-driven discovery across diverse eukaryotic pathogens and vectors.

P12. *Tg*GRP75 modulates calcium thresholds for microneme secretion and parasite egress in *Toxoplasma gondii*

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In *Toxoplasma gondii*, intracellular calcium signaling is essential for progression through the lytic cycle. The lytic cycle, comprising host cell invasion, intracellular replication, and egress, relies on precisely regulated calcium signals. During egress, parasites lyse the host cell to disseminate and sustain infection. In mammalian cells, ER-mitochondria contact sites generate high-Ca^{2+} microdomains that enable mitochondrial Ca^{2+} uptake, facilitated by IP_3R-GRP75 coupling to VDAC, and subsequent transport through the mitochondrial calcium uniporter. However, the presence and operation of a similar ER-mitochondria calcium coupling mechanism in *T. gondii* is unclear. Although orthologs of VDAC and GRP75 are present in the parasite, their roles in Ca^{2+} signaling have not been defined. Conditional depletion of *Tg*GRP75 (*iDgrp75*) in which expression is regulated by anhydrotetracycline (ATc), disrupts the lytic cycle of the parasite, specifically affecting replication and egress. *Tg*GRP75-depleted parasites also show impaired mitochondrial and ER calcium uptake. Live-cell imaging reveals significantly dampened spontaneous Ca^{2+} oscillations in the mutant, which correlates with the egress defect. However, the *iDgrp75 (*+ATc*)* mutant egresses in response to ionophore stimulation, indicating that *Tg*GRP75 functions upstream of cytosolic Ca^{2+} elevation, as Ca^{2+} ionophore treatment bypasses the egress defect. *Tg*GRP75-depleted parasites also show dysregulated microneme secretion including increased release of the adhesins MIC2 and PLP-1, consistent with altered calcium-dependent exocytosis. Reciprocal co-IP experiments of *T. gondii* lysates demonstrate a physical interaction between parasite *Tg*VDAC and *Tg*GRP75, suggesting the presence of a putative ER-mitochondria coupling complex. Together, these findings support a model in which *Tg*GRP75 through the VDAC-dependent ER-mitochondria calcium axis regulates cytosolic Ca^{2+} oscillations and tunes the threshold for parasite egress.

P13. A yeast-based system to study horizontal transposon transfer in *Saccharomyces*

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Horizontal transposon transfer (HTT) helps drive genome evolution in many species, but experimental systems to detect and characterize HTT events remain limited as evidence for HTT is usually detected by phylogenetic comparisons. We are developing cell-based assays to model interspecies HTT of the Saccharomyces retrovirus-like retrotransposon Ty1. Since *Saccharomyces* nuclei remain intact during mating and mitosis, cytoplasmic mixing in heterokaryons present an opportunity for Ty1 HTT by transfer of genomic RNA, mature virus-like particles or assembly intermediates, or chromosomes between nuclei. If different species can form heterokaryons, we can identify potential HTT events using an autonomous Ty1 containing the retromobility indicator gene *his3-AI* and distinguish *de novo* insertions in the recipient species from segmental or complete chromosome transfers containing Ty1 by DNA sequencing. Diploids formed between *MAT**a*** and *MATα* haploid yeast cells undergo separate cytoplasmic and nuclear fusion events. Nuclear fusion is partially blocked when one of the parents contain a mutation in *KAR1*, which results in a transient heterokaryon with shared cytoplasm from both parents. In such mating events, one nucleus usually enters the daughter cell during budding from the heterokaryon, allowing detection of HTT events in the absence of nuclear fusion. Here, we present genetic evidence suggesting this route of HTT occurs between a donor *S. paradoxus* strain carrying Ty1*his3-AI* (MATa his3-Δ200 trp1 Ty1his3-AI[3056] ura3) and a recipient Ty-less *S. cerevisiae* strain (MATα ade2-101 his3-Δ200 cyh2^R kar1-Δ15 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52) defective in karyogamy (*kar1-Δ15*). We will then define the Ty1 gene products responsible for HTT, and the impact of Ty1 Copy Number Control mediated by the self-encoded restriction factor p22. Also, we will assess other Ty elements implicated in HTT based on phylogenomics, develop additional yeast species as transposon donors and investigate other modes of HTT. For example, can Ty VLPs “infect” yeast cells lacking a cell wall?

P14. Optimizing excystation in *Entamoeba histolytica*

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Entamoeba histolytica is a predominantly gastrointestinal eukaryotic pathogen that causes amoebic dysentery in ~100 million people each year worldwide. *E. histolytica* has a biphasic lifestyle that transitions between the motile trophozoite that colonizes the large intestine and an infective cyst surrounded by a chitinous wall that is released to the environment to infect a new host. Historically, the related reptile pathogen *Entamoeba invadens* has served as a model for *E. histolytica* encystation and excystation because the human pathogen did not reliably convert from trophozoite into the cyst form in vitro. Our lab has established a robust, reproducible method for in vitro encystation of *E. histolytica*, allowing us now to similarly pursue a method for reliable in vitro excystation of *E. histolytica*. Here, I have conducted a systematic analysis of three parameters that may impact excystation: acid treatment, sarkosyl treatment, and cyst density. Flow cytometry was used in tandem with a chitin staining fluorescent dye (Alexa-Fluor 488) to visualize the transition between the cyst and trophozoite forms. Analysis of these and additional parameters will be used to develop and optimize a rapid and reliable excystation protocol for use in future encystation and excystation research. Flow cytometry was used in tandem with a chitin staining fluorescent dye (Alexa-Fluor 488) to visualize the transition between the cyst and trophozoite forms.

P15. The role of a vacuolar H^+-ATPase assembly factor in *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular parasite capable of infecting most warm-blooded animals. During acute infection, its tachyzoite form rapidly replicates by invading host cells, causing cell lysis and tissue damage. In extracellular tachyzoites, a unique acidic organelle known as the plant-like vacuolar compartment (PLVAC) forms. Functionally analogous to the plant vacuole, the PLVAC plays roles in ion storage, osmoregulation, and calcium homeostasis, essential for parasite survival and progression of the parasite lytic cycle. These functions rely on the organelle acidification driven by proton pumps such as the vacuolar proton-translocating ATPase (V-ATPase) and the proton-translocating pyrophosphatase (VP1), which is linked to polyphosphate (PolyP) storage. The V-ATPase is a multi-subunit enzyme that uses ATP hydrolysis to pump protons across membranes. In *T. gondii*, it localizes to the PLVAC and the plasma membrane, where it plays key roles in intracellular pH regulation and membrane potential. V-ATPase assembly occurs in the endoplasmic reticulum and involves two domains: V1 (cytoplasmic) and V0 (membrane-bound), which can associate and dissociate independently. In yeast, ER-localized proteins such as VPH2 are required for V-ATPase assembly. To investigate the function of VPH2 in *T. gondii*, we generated a conditional knockdown mutant (iΔ3HA-TgVPH2) using CRISPR/Cas9. Loss of TgVPH2 impaired parasite replication and significantly decreased PolyP levels. Localization studies confirmed that TgVPH2 resides in the PLVAC. Preliminary data suggest that TgVPH2 is important for maintaining resting cytosolic Ca^{2+} levels and regulates the Ca^{2+} content of acidic stores. Finally, TgVPH2-depleted parasites have a deficiency in proton extrusion and in the recovery of their basal intracellular pH after acid stress. In summary, TgVPH2 is essential for V-ATPase-dependent acidification of the PLVAC, and coordinates proton homeostasis, PolyP content, and calcium storage to support parasite replication.

P16. Does RON11 control rhoptry number: A cross-species question in *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular apicomplexan parasite capable of invading nearly any nucleated cell in warm-blooded hosts. The parasite utilizes specialized cytoskeletal secretory organelles at the apical complex known as rhoptries to invade host cells and establish its intracellular niche within the parasitophorous vacuole. Rhoptry neck proteins (RONs) are critical structural and functional components of this invasion machinery. In *Plasmodium falciparum* merozoites, which possess a pair of rhoptries, RON11, a seven-transmembrane, EF-hand calcium-binding protein, has been identified as essential for the generation of the second rhoptry. Depletion of PfRON11 results in merozoites with only a single rhoptry, which are unable to invade erythrocytes. To investigate whether the role of RON11 is conserved in *T. gondii*, we generated an inducible knockdown strain, TgRON11^{cKD}. We measured the number of rhoptries in *T. gondii* tachyzoites using Ultrastructure expansion microscopy. Interestingly, tachyzoites depleted of TgRON11 formed fewer rhoptries but did not show any defect in parasite growth. This preliminary study suggests that, unlike *P. falciparum*, TgRON11 may be dispensable for host cell invasion in T*. gondii* tachyzoites. Future studies will determine if there is an effect on tachyzoite invasion, as well as use CRISPR screens to identify other genes that may function in the rhoptry biogenesis pathway.

