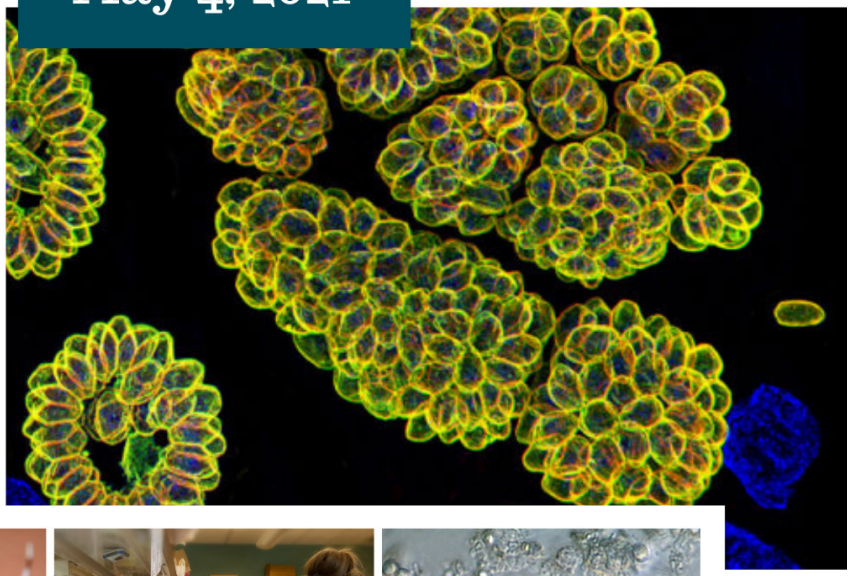
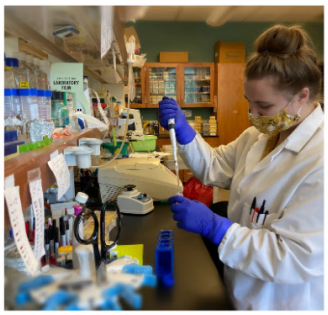
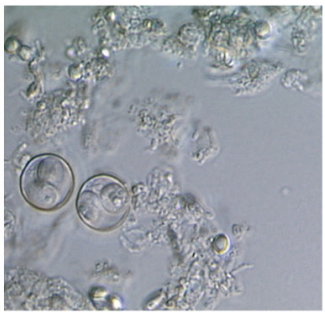
Annual Molecular Parasitology & Vector Biology Symposium

May 4, 2021







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

500 D.W. Brooks Drive, Athens, GA 30602

ctegd.uga.edu

Organizational support provided by:

Dennis Kyle, Director
David Peterson & Vasant Muralidharan, Symposium Advisers
Meeting Coordinators:
Annie Curry, David Dowless, Donna Huber, Susan Clayton

http://ctegd.uga.edu
https://www. facebook.com/CTEGD
https://twitter.com/CTEGD

To receive future announcements about the symposium, please sign up for our mailing list:
http://eepurl.com/81ZAX

Program

8:40 AM Poster Set-up

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

SESSION 1 — Alejandra Villegas & Logan Crowe

9:10 AM **Jennifer Dumaine**, Department of Pathology, University of Pennsylvania
Cryptosporidium parvum Exports Proteins into the Cytoplasm of the Epithelial Host Cell

9:30 AM **Amrita Sharma**, Department of Cellular Biology, University of Georgia
Modes of Action of NEU-4438, an Anti-Trypanosome Lead Drug

9:50 AM **INTRODUCTION OF THE EARLY CAREER SPEAKER: Dennis Kyle**

9:55 AM **Emily Derbyshire**, Assistant Professor of Chemistry, Duke University
Interdisciplinary Approaches to Reveal *Plasmodium* Parasite Vulnerabilities

10:45 AM **POSTER SESSION I**

SESSION 2 — Emma Troth & Edwin Pierre Louis

11:35 AM **Nupur Kittur**, Center for Tropical & Emerging Global Diseases, University of Georgia
ClinEpiDB.org: Lowering the Barrier for Exploratory Data Analysis of Global Health Studies

11:55 PM **Gopinath Venugopal**, Department of Microbiology & Immunology, University of Arkansas for Medical Sciences
mTOR Mediated Immune Cell Migration Leads to Immunopathology During *Leishmania major* Infection

12:15 PM **Watcharatip Dedkhad**, Center for Tropical & Emerging Global Diseases, UGA
Regulation of the Egress Proteolytic Cascade in Malaria Parasites

12:35 PM **LUNCH BREAK**

SESSION 3 — Megna Tiwari & Alona Botnar

1:05 PM **Tamanash Bhattacharya**, Department of Biology, Indiana University
Exploring the Impact of Endosymbiont-induced Viral RNA Methylation on Arbovirus Dissemination and Transmission

1:25 PM **Abigail Calixto**, CTEGD and Dept. of Microbiology, University of Georgia
A Putative Calcium Proton Exchanger of *Toxoplasma gondii*

1:45 PM **Nathan Chasen**, Center for Tropical & Emerging Global Diseases, UGA
A *Trypanosoma cruzi* Myosin Associated Regulatory Protein is Essential for Endocytosis via the Cytostome-Cytopharynx Complex

2:05 PM **POSTER SESSION II**

SESSION 4 — Melissa Sleda & Nathan Chasen

2:55 PM **Natasha Perumal**, CTEGD and Dept. of Cellular Biology, University of Georgia
cGAS-STING Pathway Activation During *Trypanosoma cruzi* Infection Leads to Tissue-dependent Parasite Control

3:15 PM **Emily Ebel**, Stanford University
Recurrent Duplication and Structural Mutation Generate Novel Antigenic Genes in the Malaria parasite *P. falciparum*

3:35 PM **INTRODUCTION OF THE KEYNOTE SPEAKER: Vasant Muralidharan**

3:40 PM **Daniel Goldberg**, Distinguished Professor of Infectious Diseases at Washington University School of Medicine in St. Louis
Malaria Parasite Plasmepsins: Not Just Plain Old Degradative Pepsins

4:40 PM ADJORN

Poster Presentations

To locate the poster: The first number of the Poster Number corresponds to the floor and the second number corresponds to table number

P4-01 **J. Antonio Baeza,** Institute of Parasitology, Slovak Academy of Sciences
A First Look at the 'Repeatome' of *Benedenia humboldti*, a Major Pathogen in Yellowtail Aquaculture: Repetitive Element Characterization, Nuclear RNA Operon Assembly, and Microsatellite Discovery

P4-02 **Nilmar Silvio Moretti**, Laboratory of Molecular Biology of Pathogens, Federal University of São Paulo
Protein Acetylation as Key Regulator of *Leishmania* Parasite Stage Differentiation

P4-03 **Sevan N. Alwan**, Departments of Biochemistry and Structural Biology, University of Texas Health Science Center
Identification of Novel Therapeutics Against Human Schistosomiasis

P4-04 **Jaquan Harley**, Albright College
The *Vorticella convallaria* Contractile Vacuole

P4-05 **Maria Teresa González**, Instituto de Ciencias Naturales Alexander von Humboldt, Universidad de Antofagasta
Mitophylogenomics Reveals a New Cryptic Species of *Benedenia* Diesing, 1858 (Monogenea: Capsalidae), a Major Pathogen Infecting the Yellowtail Kingfish *Seriola lalandi* Valenciennes, 1833 in the South-east Pacific

P4-06 **Timothy J. Nessel**, Department of Biomedical Sciences, Iowa State University
A TurboID-based Compartmental Sensor for *Plasmodium falciparum*

P4-07 **Dulani Ruwanika K. Pathirage**, Department of Parasitology, University of Colombo
Molecular Markers COI and ITS2 Reveals the Presence of Gene Flow of Sand Flies in Sri Lanka

P4-08 **Renan Weege Achjian**, Universidade de São Paulo
Design and Implementation of a Metabolic Model for the Proline-Glutamate Pathway in *Trypanosoma cruzi*

P4-09 **Bryan E. Abuchery**, Department of Chemical and Biological Sciences, São Paulo State University
Generation of *T. cruzi* Lineages Knock-out for the Kinase IP6K and Evaluation of Homologous Recombination Repair Capacity

P4-10 **John Soghigian**, North Carolina State University
Island Hopper to Globe Trotter: Evidence for the Origins of *Aedes aegypti* in the Southwestern Indian Ocean

P4-11 **A. M. Murillo**, Department of Parasitology, University of São Paulo
The Cysteine Synthase Enzyme Plays an Important Role in the Biological Cycle of *Trypanosoma cruzi*

P4-12 **Susanne Warrenfeltz**, Center for Tropical and Emerging Global Diseases, UGA
VEuPathDB: Comprehensive Informatics Support for Your Research Needs

P5-01 **Azhar Ahmad**, MCARS, Jamia Millia Islamia
Role of AGC Family Kinases in the Endocytic Processes of the Parasite *Entamoeba histolytica*

P5-02 **Victoria Mendiola**, University of Georgia
Novel Mono- and Bis-peroxide Bridged Artemisinin Dimers Show Potency Against Artemisinin-resistant *Plasmodium falciparum*

P5-03 **Arthur de O. Passos**, Dept. of Chemical and Biological Sciences, São Paulo State University
Preliminary Study of the Inositol Pyrophosphates Metabolic Pathway in Kinetoplastids: An Evolutionary Perspective

P5-04 **L. Marchese**, Department of Parasitology, University of São Paulo
Characterization of Asparagine Transport and Consumption in *Trypanosoma cruzi*

P5-05 **Gabriel A. Tafur-Gomez**, Universidad de Ciencias Aplicadas y Ambientales
Rhipicephalus sanguineus s.l Transcriptome Analysis of Questing Larvae and Engorged Larvae from Four Different Ecological Systems of Colombia

P5-06 **FPL Leite**, Center for Technological Development, UFPel/Brazil
Evaluation of the Immunomodulation Mechanism of the Th2 Response in Mice Experimentally Infected with *Toxocara canis*

P5-07 **Sabrina Marsiccobetre**, Department of Parasitology, University of São Paulo
Elucidating the Importance of Branched Chain Amino Acids Catabolic Pathway Applying CRISPR-Cas9 Technology in *Trypanosoma cruzi*

P5-08 **Emma Troth**, CTEGD and Dept. of Infectious Diseases, University of Georgia
A Novel Cytopathogenicity Assay Yields New Drug Intervention Strategies Against *Naegleria fowleri*, the Brain-eating Amoeba

P5-09 **Micaele Quintana de Moura**, Parasitology Laboratory, Universidade Federal do Rio Grande
Protective Effect of *Lactobacillus rhamnosus* (ATCC 7469) on the Intestinal Mucosa of Mice Experimentally Infected with *Toxocara canis*

P5-10 **Benjamin Hoffman**, Department of Cellular Biology, University of Georgia
A Casein Kinase Regulates DNA Synthesis and Basal Body Biogenesis in *Trypanosoma brucei*

P5-11 **Débora Carvalho Rodrigues**, Parasitology Laboratory, Universidade Federal do Rio Grande
In silico Analysis of Naphthoxyrans Molecules with Activity Against *Toxocara canis*

P5-12 **Miryam A. Hortua Triana**, Center for Tropical and Emerging Global Diseases, UGA
Characterization of an Endoplasmic Reticulum-Resident Calcium-binding Protein in *Toxoplasma gondii*

P6-01 **A. Cassiopeia Russell**, CTEGD and Dept. of Infectious Disease, University of Georgia
Characterization of the Extracellular Vesicles Secreted by *Naegleria fowleri*

P6-02 **Justin Wiedeman**, CTEGD and Dept. of Cellular, UGA
A Solution to the Challenge of Deciphering Protein Kinase Pathways in the Evolutionarily Divergent Microbe *Trypanosoma brucei*

P6-03 **Luciana F C Avila**, Postgraduate Program in Health Sciences, FURG/ Brazil
Saccharomyces boulardii Stimulates IL-17 in Mice Infected with *Toxocara canis*

P6-04 **Madelaine Usey**, CTEGD and Dept. of Cellular Biology, University of Georgia
Identifying Apicomplexan ATP Synthase Regulators Using a Förster Resonance Energy Transfer (FRET) Sensor

P6-05 **Sabrina Elizabeth Cline**, CTEGD and Dept. of Cellular Biology, University of Georgia
Elucidating the Role of Inositol-tetrakisphosphate 1-kinase in *Trypanosoma cruzi*

P6-06 **Melanie Key**, Department of Biological Sciences, Clemson University
Toxoplasma gondii Possesses a Functional Coproporphyrinogen Dehydrogenase for Its Heme Production

P6-07 **Yete G. Ferri**, Department of Chemical and Biological Sciences, São Paulo State University
Identification of the Target Proteins of Inositol Pyrophosphates in *Leishmania*: A Preliminary Study

P6-08 **Haziqah-Rashid**, Department of Evolution, Ecology and Behaviour, University of Liverpool
Do Arboviruses Manipulate Their Mosquito Vector's Thermal Preference to Increase Transmission?

P6-09 **Manuel A. Fierro**, Department of Biomedical Sciences, Iowa State University
IDentifying the Earliest Factors Required for Host Cell Subversion by *Plasmodium falciparum*

P6-10 **Mayara S. Bertolini**, CTEGD and Dept. of Cellular Biology, University of Georgia
Exploring the Relation Between MICU1 and MICU2 in *Trypanosoma cruzi* by Generation of TcMICU1-KO/TcMICU2-KO Cells

P6-11 **Edwin Pierre Louis**, CTEGD and Dept. of Cellular Biology, Univeristy of Georgia
Characterization of an Essential Golgi Localized Secreted Effector Binding Protein of *Toxoplasma gondii*

P6-12 **Logan P. Crowe**, Center for Tropical and Emerging Global Diseases, University of Georgia
Protein Polyphosphorylation and Aggregation by Inorganic Polyphosphate in Trypanosomes

P7-01 **L. Brock Thornton**, Department of Biological Sciences, Clemson University
Investigating *T. gondii* Vacuolar Compartment/Plant-like Vacuole Physiology via the Ratiometric GFP Reporter pHluorin2

P7-02 **Jillian Milanes**, EPIC and Dept. of Genetics and Biochemistry, Clemson University
Development of Transfection Approaches for Use in *Naegleria fowleri*

P7-03 **Subash Godar**, EPIC and Department of Physics and Astronomy, Clemson University
Functional Analysis Shows that Outer Dynein Arm Light Chain-2 is Essential for Directional Flagellar Motility in *Trypanosoma brucei*

P7-04 **David Anaguano-Pillajo**, University of Georgia
Identifying Proteins Required for Export of Effectors to the *Plasmodium falciparum* Infected Erythrocyte

P7-05 **Elisabet Gas-Pascual**, CTEGD and Dept. of Biochemistry & Molecular Biology, UGA
The Role of Oxygen-dependent Glycosylation on SCF (Skp1-Cullin-1-Fbox) Regulation in *Toxoplasma gondii*

P7-06 **Ruby E. Harrison**, Department of Entomology, University of Georgia
Simultaneous Ingestion of Carbohydrates and Proteins Induces Continuous Oogenesis in Mosquitoes

P7-07 **Lui P. Suzuki-Williams**, CTEGD and Department of Biochemistry and Molecular Biology, UGA
Analysis of *P. falciparum* Field Isolates for Mutations in the Chloroquine Resistance Transporter and Kelch13 Propeller Genes

P7-08 **Pooja Rani Mina**, CIMAP
Are Plant Derived Adjuvants Providing a Path in Thwarting Emerging Drug Resistant Malaria

P7-09 **Megna Tiwari**, CTEGD and Department of Biochemistry & Molecular Biology, UGA
Does a Novel *Toxoplasma gondii* O-fucosyltransferase Modulate the Localization of Target Proteins?

P7-10 **Katherine Wentworth**, EPIC and Department of Biological Sciences, Clemson University
Generation of *Trypanosoma brucei* CRISPR/Cas9 Knockouts to Understand the Role of Tubulin Post Translational Modification

P7-11 **Ifeoluwa Kayode Fagbohun**, University of Lagos
Molecular and Metabolic Resistance Mechanisms in Multiple Insecticides Resistant *Culex quinquefasciatus* Population from Lagos, Nigeria

P7-12 **Huan He**, Department of Microbiology, Molecular Genetics & Immunology, University of Kansas School of Medicine
Mechanisms of *Borrelia* Surface Lipoprotein Translocation Through the Outer Membrane

P8-01 **Jigneshkumar Mochi**, Central University of Gujarat
Adenylosuccinate Lyase and Adenylosuccinate Synthetase, Key Enzymes for Purine Salvage Pathway in *Leishmania donovani*

P8-02 **Alejandra Villegas**, University of Georgia
An Essential Fringe-Like Protein in the *Plasmodium falciparum* Asexual Life Cycle

P8-03 **Justine C. Shiau**, CTEGD and Department of Infectious Diseases, University of Georgia
In vitro hepatocyte culture of field-derived *Plasmodium falciparum*

P8-04 **Jiayan Zhang**, Dept. of Microbiology, Immunology & Molecular Genetics, Molecular Biology Institute, and California NanoSystems Institute, UCLA
Structure of the Trypanosome Paraflagellar Rod and Insights into Non-Planar Motility of Eukaryotic Cells

P8-05 **Sunil Kumar Narwa**, Div. of Molecular Parasitology & Immunology, CSIR-Central Drug Research Institute
The Malarial Stearoyl-coa Desaturase is Essential Only for Parasite Late Liver Stage Development

P8-06 **Beatriz Cristina Dias de Oliveira**, Dept. of Chemical & Biological Sciences, São Paulo State University
Study of the Effects of Telomerase RNA (TER) Knockout in *Leishmania major*

P8-07 **Charlie Franck Alfred Compaoré**, Centre International of Recherche-Développement sur l'Elevage en Zone Subhumide, Unité de Recherche sur les Maladies à Vecteurs et Biodiversité, Université Nazi Boni, Unité de Formation et de Recherche Sciences et Techniques
Analytical Sensitivity of Loopamp and Quantitative Real-time PCR on Dried Blood Spots and Their Potential Role in Monitoring Human African Trypanosomiasis Elimination

P8-08 **Ronald Andanje**, Albright College
Developing a *Crithidia* Parasite CURE (Course Undergraduate Research Experience) for Biochemistry Lab Using Metabolically Labelled 1-13C-Glucose and 13C-NMR

P8-09 **Nicholas C. Mucci**, Department of Microbiology, University of Tennessee-Knoxville
Chemical Ecology of an Apex Predator Life Cycle

P8-10 **Camila Gomez**, Department of Biology, Georgia State University
Intracellular Calcium Transport in *Crithidia fasciculata* is Regulated by the Mitochondrial Calcium Uniporter

P8-11 **Naixin Zhang**, University of Florida
Identification and Characterization of *Leishmania* PI3K Class 2 (LdPI3KC2) that Localizes to the *Leishmania* Parasitophorous Vacuole at the Host Parasite Interface

P8-12 **Aline C. A. Moreira-Souza**, Institute of Biophysics Carlos Chagas Filho Federal University of Rio de Janeiro
Contribution of Purinergic Signaling During *Toxoplasma gondii* Infection

P9-01 **Mark E. Shiburah**, Biosciences Institute of Botucatu, São Paulo State University
Regulation of Telomere Length and Telomerase Activity During the *Leishmania amazonensis* Developmental Cycle and Population Replication

P9-02 **Tahir Hussain**, Iowa State University
EXP2 is Important for Intrahepatic Parasite Development During the *Plasmodium* Liver-stage

P9-03 **Daniel Velez-Ramirez**, Department of Microbiology, Immunology & Molecular Genetics, UCLA
cAMP-dependent Phosphorylation of Flagellum Matrix Proteins in *Trypanosoma brucei*

P9-04 **Chahinez Bouguerche**, Faculté des Sciences Biologiques, Université des Sciences et de la Technologie Houari Boumediene and Institut Systématique Évolution Biodiversité, Muséum National d'Histoire Naturelle
Description of a New Species, *Microcotyle visa* (Monogenea: Microcotylidae), a Gill Parasite of *Pagrus Caeruleostictus* (Teleostei: Sparidae) Using Integrative Taxonomy

P9-05 **Eliza Lupenza**, Department of Parasitology & Medical Entomology, Muhimbili University of Health & Allied Sciences
Lymphatic Filariasis Elimination Status: *Wuchereria bancrofti* Infections in Human Populations and Factors Affecting Continued Transmission after Seven Rounds of Mass Drug Administration in Masasi District, Tanzania

P9-06 **Chahinez Bouguerche**, Faculté des Sciences Biologiques, Université des Sciences et de la Technologie Houari Boumediene and Institut Systématique Évolution Biodiversité, Muséum National d'Histoire Naturelle
Tell Me What You Eat, I Will Tell You What You Are! A Study of a Hyperparasite *Cyclocotyla bellones* (Monogenea, Platyhelminthes) Using Integrative Taxonomy

P9-07 **Anna Gioseffi**, Microbiology and Cell Science, University of Florida
Extracellular Vesicles Released by *Leishmania donovani* Infected Macrophages Contain Parasite Molecules and May Contribute to Lesion Development and Immune Modulation

P9-08 **Melissa A. Sleda**, Department of Cellular Biology, University of Georgia
Targeting the Isoprenoid Pathway of the Apicomplexan Parasite *Toxoplasma gondii*

P9-09 **Tamar Feldman**, Stanford University
Plasmodium and the Bone Marrow: Uncovering Novel Host-parasite Interactions

P9-10 **Alona Botnar**, CTEGD and Department of Infectious Diseases, University of Georgia
Investigating the Role of Gibberellic Acid on Dihydroartemisinin-induced Dormant *Plasmodium falciparum*

P9-11 **Ayelen Lizarraga**, Instituto Tecnológico Chascomús, National Scientific and Technical Research Council-National University of San Martin
Adenine DNA Methylation, 3D Genome Organization, and Gene Expression in the Parasite *Trichomonas vaginalis*

Oral Presentations

Cryptosporidium parvum Exports Proteins into the Cytoplasm of the Epithelial Host Cell

Jennifer Dumaine[1], Adam Sateriale[2], Alexis Gibson[1], Amita Reddy[3], Jaclyn Riley[1], Emma Hunter[1], Justin Boddey[4], Brad Sleebs[4], Boris Striepen[1]

Department of Pathology, University of Pennsylvania, Philadelphia, PA[1]. The Francis Crick Institute, London, UK[2]. Department of Cellular Biology, University of Georgia, Athens, GA[3]. Walter and Eliza Hall Institute of Medical Research, Parkville VIC, Australia[4]

The parasite *Cryptosporidium* is responsible for diarrheal disease in young children causing death, malnutrition, and stunted growth. *Cryptosporidium* invades enterocytes, where it develops in a specialized intracellular but extracytoplasmic niche. Infected cells exhibit profound changes in cellular morphology, physiology and transcriptional activity in response to the parasite. Host-targeted parasite effectors have been hypothesized to be driving agents of these changes; however, no such proteins have been identified. Using CRISPR/Cas9 driven homologous recombination, we epitope tagged the endogenous loci of proteins selected from highly variable regions of the *C. parvum* genome and identified MEDLE2 as the first host targeted protein. MEDLE2 localizes to the cytoplasm of infected host cells throughout the course of infection. The protein is not apparent in sporozoites but is detectable in the host cell cytoplasm as early as 6 hours post infection, suggesting a rhoptry-independent delivery system assembled by the trophozoite after invasion. The N terminus of MEDLE2 contains a host targeting motif that is processed during export, highlighting the potential for mechanistic export in *Cryptosporidium*. MEDLE2 is a multicopy gene belonging to an expanded family of predicted secreted proteins. We have demonstrated export of other members of the MEDLE gene family, albeit in less abundance than observed for MEDLE2. Additionally, we use genetic modification and chemical inhibition to map the cellular and molecular requirements for export and define a pathway that traffics proteins to the host cell in all lifestages of *Cryptosporidium*. The discovery of host targeted effectors opens the door to mechanistic understanding of host-parasite interaction in this important infection.

Modes of Action of NEU-4438, an Anti-Trypanosome Lead Drug

Amrita Sharma[1]; Michael Cipriano[4], Lori Ferrins[3]; Michael Pollastri[3]; Stephen Hajduk[4], Kojo Mensa-Wilmot[1,2]

[1]University of Georgia, Department of Cellular Biology, Athens, GA 30602 USA [2]Kennesaw State University, Department of Molecular and Cellular Biology, Kennesaw, GA 30144 USA [3]Northeastern University, Department of Chemistry & Chemical Biology, Boston, MA 02115 USA [4]University of Georgia, Department of Biochemistry and Molecular Biology, Athens, GA 30602 USA

Trypanosoma brucei causes the disease, Human African Trypanosomiasis (HAT). Current drugs for treatment of HAT have undesirable properties, so there is a need to identify safer alternatives; SCYX-7158 (acoziborole) is in clinical trials. Since *T. brucei* is a eukaryote microbe chances of resistance emerging against any drug is substantial, dictating a necessity for sustained effort to find new leads for anti-HAT drug development. We showed that NEU-4438 is an orally-bioavailable lead for HAT. To assess its potential for further development as a drug, we compared NEU-4438's modes of action to those of SCYX-7158, using a multi-disciplinary approach involving drug perturbation, hypothesis-generating proteomics, and chemical biology. Following 6 h of treating trypanosomes with pharmacologically equivalent concentrations (i.e., delayed cidal concentrations), NEU-4438 and SCYX-7158 selectively reduced steady-state levels of 68 and 92 unique proteins, respectively. Pursuing hypotheses formulated from analysis of the drug-perturbed proteomes, NEU-4438 interfered with DNA biosynthesis, and prevented trypanosome entry into S-phase. In contrast, SCYX-7158 inhibited mitosis, and blocked protein translation. Finally, SCYX-7158 prevented endocytosis of both haptoglobin-hemoglobin and transferrin, whereas NEU-448 only perturbed uptake of transferrin. We surmise that SCYX-7158 and NEU-4438 have different modes of molecular action in the trypanosome, implying that development of NEU-4438 into a drug is justifiable. Our unexpected finding of SCYX-7158 inhibition of endocytosis of proteins with GPI-anchored receptors highlights a need for new studies to fully understand drug's molecular mechanisms of action.

ClinEpiDB.org: Lowering the Barrier for Exploratory Data Analysis of Global Health Studies

Nupur Kittur[1], Danica Helb[2], David Roos[2], Steph Wever Schulman[2], Christian Stoeckert[2], Sheena Shah Tomko[2], and Jessica Kissinger[1,3] for the VEuPathDB Team

[1]CTEGD, University of Georgia, Athens, GA [2]University of Pennsylvania, Philadelphia, PA [3]Institute of Bioinformatics, University of Georgia, Athens, GA

The clinical epidemiological database ClinEpiDB.org is the newest addition to the VEuPathDB family of eukaryotic pathogen, vector and host informatics resources. Although journals and funders often require that data be made publicly available, there are significant technical, practical, and confidentiality barriers. ClinEpiDB was developed three years ago as an open-access platform to facilitate access to de-identified data from large, high-quality global health studies. This resource not only meets, but surpasses, the requirements of journals and funders by integrating study data with standardized ontologies to make data more easily reusable. ClinEpiDB hosts data from over 1.1 million participants from 29 studies representing three major domains - malaria; neglected tropical diseases; and maternal, newborn & child health. These include nine observational and experimental studies on operational research in schistosomiasis control and elimination, and 12 studies elucidating interactions between malaria parasites, mosquitos, human hosts, and public health interventions. Linked study pages provide context and study-related documentation such as consent forms, case report forms, and codebooks. The ClinEpiDB platform lowers barriers to data use with an intuitive point-and-click interface that allows users to query the data and explore and visualize associations between variables within the browser. This resource will continue to grow in the coming year with a new exploratory data analysis workspace, enhanced visualization tools, significant user outreach and education, and integration of new datasets. By facilitating appropriate data access and reusability, we hope that platforms like ClinEpiDB will be as transformational in expediting epidemiological discovery and translational impact over the next 20 years as genomics data resources have proved to be for lab-based research and translation over the past 20 years.

mTOR Mediated Immune Cell Migration Leads to Immunopathology During *Leishmania major* Infection

Gopinath Venugopal[1], Jordan Bird[2,3], Hayden Roys[1], Charity Washam[2,3], Anne Bowlin[1], Manjunath Bettadapura[1,4], Stephanie Byrum[2,3], and Tiffany Weinkopff[1]

[1]Department of Microbiology and Immunology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, 72205; [2]Department of Biochemistry and Molecular Biology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, 72205; [3]Arkansas Children's Research Institute, Little Rock, AR, 72202; [4]Department of Biology, University of Arkansas at Little Rock, Little Rock, AR, 72204

Leishmania species are the causative agents of cutaneous leishmaniasis, a parasitic disease characterized by the presence of skin lesions. During infection both the parasites and the inflammatory infiltrate contribute to disease. Bulk transcriptomic RNASeq analysis revealed pathways involved in leukocyte trans-endothelial migration, cell adhesion, and chemokine signaling were enhanced in leishmaniasis. In general, immune cell migration is mediated by blood endothelial cells (BECs) binding immune cells and guiding them across the endothelium into the inflamed tissue. However, the mechanisms by which BECs mediate cellular entry into dermal lesions during *Leishmania* infection is poorly understood. Given immunopathology contributes to disease severity, we sought to investigate the molecular mechanisms responsible for immune cell migration into the tissue. scRNASeq analyses between naïve and *L. major*-infected mice revealed cellular heterogeneity including distinct resident and recruited cell types in the skin following murine *L. major* infection. We found BECs from infected skin express elevated transcripts for selectins and adhesion molecules, while concomitantly downregulating transcripts responsible for junctional stability. During infection BECs sense hypoxic conditions in the tissue which is associated with mTOR activation. mTOR target gene expression derived from transcriptomic data reflects mTOR activation in BECs that could possibly support immune cell migration into the dermal lesions. To determine if mTOR signaling contributed to BEC activation, mice were treated with rapamycin, an mTOR inhibitor. Rapamycin treatment decreased BEC selectins and adhesion molecules such as VCAM-1 which reduced the inflammatory infiltrate leading to smaller lesions following *L. major* infection. Altogether, this comprehensive dataset shows immune cell entry into the dermal lesions is mediated by BEC mTOR signaling during leishmaniasis.