P17. Early establishment of virtual memory T cells in the neonatal lung

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$CD8^+$ T virtual memory (T_{VM}) cells are antigen-inexperienced, memory-like cells that arise early in life. To date, most T_{VM} studies have focused on adult, splenic TVM populations and failed to consider TVM function at their origin and in mucosal sites. Here, we sought to determine how T_{VM} populate the lung during early life and may impact subsequent immunity. T_{VM} in the lung tissue versus the circulation were studied using a novel intravascular staining protocol via the retro-orbital sinus that we optimized for use in neonates. Lungs were subsequently phenotyped and analyzed by flow cytometry for T_{VM} across postnatal (P) days P3–P14. To explore the contribution of cytokine signaling in neonatal lung T_{VM} establishment and localization, parallel analyses were performed in IL-15 knockout neonates. In naïve neonatal mice, T_{VM} were detected in the lung parenchyma as early as P3. While total numbers of overall lung T_{VM} increased with age, this expansion was primarily driven by circulating T_{VM}. In P5 naïve IL-15 knockout neonates, lung-resident T_{VM} were completely absent, accompanied by approximately 70% reduction in circulating lung T_{VM} and ~50% reduction in splenic T_{VM} numbers compared to wild-type controls. Our findings demonstrate that T virtual memory cells emerge in the neonatal lungs shortly after birth, establishing a stable resident pool capable independent of infection. Moreover, IL-15 may specifically contribute to the early establishment and maintenance of the T_{VM} niche in the lung parenchyma, with circulating and splenic niches only partially dependent on IL-15. Together, these data suggest that neonatal T_{VM} are regulated distinctly from adults, with IL-15 being requisite for maintaining T_{VM} at the respiratory barrier early in life.

P18. Vaccination with a *Plasmodium falciparum* MIF protein nanoparticle reduces inflammation and promotes recovery from anemia during malaria

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Malaria parasites encode a macrophage migration inhibitory factor (MIF) ortholog, *Plasmodium* MIF (pMIF), which interferes with host MIF signaling during infection. In rodent malaria models, pMIF promotes inflammation and contributes to reduced germinal center activity and diminished anti*Plasmodium* antibody responses. Vaccinationmediated neutralization of pMIF reduces inflammation and enhances anti*Plasmodium* recall responses. We hypothesized that immunization against pMIF could accelerate the development of protective malarial immunity following natural exposure to *Plasmodium falciparum* (*Pf*). To test this, we evaluated the immunogenicity, safety, and impact on infection outcomes of a *Pf*MIF protein nanoparticle vaccine in *Aotus nancymaae* compared with animals vaccinated with a control MERS nanoparticle vaccine. We show that the *Pf*MIF nanoparticle vaccine, formulated with AddaVax, is immunogenic and well tolerated, with no adverse events observed following two immunizations. Although parasitemia levels during infection were comparable between groups, *Pf*MIFvaccinated animals exhibited reduced inflammation and improved recovery from anemia following antimalarial treatment. Together, these findings demonstrate that a *Pf*MIF nanoparticle vaccine is safe, immunogenic, and confers clinical benefit in *Aotus* monkeys, supporting further evaluation of this strategy.

P19. Characterizing EXP2's assembly into the parasitophorous vacuole in *Plasmodium falciparum*

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Plasmodium parasites must rapidly remodel their red blood cell host upon invasion which is achieved by exporting proteins through the Plasmodium Translocon of Exported proteins (PTEX). EXP2, the membrane-spanning component of PTEX, inserts into the parasitophorous vacuole membrane via a N-terminal amphipathic helix to form a nutrient and protein-conductive pore. While the function of EXP2/PTEX is known, the mechanism by which EXP2 incorporates itself into the PV membrane remains unclear. Curiously, recombinant EXP2 is able to non-selectively form pores in different membranes, highlighting the need to regulate its membrane insertion to prevent premature pore-formation along the secretory pathway. One possible model stems from bacterial toxins which traffic in a soluble state until contact with membrane components promote insertion into its target membrane. We hypothesize that EXP2 traffics in a similar manner and that its transition from a soluble to a membrane inserted state is mediated by an alpha helical bundle known as the globular domain. Using a technique we developed in the lab called knockER (kER) that disrupts a target protein's traffickingvia conditional ER retention, we observed that ER retention of EXP2 led to premature parasite death compared to a full EXP2 knockout. This suggests that kER-induced parasite death is likely due to compromised ER integrity, presumably via premature membrane insertion into organellar membranes. Interestingly, parasite death was similarly observed upon ER retention of a second copy of EXP2, despite possessing a functional WT copy. More importantly, ER retention of a second copy of EXP2 lacking its amphipathic helix did not impact parasite growth, confirming the role of this region in kER-based parasite death. Using this approach, we employed knockER to express EXP2 mutants in the globular domain to assess the role of this domain in EXP2 membrane insertion. Our results show that most components of the globular domain are required for kER-mediated death and, thus, shows its importance in EXP2's membrane insertion.

P20. Assessing the role of the PEXEL motifinnon-exported proteins in *P. falciparum*

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Plasmodium falciparum is an obligate, intracellular pathogen and the deadliest causative agent of human malaria. The clinically relevant part of its lifecycle is the invasion of the host's red blood cells (RBCs) where the parasite remodels RBCs by exporting proteins into the cytoplasm of the red blood cell, enabling nutrient acquisition, immune evasion, and parasite growth. In *Plasmodium falciparum*, most of these exported proteins contain the Plasmodium Export Element (PEXEL), a pentameric motif (RxLxE/Q/D) that is typically downstream of a recessed signal sequence. While PEXEL-mediated export has been well investigated within exported proteins, our lab has shown that this processing motif is present in non-exported proteins. We presenthereanew,non-exportedproteincalledliverstageantigen3(LSA3),whichalsocontains a PEXEL motif. Using a construct containing the first 123 amino acids of LSA3, including the PEXEL sequence, fused to mNeonGreen (mNG), we observed export of mNG into the RBC space. More importantly, a secondary construct containing a key amino acid change within the PEXEL, blocked export of mNG, showing this to be a true PEXEL. Future studies will focus on understanding the role of the PEXEL motifin protein function of LSA3 and other non-exported yet PEXEL-containing proteins.

P21. Harnessing intracellular ROS production to improve the efficacy of liver-stage malaria vaccines

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Malaria is a global disease that affects hundreds of millions of people every year. *Plasmodium*, the malaria-causing parasite, undergoes obligate replication in hepatocytes before advancing to the symptomatic and transmissible blood stage. Radiation-attenuated sporozoite (RAS) is a leading vaccine candidate for malaria, relying on the inoculation of live-attenuated *Plasmodium* that produce abortive infections in the liver. Immunity generated by RAS depends on Type I Interferon (IFN-I)-induced cell-intrinsic immune responses that drive programmed cell death in hepatocytes, facilitating the delivery of RAS antigens to antigen-presenting cells (APCs) recruited to the liver. Reactive oxygen species (ROS) serve as key effectors within these IFN-I-driven responses. As exogenous IFN-I administration is not a clinically viable adjunct strategy, we explore ROS-inducing drugs as an alternative means of potentiating RAS vaccine efficacy. We hypothesize that induction of ROS in hepatocytes concurrent with RAS inoculation would enhance cell-intrinsic immunity to *Plasmodium*, augmenting RAS antigen delivery to the APCs and the resultant protective immune response. We show that suitably timed delivery of specific clinically approved inducers of ROS in hepatocytes facilitated enhanced elimination of *Plasmodium* from hepatocytes *in vitro* and *in vivo*, and improved the efficacy of RAS vaccination. These findings indicate that drugs that drive intracellular ROS production in hepatocytes can be repurposed to improve the efficacy of liver-stage malaria vaccines.

P22. Dissecting the molecular machinery of the *Trypanosoma cruzi* cytostome-cytopharynx complex

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Chagas disease, caused by *Trypanosoma cruzi*, is the most severe parasitic infection in the Americas. To fuel its rapid replication, the parasite uniquely relies on the cyto**s**tome-cyto**p**harynx **c**omplex (SPC), a highly specialized organelle responsible for the bulk endocytosis of host macromolecules. While the structural architecture of the SPC is well-defined, the molecular machinery and regulatory networks governing its function have remained a “black box”. To bridge this gap, we used TurboID proximity labeling to generate the first comprehensive SPC proteomes. By employing six distinct bait proteins (MyAP, MyoF, MyoB, MyoE, Formin3, and ESR1) localized to discrete SPC sub-regions, we identified a robust dataset of nearly 500 potential SPC-associated proteins. This analysis revealed a striking enrichment of phosphatidylinositol phosphate (PIP)-related proteins, including predicted kinases, phosphatases, and numerous hypothetical proteins possessing putative PIP-binding domains such as PH, PX, FYVE, and ENTH motifs. Furthermore, we identified a diverse cohort of signaling candidates, including atypical kinases, phosphatases, and mediators of small GTPase signaling (GEFs and GAPs). Validation of the MyAP interactome identified ~200 potential partners, the most abundant of which correctly localized to the SPC. Notably, functional studies revealed two novel kinesin motors essential for driving endocytosis. While many highly enriched hypothetical proteins with long coiled-coil domains were non-essential individually, the consistent enrichment of PIP and signaling components suggests a highly coordinated regulatory framework. These findings provide the first comprehensive molecular portrait of the SPC and uncover divergent, non-host-conserved targets for therapeutic intervention.

P23. Development and Implementation of an in vitro adhesion assay for *Acanthamoeba castellanii*

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Acanthamoeba castellanii is parasitic protozoan commonly found in all bodies of water, including public water systems, the ocean, and popular freshwater swimming holes. This parasite, resulting in eye irritation and redness, severe ocular nerve pain, and eventual blindness. This infection is known as *Acanthamoeba* Keratitis (AK). Current medical intervention consists of an onerous regimen of biguanide drops for multiple months; however, this treatment plan results in recurrent infections even after treatment. Therefore, it is important to investigate other methods of treating AK. An important goal of AK research is the discovery of methods that prevent the initial stages of infection, namely adhesion of parasites to host cells. Thus, we developed a luminescence-based adhesion assay using Promega CellTiter-Glo 2.0, which quantifies viable cells in culture by measuring ATP. Human corneal epithelial (HCE) cells were grown to confluency in opaque 96-well plates. The monolayers were fixed with paraformaldehyde (to prevent parasite-mediated lysis). Fixed monolayers were incubated for 24 hrs to dissipate residual ATP, after which, parasites were added for 30 min. The wells were then washed with PBS and the number of parasites adhering to HCE cells was determined by measuring luminescence after the addition of the CellTiter-Glo 2.0 reagent. The assay was validated using a range of concentrations of parasites and in the presence of mannose (a known inhibitor of adhesion) or glucose (negative control). We are now using this assay to test the ability of drugs (e.g., azoles) and peptides (e.g., thymosin β-4) to block adhesion.

P24. Dose-optimization of an anti-CD20 antibody to reduce B cells in owl monkeys

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Owl monkeys (*Aotus* spp.) are a valuable animal model for malaria, HIV, and other infectious diseases. These animals are particularly important in malaria research because of their susceptibility to infection with human malaria parasites. They have also contributed significantly to vaccine development, improved understanding of malarial pathogenesis, and the development of novel therapeutics. Despite their utility in preclinical and observational studies, *Aotus* usage in mechanistic investigations into malarial immunity development has been limited by the paucity of species-specific immunological reagents. To address this gap, the Nonhuman Primate Reagent Resource Center has focused on developing reagents to facilitate the use of New World monkeys in biomedical research. In this study, we tested the hypothesis that a newly developed anti-CD20 monoclonal antibody could effectively reduce peripheral B cell counts in owl monkeys following administration. In dose-optimization experiments, we evaluated whether 50 mg/kg administered as two separate infusions or 100 mg/kg administered as a single infusion was required to achieve a significant reduction in B cell numbers lasting at least 30 days. All animals exhibited a reduction of CD20 gene expression following antibody infusion, indicating that the number of circulating B cells was effectively depleted. The development and characterization of this novel reagent now enable direct experimental assessment of B cell function during human malaria parasite infections in a clinically relevant nonhuman primate model.

P25. Yeasty Boys: An in vitro system to assess the role of a *Plasmodium* protein in membrane fusion

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Malaria is a serious global health threat, with 263 million cases and 600,000 deaths annually. Clinical manifestation of the disease begins when *Plasmodium falciparum* invades red blood cells. Successful invasion is dependent upon the establishment of a parasitophorous vacuole (PV), where the parasite will reside throughout its 48hr asexual life cycle. After attachment and tight junction formation, *P. falciparum* releases various proteins through its rhoptries, which are essential secretory organelles. Prior studies from several groups have determined several key mediators of *P. falciparum* invasion into the host red blood cells. Surprisingly, the molecular mechanisms governing PV sealing and formation are completely unknown. Previously, the lab has shown that SNARON, a *P. falciparum* SNARE-like protein, localizes to rhoptries and is required for invasion. SNARE proteins are essential mediators of membrane fusion that are highly conserved across all species. Based on our preliminary data, we hypothesize that SNARON may function to fuse the RBC membrane forming the PV. Utilizing yeast as a model organism, we are investigating the potential role of SNARON in membrane fusion. Yeast strains expressing a split GFP system will be utilized in a membrane fusion assay to assess if SNARON is able to rescue vacuolar fragmentation. Using recombinantly expressed SNARON, we will also dissect the molecular determinants of SNARON function.

P26. Epidemiology and temporal trends of gastrointestinal parasites and vector-borne pathogens in golden retrievers: An eight-year cohort study

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Canine parasitic and vector-borne diseases substantially affect dog health, pose zoonotic risks to humans, and contribute to environmental contamination through pathogen shedding, thereby increasing the risk of disease transmission to other animals. However, longitudinal data describing incidence and temporal trends in companion dogs remain limited. This study aimed to estimate long-term prevalence, incidence rates, and temporal trends of gastrointestinal parasites and vector-borne pathogens in Golden Retrievers over eight years. Data for the project were extracted from the Morris Animal Foundation's Golden Retriever Lifetime Study (GRLS). A cohort of 48,705 dogs were followed over eight years. Sixteen gastrointestinal protozoa, helminths, and vector-borne pathogens were evaluated. Prevalence and incidence rates per 1000 dog-years with respective 95% confidence intervals were estimated. Temporal trends of annual prevalence of parasitic diseases were presented using time series plots, and the monotonous trends were assessed using the Mann–Kendall test and Sen's slope estimator. *Giardia* (9·1%, 95%CI:) and roundworms (8·2%, 95%CI:) were the most prevalent parasites across the eight years. Lyme disease exhibited the highest incidence rate among the parasitic diseases (4·22 (3·43–5·14), 95%CI:). Significantly increasing temporal trends were observed for the annual prevalence of *Anaplasma spp*., *Borrelia burgdorferi*, *Ehrlichia spp.,* hookworms, whipworms, and *Eimeria* ($p < 0.05$), indicating rising exposure risks among the Golden retriever dogs over time. This longitudinal cohort study demonstrates pathogen-specific increases in gastrointestinal and vector-borne infections among Golden Retrievers, reinforcing the importance of long-term canine disease surveillance for better understanding of canine health management and zoonotic transmission risks across One Health domains.

P27. Mycobacterial ATP synthase inhibitor, Bedaquiline, limits *P. falciparum* parasite replication in blood and mosquito stages

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Malaria remains a major global public health concern. One of the causative agents, Plasmodium falciparum, the most virulent form of malaria, undergoes a complex life cycle and circulates between the human host and the vectors, *Anopheline* mosquitoes. The gold-standard treatment for malaria infection is Artemisinin combination therapy (ACT); however, concerns with the rise and spread of Artemisinin-resistant parasites underscore the need to find alternative treatments. Parasite's mitochondrial ATP synthase is highly divergent from its human counterpart. Bedaquiline (BDQ), a mycobacterial ATP synthase inhibitor, has been demonstrated by others to limit asexual replication in *P. falciparum*. While its mechanism of action is unknown in *P. falciparum*, its target in mycobacteria suggests it could target the parasite's ATP synthase. This study evaluates the efficacy of BDQ against *P. falciparum* asexual and the early mosquito-stage. These findings suggest that bedaquiline possesses transmission-blocking potential, reducing the parasite burden within the mosquito. Given the divergent nature of parasite mitochondria from those of their hosts and BDQ's mechanism of action in mycobacteria, our data and further understanding of BDQ's mechanism of action in *P. falciparum* can offer novel insights into therapeutics.

P28. Characterization of two hypothetical subunits of the vacuolar transporter chaperone complex in *Toxoplasma gondii*

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Polyphosphates (PolyP) are linear polymers of inorganic phosphates that assist in metabolism, energy storage, stress responses, and growth across diverse organisms. In *Saccharomyces cerevisiae*, PolyP synthesis is mediated by the vacuolar transporter chaperone (VTC) complex, which utilizes inorganic phosphates and ATP to generate PolyP chains that are stored in the yeast vacuole. While this pathway is well characterized in yeast, the function of the VTC complex in protist parasites remains poorly defined. The eukaryotic obligate parasite *Toxoplasma gondii* infects millions of people worldwide and depends on PolyP for storing Ca^{2+} in acidic compartments like acidocalcisomes and the plant-like vacuolar compartment (PLVAC). The VTC complex consists of five subunits (VTC1-5) in yeast; however, in the *T. gondii* genome, only two homologs (TgVTC2 and TgVTC4) have been found. Previous studies demonstrated that TgVTC2 and TgVTC4 are essential for the parasite lytic cycle and acidic Ca^{2+} storage. Mass spectrometry analysis identified two hypothetical proteins, Tg239130 and Tg290225, as VTC subunit candidates. Using the CRISPR/Cas9 system, we generated double tagged parasite lines in which TGGT1_239130 and TGGT1_290225 were independently tagged at their C-terminus with a spaghetti monster Myc tag in the iΔ3HA-VTC4 background. Reciprocal immunoprecipitation assays validated the interaction between TgVTC4 and Tg239130 and Tg290225. Additionally, immunofluorescence imaging revealed that Tg239130 and Tg290225 colocalize with TgVTC4 in acidocalcisomes. We are currently generating clean knockouts of TGGT1_290225 and TGGT1_239130 to determine whether these genes are required for the parasite lytic cycle progression and PolyP synthesis, providing insight into the composition of the *T. gondii* VTC complex and PolyP metabolism.