Regulation of the Egress Proteolytic Cascade in Malaria Parasites

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

Malaria is a serious worldwide public health concern that is caused by parasites in the genus *Plasmodium* and one species, *P. falciparum*, is responsible for almost all the deaths. Clinical manifestations of malaria are associated with the asexual proliferation during the blood phase. At the end of proliferation, daughter parasites need to be released first from a parasitophorous vacuole membrane (PVM) and then from the red blood cell membrane (RBCM). A recent study from our lab has shown that the *P. falciparum* endoplasmic reticulum resident calcium binding protein PfERC is essential for the processing of the aspartic protease Plasmepsin X (PMX) in exonemes. The egress proteases are packed in secretory vesicles called exonemes. There is no published data that suggest that exonemes are acidic in nature. In addition, relatively little is known about PMX maturation. In vitro studies indicate that PMX matures via autoproteolysis and its activity requires acidic pH. Using a pH sensitive green fluorescent protein (GFP) known as super-ecliptic pHlourin (SEP) to the endogenous locus of PMX (PMXSEP) in PfERC mutants, we showed PMXSEP localizes to exonemes and partially co-localizes with PfERC. Live fluorescence microscopy data suggest that exonemes are acidic in nature and exoneme pH changes during exocytosis. In schizonts that egress during this time period, we observe several fluorescent events just prior to egress, which suggests vesicle exocytosis into the PV resulting in PMXSEP fluorescence.

Exploring the Impact of Endosymbiont-induced Viral RNA Methylation on Arbovirus Dissemination and Transmission

Tamanash Bhattacharya[1‡], Liewei Yan[2], Hani Zaher[2] Irene L.G. Newton[1*], Richard W. Hardy[1*]
[1]Department of Biology, Indiana University, Bloomington, Indiana, USA, [2]Department of Biology, Washington University, St. Louis, Missouri, USA, [‡]current address: Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

Arthropod endosymbiont *Wolbachia pipientis* is part of a global biocontrol strategy aimed at reducing the spread of mosquito-borne RNA viruses such as alphaviruses. Our prior work examining *Wolbachia*-mediated pathogen blocking has demonstrated (i) the importance of a host cytosine methyltransferase, DNMT2, in Drosophila, and (ii) viral RNA as a target through which pathogen-blocking is mediated. Here we report on the role of DNMT2 in *Wolbachia* induced virus inhibition of alphaviruses in *Aedes* sp.. Mosquito DNMT2 levels were altered in the presence of both viruses and *Wolbachia*, albeit in opposite directions. Elevated levels of DNMT2 in mosquito salivary glands induced by virus infection were suppressed in *Wolbachia* colonized animals coincident with reduction of virus replication, and decreased infectivity of progeny virus. Ectopic expression of DNMT2 in cultured *Aedes* cells was proviral increasing progeny virus infectivity, and this effect of DNMT2 on virus replication and infectivity was dependent on its methyltransferase activity. Finally, examination of the effects of *Wolbachia* on modifications of viral RNA by LC-MS showed a decrease in the amount of 5-methylcytosine modification consistent with the down-regulation of DNMT2 in *Wolbachia* colonized mosquito cells and animals. Collectively, our findings support the conclusion that disruption of 5-methylcytosine modification of viral RNA is an important mechanism operative in pathogen blocking. These data also emphasize the essential role of epitranscriptomic modifications in regulating fundamental processes of virus replication and transmission.

A Putative Calcium Proton Exchanger of *Toxoplasma gondii*

Abigail Calixto and Silvia NJ Moreno
Center for Tropical and Emerging Global Diseases and Department of Microbiology, University of Georgia, Athens, GA, 30602

Calcium (Ca^{2+}) signaling is an evolutionary conserved process among all eukaryotes. Cytosolic Ca^{2+} increases result from Ca^{2+} influx from the extracellular environment through plasma membrane channels or from release of Ca^{2+} from intracellular stores. The cytosolic Ca^{2+} concentration is highly regulated and cells express a number of mechanisms to maintain low levels. These mechanisms consist of Ca^{2+}-pumps at the plasma membrane and intracellular organelles like the endoplasmic reticulum and acidic compartments, Ca^{2+} exchangers and Ca^{2+} binding proteins that bind cytosolic calcium. In *Toxoplasma gondii*, an obligate intracellular parasite, a rise in intracellular Ca^{2+} impacts biological processes such as secretion of adhesive proteins, motility and invasion of and egress from host cells. These lytic cycle facets cause rapid and constant tissue disruption, supporting a role for Ca^{2+} signaling in pathogenesis. We characterized a putative calcium proton exchanger, Tggt1_319550 (TgCAXL1), to study its role in *T. gondii* Ca^{2+} signaling. TgCAXL1 belongs to a well-conserved family of transmembrane proteins predicted to be calcium transporters. The human and yeast orthologs localize to the golgi apparatus, whereas the Arabidopsis ortholog localizes to the chloroplast. Using CRISPR-Cas9, we introduced a multiple HA tag at the C-terminus of the TgCAXL1 and discovered that TgCAXL1 localizes to the ER and Golgi apparatus. We further used CRISPR to knockout the gene and isolated clones for phenotypic characterization. We observed that mutants have an effect on calcium release from the ER and acidic stores, most likely the golgi. Yeast complementation studies showed complementation of growth under high calcium conditions, supporting the functional role of TgCAXL1 as a calcium proton exchanger. Furthermore, we have been studying the TgCAXL1's potential role in pH homeostasis and regulation. Our work on TgCAXL1 highlights a novel player of the calcium signaling toolkit in *T. gondii*.

A *Trypanosoma cruzi* Myosin Associated Regulatory Protein is Essential for Endocytosis via the Cytostome-Cytopharynx Complex

Nathan M Chasen[1], Menna G Etheridge[1], Ronald D Etheridge[1]
[1]University of Georgia, Athens, Ga 30602

Trypanosoma cruzi is the sole pathogenic trypanosomatid that retains the unique endocytic organelle known as the cytostome-cytopharynx complex (SPC). In free-living kinetoplastids, such as Bodo saltans, this tubular feeding apparatus functions to capture and endocytose bacterial prey, but how this endocytic organelle functions at a mechanistic level and why *T. cruzi* alone retained the SPC, remains unknown. Building upon our initial work identifying the first known SPC proteins of any protozoan, we identified several SPC targeted myosins and an associated regulatory protein (MyAP1) using bioinformatic and co-immunoprecipitation methodologies. While deletion of SPC myosins in *T. cruzi* caused a significant reduction in endocytic rate, deletion of MyAP1 generated mutants completely devoid of measurable endocytosis, a phenotype that was rescued by complementation. Intriguingly, we observed that two SPC myosins (MyoF and MyoC) relied on MyAP1 for proper SPC targeting, seemingly providing an explanation for the loss of endocytic activity. However, using a mutagenized MyAP1 lacking a putative calcium binding EF-hand domain, we were able to rescue localization of the myosins without restoring endocytic activity, suggesting an additional calcium-mediated regulatory role for MyAP1 in endocytosis. Our identification of the first functional SPC components, and the generation of endocytic null mutants, has provided us with the unique capacity to dissect the mechanism behind SPC mediated endocytosis and ultimately uncover the functional role of this unusual organelle in the *T. cruzi* life cycle.

cGAS-STING Pathway Activation During *Trypanosoma cruzi* Infection Leads to Tissue-dependent Parasite Control

Natasha Perumal[1] and Rick L. Tarleton[1]
[1]Department of Cellular Biology, University of Georgia, Athens, GA, USA

Host cell invasion by *Trypanosoma cruzi* is a markedly silent process, with little evidence of induction of host transcriptional changes indicative of innate immune recognition, except for a modest and reproducible type I interferon (IFN-I) response. Here we show that *T. cruzi*- induced IFNβ production at 24 hours post-infection was nearly abolished in primary murine cGAS-/- or STING-/- macrophages. Furthermore, infection did not limit the ability of IRF-reporter macrophages to respond to other classical agonists of the cGAS- STING pathway, suggesting that the modest IFNβ induction is a consequence of limited stimulation by *T. cruzi* infection rather than parasite suppression of pathway activation. Infected cGAS-/-, STING-/- and IFNAR-/- macrophages in vitro had significantly higher numbers of amastigotes compared to WT macrophages, indicating that activation of the STING pathway constrains intracellular parasite growth through the induction of interferon-stimulated genes. However, the impact of the STING pathway during infection in vivo is more complex. Despite an initial increase in parasite growth, STING-/- and IFNAR-/- mice ultimately had lower parasite burden in footpads as compared to WT mice, demonstrating a role for IFN-I expression in potentiating parasite growth at this site. However, cGAS-STING pathway activation had little impact on parasite levels in the skeletal muscle, a site of *T. cruzi* persistence, perhaps owing to the low basal expression of STING in myofibers. In the heart, cGAS-/- and STING-/- mice, but not IFNAR-/- mice, accumulated higher acute parasite loads, suggesting a protective role of STING sensing of *T. cruzi* in the heart that was independent of IFN-I. Together, these results demonstrate that host cGAS- STING senses *T. cruzi* infection, enhancing parasite growth at the site of entry, perhaps favoring the establishment of infection, and importantly, contributing to acute parasite control in the heart.

Recurrent Duplication and Structural Mutation Generate Novel Antigenic Genes in the Malaria Parasite *P. falciparum*

Emily R Ebel[1], Bernard Y Kim[1], Marina McDew-White[2], Dmitri A Petrov[1], Elizabeth S Egan[1], Tim JC Anderson[2]
[1]Stanford University, [2]Texas Biomedical Research Institute

In *Plasmodium falciparum* malaria infection, parasite proteins encoded by var genes are exported to the surface of infected red blood cells. Each parasite genome contains ~60 distinct var genes, allowing immune invasion via antigenic switching of var expression. Furthermore, var genes derive their name from their high variability within parasite populations. No two isolates have identical var repertoires, a diversity thought to result from frequent mitotic recombination between members of the family. This complex structural diversity has complicated short-read sequencing attempts to both identify novel var genes and determine their unique mutational mechanisms. In this work, we effectively solve this long-standing problem by applying Nanopore sequencing to *P. falciparum* isolates from a 300-generation mutation accumulation experiment. We generate ultra-long reads (up to 800 kb) that completely span var clusters >70 kb in size, offering unprecedented resolution into var locus structure and dynamics. In particular, we observe multiple instances of tandem duplication that create novel, chimeric var genes by merging duplicated halves of pre-existing genes, as well as evidence of recombination-like processes that copy short segments from one var gene to another. Surprisingly, we also detect read-to-read structural variation at var loci within otherwise clonal samples, which we confirm via Southern Blot. This novel observation suggests that var mutation rates are far higher than has been observable from short read data mapped to the reference genome. Overall, this work presents direct evidence of var mutation by solving a long-standing technical challenge in the study of *P. falciparum* virulence.

Poster Presentations

P4-01. A First Look at the 'Repeatome' of *Benedenia humboldti*, a Major Pathogen in Yellowtail Aquaculture: Repetitive Element Characterization, Nuclear RNA Operon Assembly, and Microsatellite Discovery

J. Antonio Baeza[1], Natalia Leiva[2], Maria Teresa González[2]
[1]Department of Biological Sciences, 132 Long Hall, Clemson University, ClClemson, SC 29634, USA. [2]Laboratorio Eco-parasitologia y Epidemiologia Marina (LEPyEM), Instituto de Ciencias Naturales Alexander von Humboldt, Facultad de Ciencias del Mar y Recursos Biologicos, Universidad de Antofagasta, Angamos 601, Antofagasta, Chile.

The monogenean B*enedenia humboldti* is a pathogen of the yellowtail *Seriola lalandi* in the South-Eastern Pacific ocean. Using low-coverage short Illumina 150 bp pair-end reads sequencing, this study examines, for the first time, the 'repeatome' (= repetitive genomic elements), including the 45S ribosomal DNA operon and microsatellites, in *B. humboldti*. Repetitive elements comprised a large fraction of the nuclear genome and a considerable proportion of them could not be assigned to known repeat element families. Taking into account only annotated repetitive elements, the most frequent belonged to the 45S ribosomal operon or were classified as satellite DNA and Class I- Long Interspersed Nuclear Element (LINE) which were considerably more abundant than Class I - LTR elements. The ribosomal gene operon in *B. humboldti* comprises, in the following order, 5' ETS (length = 233 bp), ssrDNA (2,082 bp), ITS1 (346 bp), a 5.8S rDNA (150 bp), ITS2 (572 bp), lsrDNA (3,887 bp), and a 3' ETS (1,097 bp). A total of 15 SSRs were identified. These newly developed genomic resources will contribute to the better understanding of meta-population connectivity in this species, cryptic species in the genus, and will advance pest management strategies.