P29. Diverging from the norm: Subunit k and its unique architecture in *Toxoplasma* ATP synthase

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Mitochondria serve as a metabolic hub by utilizing the ATP synthase to harness the proton gradient generated by respiratory chain complexes for energy production. Despite its universal role, the architecture of the ATP synthase varies drastically between organisms. In apicomplexans, the enzyme has diverged from the conserved 15 subunits in yeast and mammals into a massive 32-subunit complex. This divergence is marked by 17 apicomplexan-specific proteins and unique extensions on several canonical subunits. Using *Toxoplasma gondii* as a model, our research examines subunit k; a conserved subunit that possesses an apicomplexan-specific, ubiquitin-like (UBX) extension. UBX domains in several organisms typically govern interactions with ubiquitin or modulate protein-protein interactions. However, the role of the UBX domain in subunit k has yet to be elucidated. In contrast to other ATP synthase subunits, the conditional knockdown of subunit k does not cause mitochondrial fragmentation or visible mitochondrial morphological defects. Our data indicate that this subunit is essential for the parasite lytic cycle, cristae density, the stability and assembly of the ATP synthase. Through immunoprecipitation and biochemical analysis, we tested the interaction of subunit k with ubiquitin, ultimately finding that its UBX domain lacks canonical ubiquitination activity. Future research will leverage a novel inducible complementation system to investigate how the UBX domain of subunit k contributes to ATP synthase assembly and activity. Our findings offer a basis for investigating the role of the unique UBX domain in subunit k in regulating *T. gondii* mitochondrial function and energy metabolism. Functionally characterizing the role of this subunit will provide insights into the apicomplexan biology, evolutionary adaptations, and potential therapeutic targets.

P30. Characterization of the *Toxoplasma gondii* steroidogenic acute regulatory protein-related lipid transfer domain-containing protein TgSTART2

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The Steroidogenic Acute Regulatory Protein-related Lipid Transfer (START) domain is a lipid binding protein domain conserved amongst mammalian, plant and yeast cells. Though having similar structural homology, START-domain containing proteins possess several differing functions including lipid trafficking and regulating lipid droplets. Additionally, START-domain containing proteins have been implicated in the tethering and formation of membrane contact sites (MCS), sites of contact between two membranes of differing organelles, permitting lipid exchange and signaling. While START-domain containing proteins have been characterized in other organisms, their role within *Toxoplasma gondii* is poorly understood. Previous work has identified three canonical START-domain containing proteins within *T. gondii*, in addition to five other proteins with putative START domains. Here, we focus on TgSTART2, an uncharacterized protein identified to possess a START domain on its carboxyl end. Using the CRISPR/Cas9 gene-editing system, I have tagged the C-terminus of TgSTART2, revealing a dispersed punctate pattern with vesicular morphology. However, precise localization has not been determined as TgSTART2 fails to colocalize with examined structures and compartments. Rather, points of contact have been documented with several organelles, indicating a dynamic localization and suggesting a role in vesicular transport, or as a tethering protein within MCS formation. Future work aims to C-terminally tag TgSTART2 with an eGFP marker, permitting live imaging to evaluate the possible trafficking of TgSTART2.

P31. Functional characterization of novel SAM complex subunits Sam35 and Sam37 in *Toxoplasma gondii*

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Apicomplexan parasites are obligate intracellular pathogens responsible for diseases including malaria, toxoplasmosis, and cryptosporidiosis making them important targets for therapeutic development. *Toxoplasma gondii*, a member of this phylum, has a significantly reduced mitochondrial genome which encodes for only three proteins. Therefore, *T. gondii* relies heavily on nuclear-encoded mitochondrial proteins that must be imported into the mitochondrion. This process depends on the Sorting and Assembly Machinery (SAM) complex which inserts β-barrel proteins into the outer mitochondrial membrane. While the core SAM component, SAM50, has been identified in *T. gondii*, the remaining subunits of the complex are missing from its genome. However, recent proximity biotinylation (TurboID) research conducted in our lab detected the novel presence of two potential homologs of the SAM subunits SAM35 and SAM37. In this study, SAM35 and SAM37 were epitope-tagged with a high-throughput tagging (HiT) vector strategy to assess protein expression and intracellular localization via immunofluorescence. Additionally, a CRISPR/Cas9-based construct was designed to perform conditional knockdown of SAM35 through U1-mediated mRNA destabilization, which will be used to study the function of SAM35. Together, these results confirm the mitochondrial localization of SAM35 and establish a framework for future functional characterization of the SAM complex in *T. gondii*.

P32. Establishing a protein export reporter system in *P. falciparum*

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P. falciparum exports ~10% of its proteome into the host cell which is used to extensively remodel the host RBC for parasite survival. This export is mediated by the PTEX complex, an essential translocon that is specific to the parasite. Importantly, blocking protein export via PTEX inactivation is lethal to the parasites making PTEX an excellent antimalaria drug target. While methods exist to assess protein export, current approaches such as microscopy and western blotting, are time-consuming and labor-intensive. Thus, efficient, real-time assays are needed to enable rapid and high-throughput analysis of PTEX function via protein export. We aim to develop a parasite cell line that will allow real-time measurement of protein export using the split-NanoLuc reporter system. In our approach one fragment of NanoLuc is localized at the parasitophorous vacuole membrane (PVM) facing the RBC cytosol, while the complementary fragment is fused to an exported parasite protein. Thus, when protein export occurs, the two NanoLuc fragments meet within the RBC cytosol and reconstitute an active Nano Luciferase enzyme, producing a detectable luminescent signal. The presence of luminescence then indicates successful protein export and suggest that the compound being tested does not block export. Conversely, the absence of luminescence would indicate inhibition of protein export, identifying the compound as a potential export-blocking antimalarial drug candidate. Development of this system will allow high-throughput screening of compounds against PTEX, adding to the much-needed repertoire of novel antimalarial compounds.

P33. Impact of exposure to malaria on susceptibility to respiratory viral infections

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More than half of the world's population lives in malaria-endemic areas where respiratory infections like influenza are also prevalent. Influenza causes disproportionately high mortality in malaria-endemic regions, suggesting that exposure to malaria might enhance our susceptibility to Influenza. Historical evidence from the 1918 Spanish flu pandemic suggests that individuals previously exposed to malaria suffered markedly enhanced influenza-related disease burden and mortality. Understanding how previous exposure to *Plasmodium* impacts the pathogenesis and immunity to influenza would help combat the incidence and severity of flu in malaria-endemic areas. We hypothesized that exposure to malaria induces persistent immunological and structural changes in the lung, compromising the induction and maintenance of immunity to Influenza. *Plasmodium chabaudi chabaudi* (Pcc)-experienced malaria-convalescent mice challenged with Influenza A virus (IAV; PR8-NanoLuc) exhibited significantly reduced induction of IAV-specific T and B cell and dendritic cell responses, as well as exacerbated weight loss and lung viral burdens, when compared to age-matched malaria-naïve controls. These findings indicated that malaria-convalescence durably impairs antiviral immunity to IAV in mice, establishing a model to investigate the mechanisms driving the increased vulnerability of malaria-endemic populations to acute respiratory infections.

P34. Investigating *nrps* as a putative germ cell niche regulator in tapeworms

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Tapeworms are incredibly successful parasitic flatworms that can infect almost any vertebrate host. Tapeworms produce offspring throughout adulthood, which makes them an attractive model to study reproductive processes. The germinative region (GR) is the only regeneration-competent tissue from which new proglottids containing complete male and female reproductive systems regenerate. A population of cells expressing the conserved germ cell regulator *nanos,* are detectable in the GR despite the lack of gonads, indicating that factors within the GR could be required for proper germ cell development. We ask: what cells and signals make up the tapeworm germ cell niche? Previous work implicated the gene *nonribosomal peptide synthetase* (*nrps*) in the sexual maturation of female schistosomes, a related flatworm species. We hypothesize that *nrps* and its products regulate germ cell development in the GR. In the rat intestinal tapeworm *Hymenolepis diminuta*, *nrps* expression is observed in the anterior GR, in a subset of nuclei that make up the osmoregulatory canals. The *nrps+* cells are directly adjacent to *nanos*$^{+}$ germ cells, thus *nrps* is physically positioned to act as a germ cell niche. After *nrps* knockdown by RNA interference, germ cell numbers in the GR are reduced. Tapeworm tissue contains two possible products of *nrps*: β-alanyl-tryptamine (BATT) and β-alanyl-serotonin (BAS) detectable by mass spectrophotometry. BATT supplementation increases production of undifferentiated germ cells, and preliminary results from BAS injections suggest an increase in germ cell proliferation rate. Additional functions of BATT and BAS in germ cell differentiation or germline stem cell maintenance are presently being investigated. Given these data, *nrps* plays an important role in regulating germ cell specification/maintenance, and likely represents the first germ cell niche described in tapeworms. Funding is provided by NIH 1T32GM142623.