P4-02. Protein Acetylation as Key Regulator of *Leishmania* Parasite Stage Differentiation

Suellen Rodrigues Maran[1]; Bruno Souza Bonifácio[1]; Myrna Victoria Zanchetta Costa[1]; Paulo Otávio Lourenço Moreira[2]; Rubens Lima do Monte Neto[2]; Adam A. Dowle[3]; Pegine B. Walrad[4]; Nilmar Silvio Moretti[1]
[1]Laboratory of Molecular Biology of Pathogens – Department of Microbiology, Immunology and Parasitology - Federal University of Sao Paulo (UNIFESP), Brazil; [2]René Rachou Institute – Oswaldo Cruz Foundation (Fiocruz), Brazil; [3]Metabolomics and Proteomics Lab, Department of Biology, University of York, UK; 4Centre for Immunology and Infection, Department of Biology, University of York, UK

Protein acetylation has been implicated in the regulation of essential cellular processes in diverse organisms, including protozoan. Proteomic analysis from our group revealed differential protein acetylation among the three main *Leishmania mexicana* stages, procyclic, metacyclic and amastigote, suggesting a central role of this modification during parasite differentiation. Lysine acetylation is regulated by lysine acetyltransferases (KATs), which add acetyl groups to lysines, and lysine deacetylases (KDACs) that remove the acetyl groups. The KDACs are divided into two classes: Zinc-dependent (DACs) and NAD+-dependent. Thus, to expand our knowledge on how changes in protein acetylation affects *Leishmania* differentiation, we decided to characterized the four DACs (DAC1, 3, 4 and 5) of *L. mexicana*. Using the CRISPR-Cas9 system to obtain null DACs parasites, we found that DAC1 and 3 are essential for parasite survival, while DAC4 and 5 are dispensable. Also, we demonstrated that DAC1 and 5 are cytoplasmic, while DAC3 and 4 have nuclear localization in procyclic and amastigotes. Interesting, we observed that although present in the nucleus, DAC3 is found in euchromatin, while DAC4 predominantly occupies the heterochromatin regions. Phenotype screening using the mutant parasites revealed that DAC1, 3 and 5 affects procyclic multiplication, while DAC3 and 5 affects procyclic differentiation to amastigote stage. Moreover, DAC1 and 5 directly affects differentiation of procyclic to metacyclic infective forms. Altogether, these results suggest that regulation of protein acetylation levels are crucial to *Leishmania* differentiation, opening the opportunity to explore DACs as potential drug targets. Infection assays are underway to test specific DACs inhibitors.

P4-03. Identification of Novel Therapeutics Against Human Schistosomiasis

Sevan N. Alwan[1], Alexander B. Taylor[1,3], Jayce Rhodes[4], Michael Tidwell[4], Stanton F. McHardy[4], Philip T. LoVerde[1,2]

[1]Departments of Biochemistry and Structural Biology, [2]Pathology and Laboratory Medicine, and [3]X-ray Crystallography Core Laboratory, Institutional Research Cores, the University of Texas Health Science Center, San Antonio, Texas 78229, USA. [4]Center for Innovative Drug Discovery, Department of Chemistry, University of Texas at San Antonio, San Antonio, Texas 78249

Human schistosomiasis is a neglected tropical disease caused by parasitic worms. It affects over 250 million people globally. Most human infections are caused by *S. mansoni*, *S. haematobium*, and *S. japonicum*. Currently there is only one method of treatment for human schistosomiasis, the drug praziquantel. Constant selection pressure has caused a serious concern for a rise in resistance to praziquantel leading to the necessity for additional pharmaceuticals, with a distinctly different mechanism of action, to be used in combination therapy with praziquantel. Previous treatment of *Schistosoma mansoni* included the use of oxamniquine (OXA), a prodrug that is enzymatically activated by a sulfotransferase, an enzyme produced by *S. mansoni* (SmSULT). Although sulfotransferases are produced by *S. haematobium* and *S. japonicum*, OXA is not effective against these two species. Structural data have allowed for directed drug development in reengineering oxamniquine to be effective against *S. haematobium* and *S. japonicum*. Guided by data from Xray crystallographic studies and *Schistosoma* worm killing assays on oxamniquine, our structure-based drug design approach produced a robust SAR program that identified several new lead compounds with effective worm killing. More than 300 OXA derivatives were designed and tested in vitro against the adult parasites. Currently, we were able to identify CIDD-0150610 as a very powerful derivative that kills 100 % of *S. mansoni* overnight and *S. haematobium* and *S. japonicum* in less than 7 days in vitro. A dose-dependent study demonstrated that 71 M killed 100% all three species. A study to test the effect on male and female worms showed that paired male worms, paired female worms, and single-sex mature male and female worms were highly susceptible. All of the drugs, we synthesize are racemic mixtures. CIDD-0150610 is a mixture of CIDD-0150303 and CIDD-0150302, our in vivo results show a significant reduction in worm burden when we treated infected animals with CIDD-0150303 compared to control groups. CIDD-0150610 and its enantiomer CIDD-0150303 are potential novel drugs to be used in combination with praziquantel to treat schistosomiasis.

P4-04. The *Vorticella convallaria* Contractile Vacuole

Jaquan Harley; Amy Greene; Mikhayla Reilly
Albright College

The contractile vacuole (CV) is a poorly studied organelle in osmotic regulation. A contractile vacuole is a sub-cellular structure that expels excess liquid when contracted to maintain osmoregulation. CVs are found in single-celled eukaryotes including parasitic and free-living ciliates. Examples include *Trypanosoma cruzi*, amoebas, and Leishmania parasites and in free living ciliates called *Vorticella convallaria*. *Vorticella* is a beneficial model to use while studying drug concentrations because the cycling rates can be directly measured while observing the time taken to complete a cycle. We observed *V. convallaria* CV cycling every 9.20 ± 3.05 seconds (n=5). Several drugs expected to inhibit the contractile vacuole transporters will be investigated to observe any changes in CV cycling rates. Ultimately, our results may contribute to the development of anti-parasitic drugs which target parasites' unique CV organelle.

P4-05. Mitophylogenomics Reveals a New Cryptic Species of *Benedenia* Diesing, 1858 (Monogenea: Capsalidae), a Major Pathogen Infecting the Yellowtail Kingfish *Seriola lalandi* Valenciennes, 1833 in the South-East Pacific

J. Antonio Baeza[1], Natalia Leiva[2], Maria Teresa González[3]
[1]Department of Biological Sciences, 132 Long Hall, Clemson University, Clemson, SC 29634, USA. [2]Laboratorio Eco-parasitologia y Epidemiologia Marina (LEPyEM), Instituto de Ciencias Naturales Alexander von Humboldt, Facultad de Ciencias del Mar y Recursos Biologicos, Universidad de Antofagasta, Angamos 601, Antofagasta, Chile

The monogenean *Benedenia seriolae* parasitizes fishes belonging to genus *Seriola*, represents a species complex, and causes substantial impact on fish welfare in aquaculture systems worldwide. This study reports, for the first time, the complete mitochondrial genome of *B. humboldti* n. sp., a new cryptic species from the South-East Pacific (SEP). The mitogenome of *B. humboldti* n. sp. was assembled from short Illumina 150 bp pair-end reads. The phylogenetic position of *B. humboldti* n. sp. among other closely related congeneric and confamiliar capsalids was examined using mitochondrial protein-coding genes (PCGs). Morphology of *B. humboldti* n. sp. was examined based on fixed and stained specimens. The AT-rich mitochondrial genome of *B. humboldti* is 15,455 bp in length and comprises 12 PCGs (atp8 was absent as in other monogenean genomes), 2 ribosomal RNA genes, and 22 transfer RNA genes. All protein-coding, ribosomal RNA, and transfer RNA genes are encoded on the H-strand. The gene order observed in the mitochondrial genome of *B. humboldti* n. sp. was identical to that of *B. seriolae* from Japan but different from that of *B. seriolae* from Australia. The genetic distance between *B. humboldti* n. sp. and *B. seriolae* from Japan was high. Minor but reliable differences in the shape of the penis were observed between *Benedenia humboldti* n. sp. and congeneric species. Phylogenetic analyses based on PCGs in association with differences in the shape of the penis permitted us to conclude that the material from the South-East Pacific represents a new species of *Benedenia* infecting *S. lalandi* on the coast of Chile. The discovery of this parasite represents the first step to improving our understanding of infestation dynamics and to develop control strategies for this pathogen infecting the farmed yellowtail kingfish, *Seriola lalandi*, in the South-East Pacific.

P4-06. A TurboID-based Compartmental Sensor for *Plasmodium falciparum*

Timothy J Nessel, John M Beck, Madeline Farringer, Josh R Beck
Iowa State University Department of Biomedical Sciences

Promiscuous biotin ligases have emerged as a powerful tools for proximity labeling in live cells. The predominant application has been to target these enzymes to a site or compartment of biological interest to biotinylate the surrounding environment for downstream identification of novel proteins by mass spectrometry. This approach has found wide application in protozoan parasites which generate many unique compartments and organelles and maintain genomes encoding a large proportion of novel proteins with unknown function. The most efficient promiscuous ligase available is TurboID, a variant of *E. coli* BirA engineered by directed evolution for enhanced activity. We find that the high activity of TurboID is further complemented in *P. falciparum* by the ability to grow blood stage parasites in culture without biotin, eliminating background endogenous biotin signals and allowing for pulse-chase labeling approaches. As an alternative to proteomic applications, we have adapted TurboID as a compartmental sensor to monitor biotin membrane permeation and we are currently using this strategy to study the permeability of the parasite vacuole. Additionally, a recently developed split TurboID further enables a novel approach to determine protein membrane topology that is less technically difficulty than traditional protected proteolysis strategies and offers enhanced signal over split fluorescent proteins. Collectively, this work showcases the potential for TurboID proximity labeling as a compartmental sensor to study the biology of protozoan parasites.

P4-07. Molecular Markers COI and ITS2 Reveals the Presence of Gene Flow of Sand Flies in Sri Lanka

Dulani Ruwanika K Pathirage[1], Thilini C Weeraratne[2], SHP Parakrama Karunaratne[2], Nadira D Karunaweera[1]
[1]Department of Parasitology, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka, [2]Department of Zoology, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka

Vector of leishmaniasis in Sri Lanka is *Phlebotomus argentipes* which transmit *Leishmania donovan*i and the disease is a growing health problem. A proper understanding of the population genetic structure of sand fly vectors is considered important prior to planning and implementation of a successful vector control program. Thus, the present study was conducted to determine the population genetic structure of sand fly vectors in Sri Lanka. Two mitochondrial genes viz. Cytochrome c oxidase subunit 1 (CO1) and Cytochrome b oxidase (Cytb), and the internal transcribed spacer 2 (ITS2) region from the nuclear ribosomal DNA were used for molecular characterization. Analysis was done using maximum likelihood method, Network analysis and DNA polymorphisms. The analysis revealed unique sequences of all genomic regions studied except the CO1 region in 21 flies that aligned with those from Kerala, India and Cytb region of 4 flies that aligned with those isolated earlier from Sri Lanka and 3 from Madagascar. CO1 and ITS 2 region analyses revealed gene flow between the study sites whereas Cytb gene region analysis indicated genetically distinct populations of *P. argentipes* in each of the study sites. Populations of *P. argentipes* in Sri Lanka are in most part distinct when compared to sand flies from elsewhere. Gene flow with lack of population differentiation of *P. argentipes*, even between geographically distant sites is likely to pose future challenges for leishmaniasis control due to the likelihood of gene transfer between locations. Such occurrences affecting functionally important genes such as those which confer insecticide resistance can facilitate the spread, of insecticide resistance across the country, which in turn will make vector control difficult.

P4-08. Design and Implementation of a Metabolic Model for the Proline-Glutamate Pathway in *Trypanosoma cruzi*

Renan Weege Achjian[1], Leticia Marchese[1], Karel Olavarria[2], Paul A. M. Michels[3], Ariel Mariano Silber[1]
[1]Universidade de São Paulo, Brazil. [2]Delft University of Technology, Netherlands. [3]The University of Edinburgh, Scotland

Proline is a significant amino acid in the biology of *T. cruzi*. Among other functions, it is capable of sustaining energetically the parasite, by transferring electrons to FAD and NAD+ which can further feed the Electron Transport Chain. In fact, different stages of the parasite show a vastly different steady state concentration of Proline, and the activities of enzymes involved in this pathway are also finely regulated. The objective of this work is to develop and validate an in silico model that is able to simulate and make predictions about fluxes and concentrations of the metabolites present in this pathway, in order to better understand its behavior and responses to different conditions (such as pH, NAD+/NADH pool ratios, and others). However, some enzymes and transporters have either not been characterized yet, or the characterization consisted only in determining the constants of the classical Michaelis-Menten model, which is insufficient to represent the reversibility of the reaction, or effects such as product inhibition. On the other hand, obtaining a complete description of a reversible Bi-Bi reaction for all enzymes is both experimentally difficult and time-consuming. Here, we propose the use of the generic Rohwer equation to model these reversible kinetics with fewer parameters and acceptable error. Our simulations account for Proline, Glutamate and intermediaries in two compartments (cytosol and mitochondrion), and show that this approach results in a stable system with predicted steady state concentration values for these metabolites within physiological range. Moreover, pH, a variable that is indirectly incorporated in the equations, influences the system significantly. In a next step, we will refine and validate the model by obtaining new sets of experimental data on concentrations and fluxes and by using machine learning algorithms to infer unknown variables.

P4-09. Generation of *T. cruzi* Lineages Knock-out for the Kinase IP6K and Evaluation of Homologous Recombination Repair Capacity

Bryan E. Abuchery[1], Simone G. Calderano[2], Marcelo S. da Silva[1]
[1]Department of Chemical and Biological Sciences, Biosciences Institute, São Paulo State University (UNESP), São Paulo, Brazil. [2]Cell Cycle Laboratory, Butantan Institute, São Paulo, Brasil

Trypanosomatids are one of the most important human disease-causing parasites, leading to thousands of deaths annually worldwide. In model eukaryotes, inositol pyrophosphates (PP-IPs) are involved in a wide range of processes, such as regulation of telomere length and homologous recombination (HR). However, the action mechanism of PP-IPs in pathways related to DNA metabolism is not fully understood. The PP-Ips, called IP7 and IP8, are synthesized by pathways involving the participation of IP6K and PP-IP5K kinases, respectively. Trypanosomatids, which encompass parasites of great medical relevance, such as *Trypanosoma cruzi*, synthesizes IP7 but do not synthesize IP8, which make this parasite an excellent model for the study of PP-IPs. The goal of this study is to generate IP6K KO lineages in *T. cruzi*, which will be used to investigate the possible participation of IP7 in HR. After CRISPR-Cas9 genome editing being performed, polyclonal population of IP6K KO lineages was generated and cloning isolation is being carried out. The clones will be characterized genetically (IP6K-/+ or IP6K -/-) and phenotypically, and the absence of IP7 will be checked. Episomal IP6K add-back will be performed to demonstrate the specificity of the assay and eliminate bias due to off-targets. Then, IP6K KO lineages and controls (WT and add-back) will be challenged with Ionization Radiation (IR) to induce DSBs. Growth curves and FACS analyses will be done to check the proliferation pattern and possible cell cycle arrests. Next, we will establish and compare the recruitment kinetics for HR repair pathway. This kinetics analysis will be done by immunofluorescence assays and western blot using antibodies against phosphorylated histone H2A (γH2A), RPA-1, and Rad51 (key HR player). Further analysis involving single-cell transcriptomics (scRNA-seq) of the *T. cruzi* lineages generated will help us to identify possible crypt populations that deal differently with DSBs, mainly during differentiation and infection.

P4-10. Island Hopper to Globe Trotter: Evidence for the Origins of *Aedes aegypti* in the Southwestern Indian Ocean

John Soghigian[1], Andrea Gloria-Soria[2], Jeffrey Powell[3]
[1]North Carolina State University, Raleigh NC, 27603. [2]The Connecticut Agricultural Experiment Station, New Haven CT, 06511. [3]Yale University, New Haven CT, 06511

Aedes aegypti is among the best-studied mosquitoes due to its critical role as a vector of human pathogens. Until now, this species was thought to have originated in continental Africa, and subsequently colonized much of the world following the establishment of global trade routes. However, populations of this mosquito on the islands in the southwestern Indian Ocean (SWIO), where the species occurs with its nearest relatives referred to as the Aegypti Group, have received little study. Leveraging new data from this region, we re-evaluated the evolutionary history of *Ae. aegypti* and found evidence that supports the biogeographic origins of this species were in the southwestern Indian Ocean. We uncover a cryptic species similar to *Ae. aegypti* on Madagascar, and we demonstrate that divergences among sister species correlate with island formations in the region. Finally, we document putative cases of introgression between the invasive, domestic form of this mosquito, and its sylvan relatives - including with the sister species *Ae. mascarensis*. Our results demonstrate that prior to reaching mainland Africa relatively recently, *Ae. aegypti* was widespread across islands in the southwestern Indian Ocean where its ancestors had occurred for millions of years.

P4-11. The Cysteine Synthase Enzyme Plays an Important Role in the Biological Cycle of *Trypanosoma cruzi*

Murillo, A.M.[1]; Marsiccobetre, S.[1]; Silber, A. M.[1]
Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo[1]

Trypanosoma cruzi can use amino acids as energy sources and to support several biological processes such as differentiation, resistance to stress conditions and host-cell invasion. Metabolites containing -SH groups (such glutathione, trypanothione, cysteine, and some of its intermediates such as cystathionine) are relevant to buffer the redox state of the different sub-cellular compartments of this organism. The cysteine synthase (CS) catalyze the second step in the de novo biosynthesis of cysteine, however, its role in redox homeostasis has been unexplored in *T. cruzi*. In this work, we identified a *T. cruzi* sequence encoding a functional CS (TcCS). We obtained partial knockout mutants for TcCS by using CRISPR/Cas9. TcCS-knocked out epimastigotes showed a lower proliferation rates and resistance to 30 min of exposition to 120 M to H_2O_2 when compared with the control that only express Cas9. When these parasites were submitted to nutritional stress in the presence (or not as a control) of 5 mM L-Serine, 5 mM OAS or 0.2, 0.4 or 1 mM L-Cys we observed that: i. L-Cys concentrations over of 200 M were lethal to the mutants after 48 hours and; ii. L-Ser and OAS contributed to the survival of both, mutants and wild type parasites to severe starvation. We found that TcCS parasites had diminished their ability to differentiate to metacyclic trypomastigotes. When these metacyclic trypomastigotes were evaluated for their ability to infect mammalian host cells, we observed an over rate of infection after 48 hours and an increment of trypomastigote burting with some morphological differences when compared to the control. Altogether, these data indicate that cysteine has an important role during epimastigotes proliferation, metacyclogenesis and the infection of mammalian cells. Further experiments will allow us to better understand the role of the cysteine biosynthesis de novo for the biology of *T. cruzi*

P4-12. VEuPathDB: Comprehensive Informatics Support for Your Research Needs

Susanne Warrenfeltz[1], Jessica Kissinger[1], for the VEuPathDB Team
[1]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30602, USA

VEuPathDB is an NIAID-funded bioinformatics resource center focused on eukaryotic pathogens, vectors, fungal and host informatics. It represents the merger of VectorBase and EuPathDB. VEuPathDB empowers users to leverage omics data without the need for computational programming. VEuPathDB analyzes a wide variety of omics data and couples the analysis results to advanced search capabilities, data visualizations and custom analysis tools to facilitate the discovery of meaningful relationships from large volumes of data. Available data range from basic sequence and annotation to transcript or protein expression, variation, domains, orthology, phenotypes, pathways, epigenomics and host-pathogen interactions. Data mining strategies include: records that compile all data for a single feature like a gene page, the genome browser, the search strategy system, the Galaxy interface, and the MapVEu population biology interface. Users might begin at a gene page to view tables and graphs of that gene's behaviour in an RNA-Seq experiment, then transition to the genome browser for a dynamic view of the RNA-Seq data aligned to the genome, as well as the opportunity to view nearby gene models or other data types. The search strategy system allows users to query from a genome-wide perspective, easily merge evidence from diverse data and across species, and ask questions such as 'Find genes in these 4 species that are expressed in the first half of the organism's life cycle'. Users with their own omics data can use Galaxy to privately analyze and port their results to VEuPathDB for comparison. The MapVEu tool facilitates mining of population data for traits such as insecticide resistance and infection status. Our active user support offers an email help desk, social media, tutorials, webinars, and workshops. Email us at help@veupathdb.org with questions or suggestions.

P5-01. Role of AGC Family Kinases in the Endocytic Processes of the Parasite *Entamoeba histolytica*

Azhar Ahmad and Somlata
Multidisciplinary Centre for Advanced Research and Studies(MCARS), Jamia Millia Islamia, New Delhi-110025, India

Amebiasis is a fatal diarrheal disease caused by the protozoan parasite *Entamoeba histolytica*. The burden imposed by this parasite is disproportionately large in developing countries with considerable morbidity and mortality. The mechanism of human cell killing has been unclear, though the prevalent belief was that it is a contact-dependent process where the parasite first kills the host cells and then phagocytoses them. Recently, there has been paradigm shift in understanding the pathogenicity of this organism by the recognition of a new model of host cell killing which is known as trogocytosis. During trogocytosis amoeba chews the live host cell in a stepwise manner which leads to elevation of Ca^{2+} in cytosol of the host cell, loss of its plasma membrane integrity and eventually its death. The importance of trogocytosis has now been recognized in other biological processes like antigen presentation by immune cells or in development process. In *Entamoeba histolytica*, it has been shown that EhAGC family kinases are involved in the regulation of different endocytic pathways. Specifically, EhAGCK1 is involved in the process of trogocytosis whereas EhAGCK2 is involved in all endocytic pathways including phagocytosis and trogocytosis. However, no mechanistic details are available in any other organism which differentiates trogocytosis from phagocytosis. To understand the signalling pathway regulated by these kinases we are currently focussing on their biochemical characterization. Also, in silico analysis indicates the presence of a putative but common upstream activator for the EhAGCK1 and EhAGCK2 in *Entamoeba histolytica* which is a 3-phosphoinositide-dependent kinase-1 (EhPDK1). To study the signalling cascade during endocytic processes we are creating trophozoite transfectants that will be further used for real-time imaging and protein interactions studies. We expect our observations will reveal molecular details which will differentiate similar signalling pathways and will also help in developing drug targets for treatment of amebiasis.

P5-02. Novel Mono- and Bis-peroxide Bridged Artemisinin Dimers Show Potency Against Artemisinin-resistant *Plasmodium falciparum*

Victoria Mendiola, Cynthia Lichorowic, Yingzhao Zhao, Steven Maher, Dennis Kyle, Roman Manetsch
Univeristy of Georgia, Athens, GA, 30601 ²Northeastern University, Boston, MA, 02115

The intracellular parasite *Plasmodium falciparum* is a causative agent of malaria and a major public health concern. Various drugs have been used to treat malaria at multiple stages of the *P. falciparum* life cycle but often develop resistance. One such drug, Artemisinin and its derivatives, is most commonly used treatment for malaria and often in combination with other antimalarials because of the short half-life in vivo. However, in 2008 drug resistance to Artemisinins began to emerge and spread. In vitro experiments, such as the classic ring-stage survival assay are used to examine parasite recovery for three days after drug exposure in order to identify resistant and susceptible parasite lines. Using an extended version of this assay, we examined the potency of recently synthesized bis- and mono-peroxide bridged Artemisinin dimers against Artemisinin-susceptible and -resistant asexual blood stage *P. falciparum* parasites. Tightly synchronized Artemisinin-susceptible (W2) and Artemisinin-resistant (4G) ring stage *P. falciparum* were exposed to 700 nM of dihydroartemisinin, the mono- or bis-peroxide bridged artemisinin dimer and examined for recovery for 5 days. Overall, we found that the bis-peroxide bridged dimer responds similarly to dihydroartemisinin treated parasites in both W2 and 4G strains of *P. falciparum*. Interestingly, the mono-bridged artemisinin dimer showed no recovery by day 5 post exposure while the dimer that included no bridges had similar recovery to untreated controls. This suggests that the number of bridges present in the Artemisinin dimer molecules is important to compound potency in both Artemisinin-resistant and –susceptible parasites.

P5-03. Preliminary Study of the Inositol Pyrophosphates Metabolic Pathway in Kinetoplastids: An Evolutionary Perspective

Arthur de O. Passos[1], Antônio M. Rezende[2], Marcelo S. da Silva[1]
[1]Department of Chemical and Biological Sciences, Biosciences Institute, São Paulo State University (UNESP), São Paulo, Brazil. [2]Department of Microbiology, Aggeu Magalhães Institute/FIOCRUZ, Recife, Brazil

In model eukaryotes, inositol pyrophosphates (PP-IPs), mainly IP7 and IP8, are involved in a wide range of essential cellular processes. IP7 and IP8 are synthesized through complementary pathways that involve IP6K and PP-IP5K kinases, respectively. Kinetoplastids are a group of flagellated protozoa characterized by the presence of kinetoplast, an organelle with the mitochondrial DNA. This group encompasses the Trypanosomatidae family, which includes etiological agents of human diseases. Trypanosomatids have orthologous genes for IP6K, but in *Trypanosoma cruzi* and *Trypanosoma brucei*, the gene encoding to PP-IP5K is apparently absent, i.e., they lack IP8. Thus, this work aims to investigate whether the presence of IP8 is mutually exclusive relative to parasitism within kinetoplastids. Thereunto, we performed evolutionary analyses involving IP6K and PP-IP5K to confirm the alleged loss of PP-IP5K. Using public data (amino acid sequences) available (tritrypDB.org; ncbi.nlm.nih.gov) and MEGAX software, we build phylogenetic trees using the IP6K and PP-IP5K existing sequences. Also, we estimated the percentage of amino acid residues substitution for IP6K and PP-IP5K in each analyzed organism. Predictions of the tertiary structures for each kinase were made using I-Tasser and Phyre2. After multiple analyses using hidden Markov models and amino acid sequences through the HMMER software, our findings, although preliminary, suggest that the transition from a free-living to a parasitic lifestyle has resulted in the loss of PP-IP5K. Our next steps include performing PP-IP5K knock-in (KI) (from *Bodo saltans*) in *T. cruzi*. We will perform phenotypic analyses of the generated KI lineage, in addition to conducting infection assays. We also intend to perform knock-out of PP-IP5K in Vero cells (used as receptacle in the infection assays), which will allow us to evaluate whether *T. cruzi* uses the host's IP8 during infection. This work will clarify if the PP-IPs have any relation with the parasitism developed within the order Kinetoplastida.

P5-04. Characterization of Asparagine Transport and Consumption in *Trypanosoma cruzi*

Marchese, L.[1], Alencar, M.B.[1], Souza R. O. O.[1], Pinto, A.K.[1], Biran, M.[2], Bringaud F.[3], Silber, A.M.[1]
[1]Department of Parasitology – Institute of Biomedical Sciences – University of São Paulo, São Paulo, Brasil. [2]Centre de Résonance Magnétique des Systèmes Biologiques – French National Centre for Scientific Research – University of Bordeaux, Bordeaux, France. [3]Microbiologie Fondamentale et Pathogénicité – French National Centre for Scientific Research – University of Bordeaux, Bordeaux, France.

Trypanosoma cruzi, a protozoan responsible for Chagas Disease, is well adapted to the consumption of several amino acids in the absence of glucose. One of these amino acids, aspartate (Asp), is involved in many cellular processes, such as protein biosynthesis, energy metabolism and cell differentiation. Remarkably, little is known about the biological importance of the closely related amino acid asparagine (Asn), a precursor of Asp. The aim of this study was to investigate how *T. cruzi* uptake and metabolise Asn and to unveil its possible biological roles. The uptake of Asn in *T. cruzi* epimastigotes, is an active process, dependent on the plasma membrane proton gradient. Additionally, it is inhibited by competition with alanine, serine, threonine and glycine, all of them not structurally related to Asn. Noticeably, the Asn transport is performed by a transport system, which retain the activity at unusually elevated temperatures (up to 50°C). Once inside the cell, Asn was able to trigger mitochondrial O_2 consumption, and to sustain cell viability under severe starvation. Unexpectedly, Asn was able to maintain the intracellular ATP levels of *T. cruzi* better than the intermediate Asp (an intermediate metabolite in the Asn oxidation pathway) during metabolic stress. Even more intriguing, NMR analysis of the exometabolome of parasites incubated with Asn as a sole carbon source, showed excretion of alanine as a main metabolic end-product, which was not detected in parasites incubated with Asp. These results suggest that Asn can also be catabolized by an alternative route not including the production of aspartate as an intermediate.