P35. PfDHODH is essential for mosquito-stage development of *Plasmodium falciparum*: A novel transmission blocking target

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Malaria, caused by protozoan parasites of the genus *Plasmodium* and transmitted through the bite of infected *Anopheles* mosquitoes, remains a major global threat. PfDHODH (dihydroorotate dehydrogenase), is localized in the parasite mitochondrion is essential for *de novo* pyrimidine synthesis, is a validated target against blood stage *Plasmodium* through experimental drugs such as DSM265. PfDHODH's function is dependent on the parallel action of cytochrome bc1 in the mitochondrial electron transport chain (mtETC) to provide oxidized CoQ. Inhibition of cytochrome bc1, with atovaquone (ATQ), indirectly arrests the function of DHODH. Existing antimalaria treatments target blood stage parasites, but transmission stage development in the mosquito is a bottleneck that could be exploited for disease prevention. Our prior findings show mosquito exposure to ATQ inhibits parasite development, therefore blocking and/or delaying transmission. However, the precise mechanism of ATQ-induced arrest is not known, but may be due to indirect inhibition of DHODH or inhibition of mosquito-stage specific *Plasmodium* functions. To investigate the role of DHODH during transmission, in our current study, we will employ conditional/inducible gene knock-out/down systems (DiCre/glmS) to ablate PfDHODH in a time- and development stage dependent manner during *Plasmodium* mosquito infection. In parallel we will assess the impacts of chemical ablation of DHODH using DSM265 during mosquito development. Here, we present initial findings from our chemical inhibition assays, as well as our progress towards developing genetic tools for studying *Plasmodium* gene function *in vivo* in mosquitoes.

P36. Characterizing divergent ER homeostasis mechanisms in Amicomplexa

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All eukaryotes harbor a mechanism to retain proteins in the ER via the KDEL/ERD2 receptor. This receptor acts by recognizing a C-terminal KDEL motif, and its variants, from ER proteins that have escaped to the Golgi in COPII vesicles and returning these proteins via COPI vesicles. Phylogenetic analysis shows that while all eukaryotes possess one gene encoding this receptor, Apicomplexans and plants have an expanded gene family related to this receptor called ERD2-related proteins (ERPs). Current mutagenesis screens in different *Plasmodium* species suggest both ERD2 and the only ERP found in *Plasmodium*, here called ERP1, to be essential suggesting a non-redundant function for both receptors. Moreover, while the function of the classical ERD2 receptor has been well characterized, the function of ERPs in other organisms is still unknown. Curiously, ERP1 has an additional transmembrane domain and contains mutations that would prevent it from binding the KDEL motif, suggesting that ERP1 has an additional biological function in the parasites. Thus, we will investigate the role of ERP1 using the TetR-DOZI to study its role in ER/Golgi biology. It is also known that *Plasmodium* can recognize a wide range of KDEL motifs which contrasts the narrow range used by other eukaryotes. Thus, we will investigate the role of key residues in the canonical ERD2 receptor, including one known mutation not found in the human KDEL receptor, in regulating the promiscuous recognition of KDEL variants. Together, these findings aim to uncover the KDEL receptor functions in Apicomplexans to uncover novel antimalarial targets.

P37. Establishment of a transfection system in *Naegleria fowleri* using expression vectors

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The pathogenic free-living amoeba *Naegleria fowleri* is the causative agent of primary amoebic meningoencephalitis (PAM), an infection with a mortality rate exceeding 97%. This high mortality is due, in part, to the limited availability of effective drugs, a situation exacerbated by the lack of molecular tools to study this organism. Such tools could provide critical insight into gene function and enable genetic validation of potential drug targets. Here, we report efforts to develop heritable expression vectors for transgene expression in *N. fowleri*. Through an iterative process, we have optimized vector components and transfection approaches. Our typical vector encodes the selectable marker puromycin N-acetyltransferase (*pac*), linked to open reading frames of the fluorescent proteins eYFP or mCherry via a self-cleaving T2A peptide. Both *pac* and *eYFP* were codon-optimized for *N. fowleri*. The vectors also include the 5′ and 3′ UTRs of a conserved *N. fowleri* actin gene (NfTy_067520, AmoebaDB) to drive gene expression. For transfection, we found that 40 kDa polyethylenimine (PEI-40), a cationic polymer that binds negatively charged DNA to form complex nanoparticles, enabled plasmid uptake. Following puromycin selection, we generated stable antibiotic-resistant, fluorescence-expressing lines. However, fluorescence varied across populations and was observed in only in ~10% of eYFP-expressing cells and ~25% of mCherry-expressing cells. Ongoing efforts are focused on optimizing plasmid architecture to improve the stability and uniformity of fluorescent marker expression, as well as modifying these constructs for future applications. Establishing these molecular tools will provide a foundation for addressing fundamental questions in *N. fowleri* biology and for enabling the validation of novel therapeutic targets against this devastating pathogen.

P38. Investigating the role of notch signaling on *Hymenolepis diminuta* segmentation

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Tapeworms (*Eucestoda*) are obligate endoparasitic flatworms that are a burden to both human and livestock health globally. Some species that commonly infect humans include the pork tapeworm *Taenia solium*, the fox tapeworm *Echinococcus multiocularis*, and the dwarf tapeworm *Hymenolepis nana*. Our lab uses the rat tapeworm *Hymenolepis diminuta* as a model for studying how these parasites grow, regenerate, and reproduce. The body of *H. diminuta* consists of a scolex with attachment organs, a germinative region (GR) that houses the tapeworm's regeneration potential, and a strobilated body comprised of up to thousands of hermaphroditic segments. These segments are crucial for completing the reproductive stage of the worm's life cycle; however, the mechanisms of how these segments grow is poorly understood. The goal of this project is to understand the genetics driving tapeworm segment development. Early characterizations of the tapeworm's GR revealed that both a *notch* and *delta* gene have posteriorly enriched expression, coinciding with newly formed segments. Here, we show that the tapeworm genome contains all necessary genes for canonical Notch signaling, and that the expression patterns of many components have a "stripey" pattern that points to a plausible role in segmentation. We also show preliminary data showing that gene knockdown of the two *notch* paralogs causes severe but differing effects on the tapeworm's ability to grow segments. Lastly, early investigations of a structure called the "Signaling Quartet" shows that these cells likely influence the gene expression of surrounding cells. This project is supported by NIH, NIAID: DP2AI154416.

P39. Communication is key: Investigating interorganellar communication in *Toxoplasma gondii*

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While organelles are typically thought of as individual structures in eukaryotic cells, communication between them is required to share signals and metabolites to support their functions. 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 MCSs is limited to yeast and mammals, which are members of the Opisthokont clade. *Toxoplasma gondii,* an apicomplexan, provides the unique opportunity to investigate MCSs in a divergent model eukaryote. To identify MCS protein candidates in *T. gondii*, we have taken an unbiased proximity biotinylation-based approach by generating localization handles to anchor a biotin ligase to the surface of the mitochondrion, the ER, and the apicoplast, a phylum-specific vestigial plastid organelle. Biotinylation experiments followed by mass spectrometry analysis uncovered MCS protein candidates. Candidates include several proteins with expected MCS functions, such as lipid-transfer proteins and small GTPases, as well as apicomplexan-specific proteins with no predictable functions or domains. Finally, I have endogenously tagged several candidates using CRISPR Cas9 to determine their subcellular localization and generated conditional knockdown strains, which confirmed they are essential for parasite survival. The results of this work will both expand our knowledge of membrane contact sites across the evolutionary tree, potentially uncovering apicomplexan-specific ones, and identify new therapeutic targets for apicomplexan parasites.