P5-05. *Rhipicephalus sanguineus* s.l Transcriptome Analysis of Questing Larvae and Engorged Larvae from Four Different Ecological Systems of Colombia

Gabriel A. Tafur-Gomez[1], Marco Antonio Piñeros[1], Alejandro Rodriguez[2], Danny Wilson Sanjuanelo Corredor[1], Alejandro Hoyos-Jaramillo[3], Rodrigo P. Batptista[4,5]

[1]Universidad de Ciencias Aplicadas y Ambientales - U.D.C.A, Bogotá 111166, Colombia; [2]GenXPro GmbH, Frankfurt Innovation Center Biotechnology, Frankfurt am Main, Germany; [3]Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA, US; [4]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, US; [5]Institute of Bioinformatics, University of Georgia, Athens, GA, US

The ticks belonging to the complex of *R. sanguineus* s.l group includes species worldwide distributed considered important agents for vector-borne diseases in humans and animals. Among its ecological characteristics, these tick species present a great dispersion capacity due to their elevated activity, leading to a higher population density. Fewer studies are focused on identifying the gene expression concerning to the ecological conditions and the instars of development. In our study, engorged females were collected from infested dogs belonging to four different ecological regions in Colombia to establish a laboratory colony. Newly questing larvae (QL) after hatching, and engorged larvae (EL) dropped from susceptible rabbits after the challenge were collected and conserved at -80°C. At each instars of the ticks, total RNA was extracted, and measured using Qubit and LabChip. Genome-wide gene expression profiling was performed using MACE-seq with RNA extracted from each tick instars to sequencing the 3' end portion of the mRNA. Eight transcriptome libraries were produced using the Rapid MACE-Seq kit (GenXPro GmbH, Germany), following the manufacturer's protocol using a NextSeq platform. PCR-duplicates were identified and removed from the raw data. The remaining reads were further poly (A)-trimmed, and low-quality reads were discarded. The clean reads were aligned to the *R. sanguineus* genome (ASM1333969v1), annotated and protein predicted to generate the expression hits. The libraries were normalized and compared between QL and EL at each region using DEseq2. The transcripts at each instar from all ecological regions showed a common expression of 44 upregulating transcripts with $p<0.01$. The Blastp against non-redundant proteins showed the highest expression of ribosomal transcripts and proteosome transcripts with GO terms associated with transcription and proteolysis. This approach reveals a new group of high expression transcripts involving in QL and EL instars from different ecological regions in Colombia.

P5-06. Evaluation of the Immunomodulation Mechanism of the Th2 Response in Mice Experimentally Infected with *Toxocara canis*

Borchardt JL[1], Santos AC[2], Weege GB1, Moura MQ[1], Terto WDS[1], Berne MEA, Leite FPL[2]

[1]Postgraduate Program in Microbiology and Parasitology, UFPel/Brazil; [2]Center for Technological Development, UFPel/Brazil

Toxocariasis, caused by *Toxocara canis*, is considered one of the most prevalent helmintoses in the world. During *T. canis* infection a Th2 immune response predominates and the production of Th1 lymphocytes is suppressed. The objective of this work was to evaluate the immunological modulation mediated by experimental infection of *T. canis* larvae in Balb/c mice. The profile and levels of serum immunoglobulins were evaluated by indirect ELISA, and qPCR was used for evaluating the levels of splenocytes mRNA transcription for the cytokines IL4, IL10, IL12 and YM1 protein. Infection with *T. canis* larvae was able to generate a significant ($p <0.05$) level of specific total IgG at 15 and 30 days post-infection (p.i). Analyzing the IgG isotype was observed a differentiation of the isotypes IgG1, IgG2a, IgG2b and IgG3 at significant levels ($p <0.05$). Evaluating the gene transcription, a different transcription pattern was detected. Higher transcription levels for IL4 and YM1 was observed during the chronic phase. IL10 levels were elevated in the acute phase of infection and IL12 levels were suppressed in all evaluated periods. From the data obtained, we can suggest that during the experimental infection with *T. canis* the participation of the IL4, IL10 and YM1 play an important role in the *T. canis* infection immunomodulation.

P5-07. Elucidating the Importance of Branched Chain Amino Acids Catabolic Pathway Applying CRISPR-Cas9 Technology in *Trypanosoma cruzi*

Sabrina Marsiccobetre[1], Higo F. Santos Souza[1], Ana M. Murillo[1], Ariel M. Silber[1]
[1]Laboratory of Biochemistry of Tryps—LaBTryps, Department of Parasitology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

Several studies have described the importance of amino acids and their intermediate metabolites in the biology of different protozoa, including *Trypanosoma cruzi*. Our group is interested in the metabolism of branched chain amino acids (BCAA - Leu, Ile and Val) that are essential for this parasite. Sequences encoding enzymes associated with BCAA catabolism are present in *T. cruzi* genomic banks. The first enzymatic step of this pathway is catalyzed by an aspartate or a tyrosine aminotransferase, which converts each one of the three BCAA into their correspondent ketoacids. The second step is catalyzed by a branched-chain keto acid (BCKA) dehydrogenase complex (BCKDH) which is responsible for the oxidative decarboxylation of the alpha BCKA, yielding their respective branched-chain acyl-CoA derivatives. Subsequently, acyl-CoA dehydrogenases catalyze the reversible dehydrogenation of the metabolites. The resulting products can be metabolized by an enoyl-CoA hydratase. The products of this reaction can be substrates of hydratases or dehydrogenases, which result in principle, in the production of intermediates of the tricarboxylic acids cycle. To obtain insights on the biological role of this complex pathway, we used CRISPR/Cas9 technology to obtain partial or full knockout parasites for each enzyme since the second step. We report here the obtainment of hemi and total knockouts of a hybrid strain of *T. cruzi*. Preliminary data suggest that the partial deletion of the coding sequences for isovaleryl-CoA dehydrogenase and enoyl CoA hydratase, does not lead to a significant reduction of their activities as assayed in total cell-free epimastigotes extracts. In addition, we found that complete deletion of enoyl-CoA hydratase produced a defect on the trypomastigotes bursting on infection of mammal cells and incapacity of epimastigotes to recovery normal growth after starvation in presence of valine, suggesting the accumulation of a toxic metabolite.

P5-08. A Novel Cytopathogenicity Assay Yields New Drug Intervention Strategies Against *Naegleria fowleri*, the Brain-eating Amoeba

Emma Troth[1,2], Dennis Kyle[1,2,3]
[1]Department of Infectious Diseases, University of Georgia, Athens, GA, US. [2]Center for Tropical and Emerging Global Diseases, Athens, GA, US. [3]Department of Cellular Biology, University of Georgia, Athens, GA, US

Naegleria fowleri is a free-living pathogenic amoebae that poses a substantial public health risk. *N. fowleri* is the causative agent of Primary Amebic Meningoencephalitis (PAM), a disease that has a ≥97% mortality rate. PAM is rapidly fatal, as the time from symptom onset to death is 5-12 days. There is an urgent need for the development of therapeutics against this fatal organism. *N. fowleri* secrets multiple proteases during infection that aid in the breakdown of tissue. *N. fowleri* also relies on its cytoskeleton to form food cups which allow the amoeba to perform trogocytosis. Current therapeutics do not target either of these destructive amoebic functions. In this study, we aimed to develop a new assay to aid the discovery of novel compounds that block secreted proteases and/or food cup formation and slow down the rate of tissue destruction. We have developed an innovative cytopathogenicity assay with which we can identify compounds capable of inhibiting *N. fowleri* – mediated destruction of mammalian cells. We have utilized live-cell imaging using CellTrace staining as endpoints for quantitative analysis. Using automated microscopy, we are able to measure *N. fowleri*-mediated degradation and report inhibitory values for compounds tested in the assay. This assay also allows for plaque measurement. Herein, we assessed currently used PAM therapeutics and a series of protease and actin inhibitors, both alone and in combination, for their ability to inhibit *N. fowleri* replication and ability to hinder *N. fowleri* – mediated monolayer degradation.

P5-09. Protective Effect of *Lactobacillus rhamnosus* (ATCC 7469) on the Intestinal Mucosa of Mice Experimentally Infected with *Toxocara canis*

Micaele Quintana de Moura[1], Carolina Netto de Oliveira da Cunha[1], Nicholas Frota Gonçalves de Souza[2], Luciana Farias da Costa Ávila[1], Carlos James Scaini[1]

[1]Post-Graduate Program in Health Sciences – Parasitology Laboratory, Universidade Federal do Rio Grande (FURG), Academic Area of the University Hospital – FURG, Rio Grande, Rio Grande do Sul, Brazil. [2]Graduate Program in Medicine – Faculty of Medicine, Universidade Federal do Rio Grande (FURG), Rio Grande, Rio Grande do Sul, Brazil

Toxocariasis is considered a neglected anthropozoonosis with wide geographical distribution. The therapy for toxocariasis is not fully effective, so alternative therapies and prevention methods have been studied, among them probiotics. The objective of this study was to evaluate the protective effect of the probiotic *Lactobacillus rhamnosus* and to investigate its immunomodulatory action on the intestinal mucosa of mice experimental infected with *Toxocara canis*. For that, 20 female Swiss mice were supplemented by *L. rhamnosus* (1x107 UFC/mL) for 15 days before the inoculation of 100 embryonated *Toxocara cani*s eggs through gavage. The animals were divided into groups: Tx: infected with *T. canis*; Lc: supplemented by the probiotic; LcTx: supplemented and infected; Ct: negative control. The animals were euthanized 48 h post infection and had brain, liver and lungs removed for larvae recovery. The cells of the intestinal mucosa of the duodenum were collected, stored in TRIreagente sigmal® for subsequent RNA extraction, cDNA synthesis and qPCR for analysis of the transcription of the IFN-γ and IL-13. There was 50.95% of reduction in the intensity of *T. canis* infection in animals supplemented with probiotic. In the intestinal mucosa, IFN-γ gene transcription is suppressed in all evaluated groups, while the IL-13 transcription had a 10x increase in the Lc group. The protective effect of *L. rhamnosus* against *T. canis* infection seems to have different mechanisms than those induced by *Saccharomyces boulardii*, which also had a protective effect in the acute phase of toxocariasis. However, *S. boulardii* was associated with stimulating of IFN-γ and IL-12, including intestinal mucosa cells (IL-12). Despite the increase in IL-13 transcription in the Lc group, this was not observed in the LcTx group, indicating that the protective mechanism of *L. rhamnosus* does not involve this interleukin either. Future studies are needed to elucidate the mechanisms of action of *L. rhamnosus*.

P5-10. A Casein Kinase Regulates DNA Synthesis and Basal Body Biogenesis in T*rypanosoma brucei*

Benjamin Hoffman[1], Catherine Sullenberger[1], Kojo Mensa-Wilmot[1,2]

[1]Department of Cellular Biology, University of Georgia, Athens, GA. [2]Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA

TbCK1.2 from the eukaryotic microbe *Trypanosoma brucei* is a homologue of casein kinase 1. However, biological pathways regulated by TbCK1.2 cannot be inferred from those described in humans and yeasts using bioinformatic analysis, due to extensive divergence of the proteome of the trypanosome from other eukaryotes. In bloodstream *T. brucei* TbCK1.2 is essential for viability, most likely as a regulator of cytokinesis and division of mitochondrial DNA (kinetoplast DNA (kDNA)). We report here new roles for TbCK1.2 in DNA synthesis, and basal body copy number control. Knockdown of TbCK1.2 triggers DNA synthesis in post-mitotic nuclei, and in kinetoplasts that have divided kDNA in two (in G2 and M phases). The copy number of basal bodies, whose duplication occurs in S phase of the cell cycle, increases above that observed in control cells. Finally, the location of p197 in the kinetoflagellar zone is altered after knockdown of TbCK1.2. These observations are consistent with unanticipated roles for TbCK1.2 in *T. brucei* entry into the S-phase of the cell cycle.

P5-11. In silico Analysis of Naphthoxyrans Molecules with Activity Against Toxocara canis

Débora Carvalho Rodrigues[1], Tais Mata-Santos[1], Hilton Antônio Mata-Santos[2], Paula Fernandes Carneiro[3], Maria do Carmo Freire Ribeiro Pinto[3], Vitor Francisco Ferreira[3], Lourdes Rodrigues Martins[1], Luciana Farias da Costa Avila[1], Pedro Eduardo Almeida da Silva[5], Daniela Fernandes Ramos[4], Carlos James Scaini[1]
[1]Parasitology Laboratory, Medical College, Universidade Federal do Rio Grande (FURG), Rio Grande, Rio Grande do Sul, Brazil, [2]Department of Clinical and Toxicological Analysis, Pharmacy College, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [3]Natural Products Research Institute, Pharmacy College, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [4]Development Center of New Drugs, Medical College, Universidade Federal do Rio Grande (FURG), Rio Grande, Rio Grande do Sul, Brazil, [5]Mycobacteria Laboratory, Medical College, Universidade Federal do Rio Grande (FURG), Rio Grande, Rio Grande do Sul, Brazil

Human toxocariasis is a neglected parasitosis, treated with administration of anthelmintics, which have low to moderate efficacy due to difficulties in action at tissue level. In view of the pharmacological potential of naphthoquinones and their derivatives, such as anti-inflammatory, antibacterial, trypanocidal, antifungal and antitumor properties, studies have suggested that these molecules may be precursors for the development of new drugs. A recent study evaluated the activity of four naphthoxyrans (derived from naphthoquinones), selecting two, which showed low cytotoxicity and efficiency in in vitro tests with *Toxocara canis* larvae. The purpose of this study was to verify the pharmacokinetic parameters and physicochemical properties of these two molecules, $C_{16}H_{20}O_3$ and $C_{16}H_{16}O_3$. For the in silico test, two softwares were used, Swiss ADME and Mol inspiration, and the results were observed following the guidelines of the "rule of five" by Lipinski. Analyzes revealed that molecules do not violate the pre-established guidelines, presenting very similar physico-chemical properties: molecular weight of 260.33 g/mol and miLogP: 3.01 ($C_{16}H_{20}O_3$) and molecular weight of 256.30 g/mol and miLogP: 3.10 ($C_{16}H_{16}O_3$). Molecular weight less than 500 g/mol indicates a greater ease of transport by proteins, interfering positively in the absorption and metabolization, while miLogP is an indicator of lipophilicity, a measure that establishes the ability of a compound to be absorbed in oils, the lower this value the greater the solubility of the compound in water. In addition, both had zero donors and three hydrogen acceptors, indicating the number of rotating connections made, the fewer rotations, the greater will be the interaction of the drug with its target. Finally, the molecules also demonstrated a high gastrointestinal absorption capacity and the ability to permeate the blood-brain barrier. Thus, the results obtained by this analysis, added with those from other studies, demonstrate the high therapeutic potential of these naphthoxyrans.

P5-12. Characterization of an Endoplasmic Reticulum-Resident Calcium-binding Protein in *Toxoplasma gondii*

Miryam A. Hortua Triana[1], Stephen Vella1, Catherine Li[1], Silvia N. Moreno[1]
[1]Center for Tropical and Emerging Global Diseases, University of Georgia, UGA

Calcium signaling is universal and impacts almost every aspect of cellular life. In *T. gondii* Ca^{2+} signaling stimulates specific features of the parasite infection cycle and a number of known Ca^{2+} signaling elements are important for its parasitic cycle. Initiation of motility and the subsequent invasion and egress events have been shown to be activated by the release of intracellular Ca^{2+} stores. The endoplasmic reticulum (ER), likely the main Ca^{2+} store in the cell is important for both Ca^{2+} homeostasis and signaling. We identified a calcium binding protein (Tg229480), an orthologue of the *Plasmodium* ER Calcium Binding Protein, or TgERC that localized to the *T. gondii* ER. Deletion of the TgERC gene resulted in mutants with reduced capacity to store Ca^{2+} in the ER. Using chemical Ca^{2+} indicators showed that when exposed to high extracellular Ca^{2+} ($[Ca^{2+}]$ ~1.8 mM) the ER of the mutant parasites is unable to retain Ca^{2+}. Phenotypic analysis of TgERC showed slow growth. Further analysis with mutants expressing GCaMP6f showed a delayed egress upon saponin addition compared to wild type, and the typical spike of Ca^{2+} that precedes egress was no longer high and constant. Overall, TgERC play an important role in Ca^{2+} storage that lead to lytic cycle defects. Further analysis of TgERC partners at the ER and the effectors of this intracellular store Ca^{2+} release is ongoing.