P40. Genetic basis for hypnozoite formation in *Plasmodium vivax*

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Plasmodium vivax remains a major global health threat, contributing substantially to malaria morbidity and presenting a significant barrier to elimination. A key challenge is its ability to generate relapsing infections through the formation of dormant liverstage hypnozoites. Following mosquito inoculation, *P. vivax* sporozoites invade hepatocytes and differentiate into either rapidly replicating schizonts or longlived hypnozoites capable of persisting for months to years, evading immune clearance and standard diagnostics before reactivating to cause relapsing infection. The molecular mechanisms governing the decision between replication and hypnozoite dormancy remain largely unresolved, though previous studies implicate epigenetic regulation and transcriptional control. Notably, two *P. vivax* isolates; North Korea and Chesson, exhibit distinct and reproducible hypnozoite formation phenotypes independent of host background. Here we present highquality genome assemblies for these isolates and investigate genetic and regulatory differences that may underlie their contrasting hypnozoite ratios. Our analyses reveal divergence in promoter regions and multicopy gene families, highlighting candidate loci that may influence developmental fate decisions. These findings provide new avenues for dissecting the biology of hypnozoite formation and may inform the development of improved antimalarials for achieving radical cure.

P41. Evaluating differential responses to *Naegleria fowleri* challenge

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Naegleria fowleri, the brain-eating amoeba, causes Primary Amoebic Meningoencephalitis (PAM) when amoebae enter the nasal passages through contaminated water and migrate to the brain, where they feed on neural tissue and induce a highly pro-inflammatory immune response. With only about 5 annual U.S. cases and a 97% mortality rate, how is it that *N. fowleri* has this highly unusual infection pattern with such dire disease outcomes in humans? We hypothesize that genetic differences in key host factors correlate with susceptibility and refractory responses to *N.fowleri* infection. We infected 45 mouselines from the Collaborative Cross (CC), a mouse model of genetic diversity, with *N. fowleri* and determined their survival rates. We are quantifying amoebae burden over time using two sample types: urine collected daily and plasma collected once a mouse met pre-determined end-stage criteria. Both of these samples were run through an RT-qPCR assay, which quantifies the amount of an *N.fowleri*-specific small RNA as a measure of relative amoebae burden. In control C57/Bl6J mice, detectable small RNA gradually increases over time in the urine, with the highest burden detected at the end stage. Varying in genetic background and susceptibility, a series of mouse lines, such as CC039, CC003, and CC032, were investigated. The ultimate objective is to quantify the amoebae burdens in samples collected, which can be used for a downstream Quantitative Trait Loci (QTL) mapping analysis to identify genetic determinants that are associated with these phenotypes.

P42. Humoral immunity leads to control of chronic *Plasmodium* infections

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Chronic *Plasmodium falciparum* (*Pf*) infections are widespread in endemic areas, increase chances of severe disease, and may reduce malaria vaccine efficacy. Understanding the immune mechanisms that establish chronicity may help improved clinical outcomes and vaccine efficacy. Here, we used *Plasmodium coatneyi* infection in rhesus macaques, a validated *Pf* model, to define host immune responses related to parasite control in chronic infection. B cell transcriptional pathways were upregulated and correlated with parasite and symptom control in chronic infections. IgG and IgM antibodies against MSP-1_{19} peaked coincident with parasite control and remained elevated, and plasma from these same time points inhibited parasite growth *in vitro*. MSP-1_{19}-specific B cells were found in switched memory ($IgD^{-}CD27^{+}$), unswitched memory ($IgD^{+}CD27^{+}$), and activated naïve ($IgD^{+}CD27^{-}CD21^{-}$) populations. Notably, the extrafollicular DN2 B cells ($IgD^{-}CD27^{-}CD11c^{+}$) expanded without an increase in MSP-1_{19}-specific B cells and were surprisingly $CXCR5^{+}$. Unswitched memory B cells were also $CXCR5^{+}$ yet stayed IgM^{+}, implying limited class switching. Transcriptionally, unswitched memory and DN2 B cells were similar and highly correlated with atypical B cell ($CD21^{-}CD27^{-}$) transcriptomes in malaria-exposed humans. Together, these data show that humoral immunity drives parasite and symptom control in chronic *Plasmodium* infections and highlights the complexities of B cell subsets generated by these infections.

P43. Immune mechanisms regulating *Trypanosoma cruzi* control and persistence in muscle

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*Trypanosoma cruz*i (*T. cruzi*) elicits a strong immune response that limits but does not eliminate infection, enabling chronic parasite persistence and associated muscle pathology. Highresolution microscopy shows that amastigotes can silently infect and replicate within myocytes in the absence of local inflammation. Only after myofiber rupture and parasite release are T cells and macrophages recruited, suggesting a delayed and inefficient containment of parasite replication. Given the essential role of T cells and interferongamma (IFNγ) in parasite control, we examined IFNγdependent pathways in myocytes. Using conditional knockout mouse models and RNA sequencing, we found that IFNγ signaling does not trigger intrinsic parasitekilling mechanisms in myocytes, but is essential for parasite control by increasing antigen presentation (class I MHC) to sustain $CD8^+$ T cell IFNγ production and by inducing secretion of chemokines (such as CCL2) that recruit macrophages. To directly assess whether recruited macrophages mediate parasite elimination, we generated mice with macrophagespecific deletion of the IFNγ receptor. These IFNγunresponsive macrophages were still recruited to lesion sites but failed to eliminate released trypomastigotes, resulting in markedly increased tissue parasite burden. Future studies will dissect the mechanisms by which macrophages kill T. cruzi and determine how antibodymediated opsonization enhances macrophage effector function. This work will clarify whether Fc receptor–dependent pathways can be leveraged to improve therapeutic antibody strategies.

P44. Protein glycosylation in pathogenic protist parasites - a few pathologically relevant examples

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Protein glycosylation of protists is understudied, pathologically relevant, and no less important than in their animal or plant counterparts. Here we present a few examples from recent collaborations. *Trypanosoma cruzi* metacyclic forms exhibit stage-specific terminal α3-Gal O-glycan modifications, which are detectable serologically and have been suggested as pro-infection, pro-inflammatory signals. With Carolina Koeller and Norton Heise, we predicted the responsible glycosyltransferase and generated KOs. With Robert Amos, nLC/MS of alkali-released O-glycans in conjunction with ^{13}C-standards verified the presence of a trisaccharide sensitive to α-galactosidase treatment, and absence of the trisaccharide in the mutants. Mutants exhibit deficits in parasite ability both to infect and emerge from host cells. GPI anchors and GIPLs in *Toxoplasma gondii* are characterized by a distinctive disaccharide sidechain. In collaboration with Kirk Jensen, we predicted the responsible glycosyltransferases and confirmed their role by nLC/MS analysis of GPIs and GIPLs from KO strains, allowing for functional analysis of the disaccharide-free forms. KO strains exhibited increased virulence in mouse infection models.O-Fuc in *Acanthamoeba castellanii*. Probing permeabilized trophozoites suggested presence of fucose in their nuclei, confirmed to be O-Fuc using specialized anti-O-Fuc pAbs. In collaboration with John Samuelson, a proteomics survey of affinity-purified material suggests O-fucosylation of a range of nucleocytoplasmic proteins, which parallels findings in the social amoeba *Dictyostelium discoideum*.

P45. Collaborative cross mice yield genetic modifiers for sensitivity to infection with the brain eating amoeba *Naegleria fowleri*

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The free-living amoeba, *Naegleria fowleri* (*Nf*), is the causative agent of primary amoebic meningoencephalitis (PAM) and results in a fatal outcome in >97% of cases. Infection occurs following instillation of water containing amoebae into the nasal passages. *N. fowleri* then crosses the olfactory epithelium and migrates along the olfactory nerves to the frontal lobes of the brain where it causes extensive inflammation and pathology by degrading neural tissue. The infection progresses rapidly with onset of symptoms within 5-10 days and death by 14-17 days post-infection. Surveillance efforts suggest that *Nf* lives ubiquitously in freshwater across the globe, putting millions of individuals at risk every year for PAM. However, only 5 PAM cases on average are documented in the U.S. annually and very little is known as to why so few people are infected by *Nf* when it is likely that millions are exposed to the amoebae each year. We hypothesize that genetic differences in key host factors contribute significantly to susceptibility of *Nf* infection. To test this, we utilized Quantitative Trait Loci (QTL) mapping and susceptibility phenotypes of 45 progeny and 8 founder lines from the Collaborative Cross mouse series, a model of genetic diversity that represents over 90% of the common genetic variations found in mice. Susceptibility was quantified as mean survival time in days post-infection, as well as amoebae burden measured by levels of amoebae derived RNA in urine and plasma samples. QTL analysis of survival time revealed one significant QTL and two suggestive QTL, each with high priority candidate genes suitable for further investigation and confirmation of their effect on host susceptibility.