P6-01. Characterization of the Extracellular Vesicles Secreted by *Naegleria fowleri*

A. Cassiopeia Russell[1,2] and Dennis Kyle[1,2,3]

[1]Center for Tropical and Emerging Global Diseases, Athens, GA 30602, USA. [2]Department of Infectious Diseases, University of Georgia, Athens, GA 30602, USA. [3]Department of Cellular Biology, University of Georgia, Athens, GA 30602, USA

The pathogenic free-living amoeba, *Naegleria fowle*ri is the causative agent for primary amoebic meningoencephalitis, an acute brain disease with a case mortality rate of >97%. Several factors contribute to this considerable degree of mortality including delayed diagnosis, ineffective therapeutics and a lack of understanding of the amoebic pathogenesis. Recently, there have been notable advances in the study of the parasite biology, but the genome still remains poorly annotated and the understanding of the molecular basis for parasite-host interactions is lacking. Major players known as extracellular vesicles (EVs) have recently been implicated in the field of intercellular communication. These EVs are secreted by originating cells and house various signaling molecules including proteins and nucleic acids that elicit a response in recipient cells. One class of molecules that can be found in these vesicles are microRNAs (miRNAs), which are small secretory molecules that can be detected in many bodily fluids. These have been implicated as regulators of gene expression, can modulate host immune responses, and are currently used as biomarkers for the diagnosis and prognosis of various diseases such as cancer. As such, based upon prior research that indicates that *N. fowleri* and other parasitic free-living amoebae can undergo membrane vesiculation and produce EVs, we reasoned that *N. fowleri* might also produce miRNAs and package them into these EVs to be secreted for similar purposes. We show that EVs are secreted by N. fowleri and Nanoparticle Tracking Analysis has been applied for enumeration as well as size determination. Deep-sequencing of the RNA contents of these EVs followed by computational predictions has uncovered 10 novel miRNAs whose presence we are validating with qPCR in several *N. fowleri* clinical isolates both in vitro and in vivo.

P6-02. A Solution to the Challenge of Deciphering Protein Kinase Pathways in the Evolutionarily Divergent Microbe *Trypanosoma brucei*

Justin Wiedeman[1,2], Catherine Sullenberger[1,2], Kojo Mensa-Wilmot[1,2,3]

[1]Departent of Cellular Biology, University of Georgia, Athens, GA, [2]Center for Tropical and Emerging Global Diseases, Athens, GA, [3]Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA

Trypanosoma brucei is a protozoan responsible for the disease Human African Trypanosomiasis. Trypanosome casein kinase TbCK1.2 regulates kinetoplast division and cytokinesis, and is essential for cell viability. Delineation of protein kinase signaling pathways in the trypanosome is hampered by several factors. First, 60% of proteins in *T. brucei* lack homologs in model eukaryotes. Second, substrates for *T. brucei* kinases have not been defined experimentally. Combined, these facts preclude bioinformatic approaches for kinase pathway prediction. Here, we present a bottom-up, biology-guided approach to characterize the signaling pathways of protein kinases and identify endogenous kinase substrates. First, we identify early phenotypic changes associated with TbCK1.2 knockdown or small molecule inhibition. Second, we discover proteins that are dephosphorylated following TbCK1.2 knockdown. Third, we show that a subset of these proteins (termed pathway proteins) are substrates for purified TbCK1.2 in vitro. Fourth, we show that three novel substrates of TbCK1.2 (namely, TbLRRP1, TbBBP59, and Tb427.10.15310) are involved with kinetoplast division or cytokinesis. Finally, we identify sites on TbBBP59 that are phosphorylated by TbCK1.2, and investigate contributions to cytokinesis and kinetoplast division, two pathways regulated by TbCK1.2. The strategies presented in this study may find general use for identifying proteins involved in pathways regulated by protein kinases in other evolutionarily divergent eukaryotes.

P6-03. *Saccharomyces boulardii* Stimulates IL-17 in Mice Infected with *Toxocara canis*

Luciana F C Avila[1]; Micaele Quintana Moura[2]; Maria Elisabeth Aires Berne[2]; Carlos James Scaini[1]; Fábio Leivas Leite[3]

[1]Postgraduate Program in Health Sciences, FURG/ Brazil; [2]Postgraduate Program in Microbiology and Parasitology, UFPel/Brazil; 3Center for Technological Development, UFPel/Brazil

Human toxocariasis is considered a public health problem in Latin America. Studies reveal that *Saccharomyces boulardii* promotes a reduction in the intensity of the infection by *Toxocara canis* in mice. Considering that the establishment of chronic infection by this nematode in the brain of mice is related to the suppression of IL-17, the aim of this study was to evaluate the role of *S. boulardii* on the levels of IL-17 mRNA in infected mice. Two groups of fifteen Swiss mice were formed: G1 received feed supplemented with *S. boulardii* (107 CFU/g) while G2 received feed without supplementation. After 15 days, G1 and G2 were inoculated with 100 embryonic *T. canis* eggs and euthanized with 24h of infection. Splenocytes were collected and IL-17 mRNA levels were evaluated using qPCR. In the present study, it was possible to observe a significant increase of 35% in the levels of IL-17 mRNA in the splenocytes of the animals that received feed supplemented with the probiotic, when compared to those without the probiotic. When splenocytes were stimulated in vitro with *S. boulardii* (107 CFU / mL), it was possible to observe even more pronounced levels of IL-17 mRNA in G1, an increase of 57% in relation to G2. However, when *T. canis* larva (2 larvae / mL) in vitro stimulation was performed, it was possible to confirm the suppression characteristic of the parasite, since the levels of IL-17 mRNA decreased by approximately 4x. From the data obtained, we can suggest the participation of the pro-inflammatory cytokine IL-17 as one of the factors responsible for the protection mediated by *S. boulardii* in the infection by *T. canis*.

P6-04. Identifying Apicomplexan ATP Synthase Regulators Using a Förster Resonance Energy Transfer (FRET) Sensor

Madelaine Usey[1,2], Diego Huet[1,2,3]

[1]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30605. [2]Department of Cellular Biology, University of Georgia, Athens, GA 30605. [3]Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA 30605

Apicomplexan parasites are the causative agents of significant human diseases including malaria, cryptosporidiosis, and toxoplasmosis. Throughout their complex life cycles, these parasites encounter a wide range of nutrient availabilities and must regulate their metabolism accordingly. In particular, mitochondria play a critical role in such adaptations. The ATP synthase is a mitochondrial multimeric complex that generates ATP to power cellular processes, and previous work has shown that the subunit composition of the apicomplexan ATP synthase differs significantly from that of its mammalian counterpart. In many organisms, ATP synthase activity is tightly regulated by numerous mechanisms, including via binding of interactor proteins. However, regulatory mechanisms which govern ATP synthase activity remain largely uncharacterized in the apicomplexan phylum. Further complicating investigation of factors regulating the apicomplexan ATP synthase is a lack of tools for directly measuring mitochondrial ATP production in these parasites. As apicomplexans are exposed to highly variable environmental conditions, we hypothesize that their ATP synthase activity must also be tightly regulated. To create a tool allowing for direct measurements of *T. gondii* ATP synthase activity, we have targeted a Förster Resonance Energy Transfer (FRET) ATP sensor to the parasite mitochondrion. This fluorescent probe will allow for observations of real-time changes in mitochondrial ATP production using live-cell imaging. As previous work has shown that FRET measurements can be quantified in a high-throughput manner via fluorescence activated cell sorting (FACS), we will utilize this sensor to conduct a FACS-based CRISPR screen for genes involved in the regulation of ATP synthase activity in *T. gondii*. Since the ATP synthase is essential for survival of the T. gondii stage responsible for acute toxoplasmosis, as well as for *Plasmodium* mosquito-stage parasites critical for malaria transmission, identification of factors which regulate the unique apicomplexan ATP synthase could reveal targets for novel therapeutic interventions against these parasites

P6-05. Elucidating the Role of Inositol-tetrakisphosphate 1-kinase in *Trypanosoma cruzi*

Sabrina Elizabeth Cline[1], Miguel A. Chiurillo[2], Adolfo Saiardi[3], & Roberto Docampo[1]
[1]Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA 30602-7400, USA. [2]Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221, USA. [3]Medical Research Council Laboratory for Cell Biology, University College London, WC1E 6BT London, United Kingdom

Trypanosoma cruzi is an obligate intracellular parasite that is the causative agent for Chagas Disease, a chronic bloodborne infection that has infected eight million individuals and puts another eighty million at risk of infection in the Americas. Classically, inositol polyphosphate (IPP) synthesis occurs through the phosphoinositide phospholipase C (PLC)-catalyzed hydrolysis of PIP2 into IP3 and diacylglycerol and further IP3 phosphorylation by additional kinases. Recently, inositol-tetrakisphosphate 1-kinase (ITPK1) has been described as an enzyme that may mediate a lipid-independent IP6 soluble synthesis pathway through phosphorylation of IP1 and other intermediates in the cytosol. The role of ITPK1 in the protozoan parasite, *Trypanosoma cruzi*, is unknown. Preliminary bioinformatics research has identified hypothetical protein TcCLB.503885.50 as a potential *T. cruzi* ITPK1 (TcITPK1) homolog. Further phylogenetic studies have demonstrated this hypothetical protein to be an ortholog of human ITPK1, a protein previously established to follow the lipid-independent IPP synthesis pathway. The ability of TcITPK1 to act as the mediator for this alternative pathway has been established through plc1Δ yeast complementation assays and SAX-HPLC analysis of radioactively labeled inositol in the complemented yeast. TcITPK1 has been endogenously tagged using CRISPR/Cas9 genetic manipulation techniques to allow for subcellular localization studies within the parasite. Immunofluorescent assays have shown this protein to localize to the cytosol, the site of the lipid-independent IPP synthesis pathway. Future experiments related to enzymatic activity and CRISPR/Cas9 knockdown will aid in advancing the characterization of TcITPK1 and the role of lipid-independent IPP synthesis in the parasitic life cycle.

P6-06. *Toxoplasma gondii* Possesses a Functional Coproporphyrinogen Dehydrogenase for Its Heme Production

Melanie Key[1], Katherine Floyd[1], Zhicheng Dou[1]
[1]Department of Biological Sciences, Clemson University, Clemson, SC 29634

Toxoplasma gondii is an apicomplexan parasite that causes opportunistic infections in immunocompromised individuals and pregnant women. Due to its obligate intracellular nature, *T. gondii* requires vital nutrients either scavenged from the host or synthesized de novo. Heme, a vital nutrient across all domains of life, is synthesized by parasites de novo and influences *T. gondii* growth and virulence. While some strains genetically ablated of heme synthesis enzymes still remain viable in culture, the mechanism in which these strains maintain their vitality upon heme deficits is unknown. One possibility is through the non-canonical isozyme of the heme synthesis enzyme coproporphyrinogen III oxidase (TgCPOX), coproporphyrinogen dehydrogenase (TgCPDH). TgCPDH is an oxygen-independent enzyme that actively participates in synthesis of heme. In our study, we successfully complemented TgCPDH in the *Salmonella* TE3006 strain that lacks the bacterial orthologs of CPDH and CPOX. The resulting trans-genera complementation strain restored its growth in heme-free medium, supporting that TgCPDH is an active enzyme. In addition, TgCPDH is upregulated ~2-fold in the knockout strain for its isozyme, Δcpox. With this logic, we sought to determine if overexpression of TgCPDH in Δcpox would improve its growth rate, which has been previously shown to be attenuated by 50%. Here, we show that overexpression of TgCPDH in Δcpox increases its growth rate, further highlighting its role in heme synthesis of *Toxoplasma gondii*.

P6-07. Identification of the Target Proteins of Inositol Pyrophosphates in *Leishmania*: A Preliminary Study

Yete G. Ferri[1], Jennifer A. Black[2,3], Luiz R. O. Tosi[3], Marcelo S. da Silva[1]
[1]Institute of Biosciences - Department of Chemical and Biological Sciences, São Paulo State University (UNESP), Botucatu, Brazil. [2]University of Glasgow, Glasgow, United Kingdom. [3]Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil

In model eukaryotes, inositol pyrophosphates (PP-IPs) – mainly PP-IP4, IP7, and IP8 – are involved in a wide range of cellular processes, such as telomere length regulation and homologous recombination. However, putative target proteins of PP-IPs, as well as their mechanisms of action, are still poorly understood. PP-IP4, IP7, and IP8 are synthesized through complementary pathways requiring the kinases IP6K and PP-IP5K. Trypanosomatids, a group of single-celled eukaryotes containing parasites of medical importance (e.g., *Leishmania* spp. and *Trypanosoma* spp.), have an ortholog of IP6K, but lack orthologs of PP-IP5K, meaning they are unable to synthesize IP8 and making these parasites excellent models to study the functions of IP7. Thus, in this work, we will express recombinant IP6K from *Leishmania braziliensis* allowing us to produce IP7 in vitro labeled with γ-(Propargil)-imido on the β-phosphate moiety (IP7-labeled). This approach will allow us to selectively conjugation biotin, via a click chemistry reaction, to proteins which have receive the β-phosphate from IP7-labeled. We can then isolate biotin labeled proteins using strepdavidin immunoprecipitation and identify them with mass spectrometry. We will use our approach to track pyrophosphorylated proteins in *L. braziliensis* protein extracts. We will examine protein extracts from WT cells and in an *L. braziliensis* mutant lacking IP6K, which we have previously challenged with genotoxic agents to investigate pyrophosphorylation during the *Leishmania* DNA damage response. Given pyrophosphorylation by PP-IPs is apparently not enzymatic and still a poorly understood mechanism of intracellular signaling, our work may uncover new avenues for drug development and therefore the treatment of leishmaniasis. In addition, understanding this mechanism can help to transpose these analyses for other organisms or cell types in which pyrophosphorylation by PP-IPs may play a key role in disease development, such as cancer.