P46. The role of putative ATP synthase subunits during sexual conversion of malaria parasites

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The human malaria parasite, *Plasmodium falciparum*, undergoes energetic changes as it moves from the human bloodstream to the mosquito vector. It is hypothesized to shift from dependence on glycolysis to relying on oxidative phosphorylation (OXPHOS) to meet its energetic needs within the mosquito host. This metabolic change is accompanied by the formation of tubular cristae, invaginations to the inner mitochondrial membrane that may be associated with increased metabolic flux. In the apicomplexan parasite *Toxoplasma gondii,* cristae morphology is maintained by interactions between phylum-specific subunits of ATP synthase which are partially conserved, but uncharacterized in *Plasmodium.* Reverse genetic studies revealed that knocking out one subunit, ATPTG11 was associated with a loss of *T. gondii* virulence *in vivo*. RNA-sequencing data indicates that transcription of *P. falciparum* orthologues of *T.gondii* ATP synthase subunits are significantly upregulated as malaria parasites undergoing sexual conversion to produce gametocytes, their mosquito-transmissible form. Taken with date from our lab showing that *P. falciparum* is vulnerable to ATPS inhibition during mosquito infection, we hypothesize that mitochondrial cristae formation is 1) essential for the transmission of *P. falciparum* through the mosquito and 2) as in *T. gondii,* facilitated by polymerization of ATPS via interactions with these apicomplexan-specific subunits. Here we present preliminary data examining expression patterns of target genes of interest during sexual convergence, and initial work towards generating *Plasmodium* parasites expressing target genes under inducible/conditional knockdown control.

P47. Characterization of the protein phosphatase calcineurin (CN) network in *Toxoplasma gondii*

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Calcium (Ca^{2+}) signaling is a universal regulator of diverse cellular processes in eukaryotic cells. One key Ca^{2+} responsive factor is calcineurin, a conserved serine/threonine phosphatase that links Ca^{2+} signaling to dephosphorylation events to regulate a wide variety of cellular processes across different organisms, including mammals, fungi, and parasites. In yeast, calcineurin is required for stress response, virulence, and pathogenesis, while in mammals it plays an important role in the nervous, cardiovascular, and immune systems. In the apicomplexan parasite *Toxoplasma gondii,* Ca^{2+} signaling is essential for motility, host cell invasion and egress, key processes that are essential for its virulence. *T. gondii* encodes a conserved calcineurin complex essential for its lytic cycle. However, most of what is known about calcineurin comes from yeast and mammalian systems, and its role and molecular mechanism, including its substrates, remain poorly understood in apicomplexan parasites. Here, we characterize the calcineurin complex, define its role in calcium signaling, and identify its network of interacting partners in *T. gondii*. CRISPR/Cas9-mediated knockdown of calcineurin in *T. gondii* results in reduced cytosolic calcium levels and decreased calcium entry, suggesting a role in calcium homeostasis. Using co-immunoprecipitation with crosslinking mass spectrometry under Ca^{2+} and EGTA conditions identified candidate interactors involved in invasion processes, calcium signaling, metabolism, and cytoskeletal organization, suggesting that calcineurin regulates multiple pathways. Additionally, pharmacological inhibition of calcineurin using cyclosporin A (CsA) and derived analogs showed inhibition against tachyzoites, with dhCsA showing the strongest inhibitory effect (average EC_{50} ~0.53 μM), supporting the functional importance of calcineurin activity in the parasite.

P48. Developing transgenic systems for studying relapsing malaria parasite biology

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Understanding the molecular features of *Plasmodium vivax (Pv*) dormant liver stages is essential for developing new therapies to prevent disease and reduce transmission. However, the lack of genetic tools for *Pv* has limited progress in this area. Here, we use a closely-related parasite *P. cynomolgi* (*Pcy)* to test whether the S1 locus is dispensable throughout the life-cycle with the goal of inserting reporter constructs into this locus. *Pcy* M/B strain parasites were isolated from an infected macaque and transfected with a linearized DNA construct that would knock out the S1 loci with a drug selection cassette and firefly luciferase. After transfection, multiple rounds of pyrimethamine treatments were performed, and eventually recrudescent parasites were recovered that had high luciferase signal. Luciferase was detected throughout the sporozoite stage but was lost after passaging into new animals. PCR showed that the S1 locus was successfully disrupted in the blood stages, but the population of mutants was small, indicating that pyrimethamine resistant, wild type parasites emerge quickly. These data suggest the S1 locus may be dispensable in *Pcy* but future studies must optimize the pyrimethamine treatment regimen to obtain homogenous populations of transgenic parasites.

P49. RNA-seq analysis of gene regulation during early encystation in *Entamoeba histolytica*

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Entamoeba histolytica, a human parasite responsible for approximately 100 million cases of amoebiasis annually, transmits the disease through durable cysts. Encystation, the conversion of the growing trophozoite to infectious cyst, can be induced in laboratory conditions via glucose starvation and is significantly accelerated by acetate, a short-chain fatty acid abundant in the human colon. In contrast, acetate inhibits encystation of the reptile pathogen *Entamoeba invadens*, which was previously the primary model for studying encystation until our lab developed reproducible encystation of *E. histolytica*. We used RNA-seq analysis to investigate transcriptome changes in early encystation in *E. histolytica*, before formation of the chitin cell wall. We compared gene expression levels during standard trophozoite growth on glucose medium to 24-, 36-, and 48-hr encystation in basal medium lacking added glucose (standard encystation) as well as 24-hr encystation in basal medium containing acetate (rapid encystation). An overview of our results is presented here.

P50. Automated detection and quantification of *Plasmodium cynomolgi* with the Sysmex XN-30 hematology analyzer

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The automated hematology analyzer XN-30 from Sysmex was developed to detect malaria parasitemia, parasite staging, and hematological parameters in human blood from malaria endemic regions. Although optimized for *ex vivo* detection of *P. vivax* and *P. falciparum* in patient samples, the XN-30 can be used to detect and quantify rodent malaria parasites and *P. falciparum* from culture. Nonhuman primate models of malaria are used to study the basic biology of malaria parasites in addition to applied research in drug discovery and vaccine development. These studies traditionally rely on microscopy, which is time and labor intensive and can be biased even with expert malaria microscopists. Whether the Sysmex XN-30 analyzer could address these limitations of microscopy with nonhuman primate samples is unknown. This study aimed to test the hypothesis that the Sysmex XN-30 analyzer could be used to detect and quantify *P. cynomolgi* from *ex vivo* and *in vitro* samples. *Ex vivo* blood samples from Rhesus or Japanese macaques infected with *P. cynomolgi* or *in vitro* culture samples were measured by XN30 and microscopy for total parasitemia and parasite differentials. Using microscopy as the standard, sensitivity was measured for the XN-30, and statistical correlations were calculated for parasitemia and staging. Sensitivity for macaques was 10/ul by microscopy and 30-50/ul on the XN-30, depending on the mode selected. Total parasitemia was significantly correlated with microscopy results. Parasite staging was significantly correlated for rings and trophozoites but not for gametocytes and schizonts, likely due to low frequencies of these stages and difficulty in morphologically identifying gametocytes. Interestingly, some macaques were flagged at baseline as suspect scattergrams, associated with potential hemoglobinopathies. Overall, the XN-30 is a suitable alternative to microscopy for *P. cynomolgi* samples from macaques or culture and will reduce personnel time and microscopy bias when performing *P. cynomolgi* studies.

P51. Chemoproteomics-driven target discovery for the novel antimalarial PRC1910

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Malaria is a devastating disease that disproportionately impacts children in sub-Saharan Africa. The global emergence of resistance to frontline antimalarials highlights the urgent need for new compounds with novel mechanisms of action and high barriers to resistance. Our group identified PRC1910 as a lead antimalarial that is potent against the asexual erythrocytic life cycle stages of *Plasmodium falciparum*, exhibiting sub-100 nM potency, rapid ring-stage killing comparable to artemisinin, excellent oral pharmacokinetics, single-dose curative efficacy in mice, and a high barrier to resistance *in vitro*. In addition, PRC1910 also kills artemisinin-induced dormant parasites. Despite being an advanced lead, the molecular target of PRC1910 remains unknown. To identify its potential molecular target(s), we developed a photoaffinity probe, PRC2545, to use in our established chemoproteomics competition assay. We have selected three essential potential molecular targets of PRC1910 to prioritize for further validation and target deconvolution. Orthogonal validation approaches such as measuring direct drug-target binding through thermal shift stability assays and the generation of inducible knockdown and knockout strains to assess shifts in drug sensitivity will confirm direct target engagement. Defining the molecular target of PRC1910 will provide insight into a novel resistance-refractory antimalarial scaffold and may uncover a previously unknown drug target.

P52. Optimizing treatment for primary amoebic meningoencephalitis caused by *Naegleria fowleri*

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Naegleria fowleri is a pathogenic free-living amoeba that causes primary amoebic meningoencephalitis (PAM). PAM is a rare but rapidly progressing central nervous system infection with a fatality rate exceeding 97% and a median time from symptom onset to death of approximately five days. Treatment currently relies on a multidrug regimen comprising up to seven antimicrobial, antifungal, and antiparasitic agents, largely selected empirically based on either survivor data or activity against other pathogens, rather than on systematic evaluation against *N. fowleri*. The current standard of care, informed by the most recent documented survivor in the United States, includes amphotericin B, miltefosine, azithromycin, fluconazole, rifampin, dexamethasone, and nitroxoline. The goal of this study is to characterize the drug-drug interaction profiles of compounds included in this regimen. Using *in vitro* viability-based assays and two-drug checkerboard combination testing, we quantify individual drug toxicity against *N. fowleri* trophozoites and assess combinatorial effects to identify toxic, additive, or synergistic interactions that may enhance or compromise therapeutic safety.

P53. Investigating the role of CDC42 in early encystation and stress response in *Entamoeba histolytica*

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Entamoeba histolytica is a parasitic protozoan responsible for amoebiasis, a disease affecting nearly 100 million people annually and causing approximately 100,000 deaths worldwide. The parasite exists in two distinct forms: the actively proliferating trophozoite and the infectious cyst. Cysts are ingested and excyst to the trophozoite form in the small intestine. Trophozoites then migrate to and colonize the large intestine, where they can differentiate back into cysts. Encystation in *E. histolytica* is induced by glucose starvation and results in a mature, tetranucleate cyst protected by a chitin cell wall. We used RNAseq analysis to identify genes that are upregulated early in encystation, before formation of the chitin cell wall. EHI_154270 encodes cell division control protein 42 (CDC42) in *E. histolytica* and is upregulated significantly by 24 hours into encystation. Our preliminary results indicate that a *cdc42* RNAi knockdown strain is unable to encyst. We have shown that *cdc42* expression is also upregulated by heat shock and serum stress, suggesting this gene may play a role in a general stress response as well as encystation.

P54. Targeting tissue cysts in chronic *Toxoplasma gondii*: Mitochondrial inhibition with 4(*1H*)-Quinolones

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Current clinical therapies for *T. gondii* are effective against the acute tachyzoite stage but have limited activity against bradyzoites in tissue cysts, underscoring the need for treatments targeting chronic infection. The mitochondrion is a validated target, and the clinically used antiparasitic, Atovaquone, inhibits the electron transport chain (ETC) through inhibition of the coenzyme Q:cytochrome c oxidoreductase (cytochrome bc_1) complex. 4(*1H*)-quinolones have been validated as antimalarials targeting the cytochrome bc_1 complex. Here, we evaluate two classes of 4(*1H*)-quinolones, Tetrahydroacridinones (THAs) and Quinolines (PEQs), and demonstrate picomolar to nanomolar potency against tachyzoites. To enable testing of larger numbers of compounds directly against chronic stages, we established a scalable, quantitative ex vivo screening platform using in vivo-derived cysts, allowing systematic evaluation of 160 compounds for dose response. Multiple PEQ and THA analogs retained subnanomolar EC_{50} values against bradyzoites, demonstrating that potent ETC inhibition extends to the chronic stage. Mechanistically, lead compounds significantly reduced cytochrome c reduction in parasite lysates and disrupted mitochondrial membrane potential, consistent with inhibition of mitochondrial ETC function. Pharmacokinetic analysis of the lead compounds supports their observed *in vitro* and *ex vivo* efficacy and suitability for *in vivo* evaluation. Two lead compounds, provided complete protection in an acute mouse model at doses as low as 1 mg/kg/day. Both compounds reduced cyst size *in vitro* and exhibited subnanomolar EC_{50}s against *in vivo*-derived bradyzoites. Preliminary studies in chronically infected mice further indicate a reduction in overall cyst burden. Together, these findings integrate a scalable bradyzoite screening platform with potent mitochondrial inhibitors to establish 4(*1H*)-quinolones as strong translational candidates for the treatment of chronic toxoplasmosis.

P55. Investigating the role of *Adenyl Cyclase beta (ACβ)* and *Armadillo Interacting Protein (AIP)* in biogenesis of *Plasmodium* rhoptries

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Malaria is one of the leading causes of death in the world, which accounts for up to 610,000 deaths, around which 95% of them are caused by Plasmodium falciparum. The clinical manifestation of this disease occurs when the parasite multiplies inside, egresses, and invades a new red blood cell (RBC). The invasion mechanism of a merozoite begins with the secretion of a pair of club-shaped secretory organelles called rhoptries. In a previous work, we showed that Rhoptry Neck Protein, RON11, is essential for the biogenesis of the new rhoptry in merozoites. Conditional knockdown of RON11 ($RON11^{cKD}$) led to one rhoptry per merozoite that were able to secrete their contents into the host RBC. However, these single-rhoptry merozoites failed to invade the host RBC. To determine why these single rhoptry-merozoites failed to invade, we used comparative whole cell proteomics to identify proteins that were absent in the single-rhoptry $RON11^{cKD}$ merozoites. The quantitative proteomics identified two proteins, *Adenyl Cyclase beta* (*ACβ*) and *Armadillo Interacting Protein (AIP)*, that were absent in single-rhoptry merozoites. Both ACβ and AIP have been shown to be required for merozoite invasion into RBCs. Conditional knockdown of ACβ and AIP phenocopies the conditional knockdown of RON11. However, their roles in rhoptry biogenesis as well as their interaction, if any, with RON11 remains unknown. We have tagged both ACβ and AIP in $RON11^{cKD}$ parasites. Using ultra-structure expansion microscopy and biochemical tools, we will investigate the effect of RON11 knockdown on the localization as well as expression of ACβ and AIP. Future studies employing conditional knockdowns of ACβ and AIP will investigate their roles in rhoptry biogenesis.

P56. Determining the mechanism of antigen-presenting cell recruitment during liver-stage malaria

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Plasmodium parasites have to undergo development in the liver before progressing to the potentially lethal blood stage of malaria. Therefore, the liver stage of malaria represents an ideal target for vaccine development. Vaccination with attenuated *Plasmodium* sporozoites, such as radiation-attenuated sporozoites (RAS), induces robust CD8 T cell responses that target and clear the infected hepatocytes during subsequent infections, impeding the onset of blood-stage malaria. RAS establish abortive infections in hepatocytes, triggering pyroptotic cell death and release of *Plasmodium* antigens into the extracellular environment of the liver. $CSF1R^+$ antigen-presenting cells (APCs) recruited to the liver capture these antigens and prime CD8 T cell response in the liver-draining lymph nodes. The strength of $CSF1R^+$ APC recruitment is a key determinant of the magnitude of CD8 T cell responses and RAS-mediated protection against malaria. However, the mechanism of such APC recruitment remains unknown. We show that the liver-resident macrophages, Kupffer cells (KC), acquire inflammasome complexes released from pyroptotic hepatocytes and drive adoptive-inflammasome activation (AIA), generating mature IL-1β. This IL-1β in turn induces CCL2 and CCL7 in specific liver non-parenchymal cells, driving CCR2-mediated recruitment of $CSF1R^+$ APC to the liver from circulation. In addition to advancing our current understanding of immune cell dynamics in the liver, we expect this work to inform the generation of more immunogenic malaria vaccines.

P57. Harnessing anti-α-Gal immunity: A cross-species virus-like particle vaccine platform against cutaneous leishmaniasis

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Leishmaniasis infects over one billion people globally and kills roughly 30,000 each year from its most severe form, visceral leishmaniasis — yet no licensed human vaccine exists. Current drugs are toxic, require hospitalization, and are failing as resistance spreads. A fundamentally new approach is needed, and humans may already carry the key to one. Every human naturally produces high-titer antibodies against α-galactosyl (α-Gal) epitopes — carbohydrate structures present on *Leishmania* parasites but absent from human cells due to an ancient evolutionary gene deletion. We engineered Qβ bacteriophage virus-like particles (VLPs) displaying α-Gal epitopes to redirect this pre-existing immune arsenal directly against the parasite, bypassing the need for de novo immune priming and eliminating the need for adjuvants. Using α-1,3-galactosyltransferase knockout C57BL/6 mice — which mirror the human immunological context — we vaccinated animals with Qβ-αGal VLPs, empty Qβ controls, PBS, or left them naïve, then challenged them with metacyclic promastigotes of *Leishmania braziliensis* and *L. amazonensis* separately, monitoring disease progression over 18 weeks. Qβ-αGal–vaccinated mice maintained near-normal footpad thickness throughout the study, while all control groups developed progressive swelling and tissue destruction. Electron microscopy and histology confirmed dramatically reduced amastigote burden and preserved tissue architecture in vaccinated animals. Critically, protection held across both parasite species, demonstrating cross-species vaccine efficacy — a rarely achieved benchmark in leishmaniasis research. Serologic analysis confirmed robust anti-α-Gal IgG induction exclusive to the Qβ-αGal group, and ongoing cytokine profiling (IFN-γ, TNF-α, IL-4) supports activation of macrophage-mediated parasite killing. These results establish α-Gal–decorated Qβ VLPs as a cross-species, adjuvant-free vaccine platform that arrests cutaneous leishmaniasis progression at the cellular level. By weaponizing an immune response humans already have, this approach offers a scalable, cost-effective path toward the first licensed human leishmaniasis vaccine.