P6-08. Do Arboviruses Manipulate Their Mosquito Vector's Thermal Preference to Increase Transmission?

Haziqah-Rashid[1], Marcus S.C Blagrove[1], Matthew Baylis[2,3]
[1]Department of Evolution, Ecology and Behaviour, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Brownlow Hill, Liverpool L3 5RF, UK, [2]Health Protection Research Unit in Emerging and Zoonotic Infections, University of Liverpool, UK, [3]Department of Livestock and One Health, Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Brownlow Hill, Liverpool L3 5RF, UK

There is a conflict of interests between the mosquito and arboviruses thermal preferences where the mosquito generally has a much lower optimal temperature than the arbovirus. For instance, Dengue virus is known to infect the brain of the mosquito, and showed some evidence of causing behavioural changes in infected mosquitoes. Thus, this project aims to study the preference of a higher temperature in the mosquitoes upon infection with arboviruses. The mosquitoes will be infected with arboviruses orally and preferences of the mosquitoes towards different temperatures will be observed using a two chamber test, where the mosquitoes have a free choice of two conditions. Result from this study can be used in predicting areas in which the risk posed has been significantly underestimated. For example, in cooler climates where warm microclimates are frequent; here, infected mosquitoes could seek out warmer resting places to speed up virus development.

P6-09. IDentifying the Earliest Factors Required for Host Cell Subversion by *Plasmodium falciparum*

Manuel A. Fierro[1], Timothy Nessel[1], Josh R. Beck[1]
[1]Dept. Biomedical Sciences, Iowa State University, Ames, IA 50011

As an obligate intracellular parasite, *Plasmodium falciparum* establishes a niche within its host cell by translocating an arsenal of effector proteins across an encasing parasite vacuole (PV) and into the host cytosol. Exported proteins initially reach the PV via secretory organelle release immediately after invasion and subsequently through the default secretory pathway during intraerythrocytic development. While many exported proteins have been identified and characterized, little is known about the earliest PV machinery and effectors that the parasite requires to establish residency within RBCs. The dense granules (DGs) are discharged immediately after invasion, delivering the PTEX translocon into the PV membrane along with the earliest known exported effector RESA. Although to date only 6 proteins have been localized to the DGs, we hypothesize that more factors are released by these organelles for host manipulation. We aim to identify these early effectors by harnessing the power of the promiscuous biotin ligase TurboID. To resolve the contents of DGs, we developed a strategy to conditionally fuse TurboID to the C-terminus of several dense granule proteins using the dimerizable Cre recombinase. This approach enables selective targeting of TurboID to forming merozoite DGs but not the mother PV, allowing selective biotinylation of the DG compartment. In a complementary approach we are determining a time-resolved proteome of the nascent PV from the time of formation through the ring-stage. This approach is carried out in the context of a parasite line that allows for conditional inactivation of PTEX function, trapping early effectors in the PV to aid in their identification. We show that TurboID is active in the early PV and inactivation of PTEX reveals differential biotinylation patterns by western blot. Together, these proteomic approaches will enable discovery of the early exportome and PV machinery of *P. falciparum*, shedding light on the earliest events in RBC subversion.

P6-10. Exploring the Relation Between MICU1 and MICU2 in *Trypanosoma cruzi* by Generation of TcMICU1-KO/TcMICU2-KO Cells

Mayara S. Bertolini and Roberto Docampo
Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, Georgia, United States

The mitochondrial calcium uptake in trypanosomatids, which belong to the eukaryotic supergroup Discoba, shares biochemical characteristics with that of animals, which, together with fungi, belong to the supergroup Opisthokonta. However, the composition of the mitochondrial calcium uniporter (MCU) complex in trypanosomatids is quite peculiar, suggesting lineage-specific adaptations. The role of regulatory subunits mitochondrial calcium uptake 1 (MICU1) and 2 (MICU2) proteins present in this complex was recently described by our group using *T. cruzi*. TcMICU1 and TcMICU2 have mitochondrial targeting signals, two canonical EF-hand calcium binding domains, and localize to the mitochondria. Ablation of either TcMICU1 or TcMICU2 showed a significantly reduced mitochondrial calcium uptake in permeabilized epimastigotes without dissipation of the mitochondrial membrane potential. In mammalian cells the expression levels of the MICU1 and MICU2 subunits are dependent of each other and they act together to establish the cytosolic calcium response threshold. However, none of these proteins had a gatekeeper function at low calcium concentrations, as occurs with their mammalian orthologs. In this work, using the CRISPR/Cas9 system, we designed a strategy to generated TcMICU1/TcMICU2 knockout cells. Ablation of TcMICU1 and TcMICU2 showed a reduced mitochondrial calcium uptake in permeabilized epimastigotes without dissipation of the mitochondrial membrane potential, as TcMICU1-KO or TcMICU2-KO cells. Further expression analysis of TcMICU1 or TcMICU2 using TcMICU1-KO or TcMICU2-KO cell lines generated before will be performed to clarify whether the expression level of these proteins is related to each other.

P6-11. Characterization of an Essential Golgi Localized Secreted Effector Binding Protein of *Toxoplasma gondii*

Edwin Pierre Louis[1&2], Menna Etheridge[1&2], R Drew Etheridge[1&2]
[1]Cellular Biology, University of Georgia, GA 30602. [2]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30602

The apicomplexan parasite *Toxoplasma gondii* contains three unique organelles known as micronemes, rhoptries and dense granules (DG) that form an intricate secretory system critical for its life cycle. *T. gondii* relies on the sequential release of these organelles to invade a host cell and establish a parasitophorous vacuole (PV). The microneme (MIC) and rhoptry (ROP) proteins are first discharged to allow parasite motility, attachment, and host cell invasion respectively. Upon invasion of the host cell, *T. gondii* forms a PV from the host plasma membrane and releases its dense granules (GRA) proteins to reshape this PV structure using a large repertoire of secreted GRAs that manipulate the host cell. Proteins destined for secretion translocate from the endoplasmic reticulum (ER) to the Golgi complex, which coordinates the maturation of these proteins and sorts them to their appropriate secretory organelle. Previously, extensive studies in *T. gondii* protein secretion have demonstrated the regulatory mechanisms necessary for transporting MIC and ROP proteins from the Golgi to their distinct secretory vesicles. However, it remains largely unknown how the GRA proteins are sorted to the dense granules. Here, we mined the interactome of the previously characterized DG secreted effector TgIST and found an essential hypothetical Golgi resident protein with a predicted type I transmembrane topology. Through immunoprecipitation and mass spectrometry analyses, we identified a number of previously characterized dense granule targeted secreted effectors that also associate with this protein. Additionally, several highly conserved vesicular trafficking proteins, were also identified as well as a number of hypothetical proteins. We subsequently knocked down this secreted effector binding protein 1, (SEB1), and observed a significant growth impairment in vitro. Through endogenous tagging of interacting hypothetical proteins we have identified a number of novel dense granule proteins secreted into the parasitophorous vacuole. Altogether, this work, seeks to determine the role of SEB1 as a potential cargo receptor required for the proper targeting of GRA proteins to their target vesicles.

P6-12. Protein Polyphosphorylation and Aggregation by Inorganic Polyphosphate in Trypanosomes

Logan P. Crowe[1], Raquel Negreiros[1] and Roberto Docampo[1,2]
[1]Center for Tropical and Emerging Global Diseases and [2]Department of Cellular Biology, University of Georgia, Athens, GA 30602

Inorganic polyphosphate (polyP) is a linear polymer of orthophosphates that can range from 3 to several hundred subunits that are found in all cells. Polyphosphate has been implicated in many processes from serving as an energy source to acting as a post-translational modification (PTM) and regulating gene expression. In the trypanosomatid parasites, *Trypanosoma cruzi* and *Trypanosoma brucei*, polyP was first found in acidocalcisomes; however, recent work from our lab has identified the presence of polyP in glycosomes and the nucleolus. Using a combination of biotinylated polyP and a bioinformatics approach, we have identified several glycolytic/gluconeogenic proteins, ribosomal proteins, and other nucleolar proteins that putatively interact with polyP. Recombinant enzyme assays have demonstrated the ability for polyP to cause protein aggregation of glycosomal proteins and inhibition of enzyme activity, suggesting a possible role for polyP in regulation of glycolysis and gluconeogenesis. Additionally, we have observed non-enzymatic polyphosphorylation of recombinant proteins in vitro and in vivo via mobility shift assays. In this work, we present evidence that polyP may act as a regulator of protein activity either through direct interaction as a PTM or as an inhibitor/activator in enzyme activity. Furthermore, we explore other proteins harboring PASK domains that may interact with polyphosphate that were identified in our bioinformatics screen.

P7-01. Investigating *T. gondii* Vacuolar Compartment/Plant-like Vacuole Physiology via the Ratiometric GFP Reporter pHluorin2

L. Brock Thornton[1], Davis Osborn[1], Amy Bergmann[1], Zhicheng Dou[1]
[1]Department of Biological Sciences, Clemson University, Clemson, SC 29631

Toxoplasma gondii is an obligate intracellular parasite, which infects approximately one-third of the human population. Within *T. gondii*, the lysosome-like vacuolar compartment/plant-like vacuole (VAC/PLV) is an important organelle that houses proteolytic enzymes which require an acidic pH in order to function efficiently. The endolysosomal system within *T. gondii* parasites includes organelles such as the VAC as well as the endosome-like compartment (ELC), which are involved in the endocytosis and digestion of host proteins. Previously, we observed an aberrant overlap of the swollen VAC and the ELC within a mutant strain of parasites which lack the VAC-residing transmembrane chloroquine resistance transporter (CRT; Δcrt). This aberrant colocalization of the VAC and ELC was not observed in the WT and complement (ΔcrtCRT) strains. We hypothesize that the overlap of these two organelles is likely to alter the physiology of the VAC and ultimately inhibit proper function of the organelle. In order to investigate possible perturbation of the vacuolar pH within during the aberrantly colocalized state observed in Δcrt, we introduced the pH-sensitive GFP biosensor, pHluorin2, into WT, Δcrt, and ΔcrtCRT parasites to quantify both the cytosolic and the vacuolar pH within these strains. We found that there is a consistent increase in the I405/I485 ratio of emission intensities at 528 nm within Δcrt indicating that there is also an increase in the vacuolar pH within the mutant. Furthermore, we showed that we can utilize various drugs which function to neutralize the normally acidic VAC/PLV to induce measurable increases in the vacuolar pH. Collectively, our work will provide a better understanding of how the physiology of the lysosome-like VAC in *Toxoplasma* is altered within a disorganized endolysosomal system and may shed light on possible mechanisms for disrupting the ability of *T. gondii* to regulate the physiology of this essential organelle.

P7-02. Development of Transfection Approaches for Use in *Naegleria fowleri*

Jillian Milanes, Alexander Richardson, Sarah Grace McAlpine, James Morris
Clemson University Eukaryotic Pathogens Innovation Center. Department of Genetics and Biochemistry, Clemson University, Clemson, SC

The pathogenic free-living amoeba, *Naegleria fowleri*, is the causative agent of primary amoebic meningoencephalitis (PAM), a rare yet usually lethal infection of the brain. In an effort to identify new targets for therapeutic intervention, we are working to develop molecular tools for use in *N. fowleri* to assess the importance of potential target genes. We have developed a series of expression vectors for use in the amoebae and in parallel have been using a series of modified approaches used in other parasite systems for plasmid delivery including the transfection reagent SuperFect, Amaxa Nucleofector technologies, and varying electroporation settings. Transfection of *N. fowleri* flagellates with 5g of the plasmid pJMJM1, containing 858bp of the 5'UTR of actin 1 (NF0111190) upstream of both eYFP and a hygromycin resistance gene by electroporation (100V, 500µF, 400Ω) yielded a population of fluorescent cells 7 days after being treated with 300µg/mL Hygromycin. The expression of eYFP diminished through time and by day 15, the transfected, drug-resistant culture was indistinguishable by fluorescence microscopy from the control. Currently, we are modifying pJMJM1 to promote stable expression of both markers. In addition, we have started to explore the possibilities of using RNA interference in the amoeba. We have cloned ~200bp of target genes into pZJM, a vector that harbors opposing T7 promoters that flank the insert. We have transformed pZJM into bacteria that harbor inducible T7 polymerase and have isolated dsRNA for delivery by soaking and electroporation into *N. fowleri*. Initial experiments suggest that soaking amoebae with dsRNA may be useful, as treated cells have reduced growth. We anticipate that the tools developed here will be useful for uncovering potential genes that can be targeted for drug discovery.

P7-03. Functional Analysis Shows that Outer Dynein Arm Light Chain-2 is Essential for Directional Flagellar Motility in *Trypanosoma brucei*

Subash Godar[1,4], James Oristian[2,3], Valerie Hinsch[2], and Joshua Alper[1,3,4]
[1]Department of Physics and Astronomy, Clemson University, Clemson, SC 29631, [2]Department of Genetics and Biochemistry, Clemson University, Clemson, SC 29631, [3]Department of Biological Sciences, Clemson University, Clemson, SC 29631, [4]Eukaryotic Pathogen Innovation Center, Clemson University, Clemson, SC 29631

The flagellar motility of kinetoplastids, which is essential for viability, virulence, cell morphology, and cytokinesis, exhibits a bending wave that uniquely propagates from the flagellum's tip to its base, rather than base-to-tip as in all other eukaryotes (including humans), driven by thousands of dynein arms decorated along the axoneme. Much evidence suggests that dynein-associated light and intermediate chains, including specific evidence from outer arm dynein (OAD) light chain 2 (LC2) in *Chlamydomonas reinhardtii*, regulate the biophysical mechanisms of OADs leading to specific flagellar bending wave propagation direction, amplitude, and frequency. However, dynein light chains' role in determining tip-to-base motility of flagella in disease-causing kinetoplastid parasites like *Trypanosoma brucei* remains unknown. We identified an LC2 homolog in *T. brucei* procyclic form (Tb927.9.12820, TbLC2), knocked down its expression in wild-type and a flagellar attachment zone protein (Flam3) knockdown cells using RNA interference (RNAi), and rescued the knockdown phenotypes with eGFP-tagged TbLC2. We found that TbLC2 localized along the axoneme and that LC2 knocked down cells showed highly non-directional swimming behavior at a reduced velocity and exhibited a flagellar oscillation characterized by an asymmetric beat at a significantly higher frequency. Together, these findings suggest that TbLC2 regulates OAD-based tip-to-base forward flagellar motility in *T. brucei*. Current treatments for kinetoplastid diseases show limited efficacy and difficulty in administration. Therefore, we expect that characterization of the regulatory roles of multi-subunit structures like LC2 in understanding the primary mechanisms underlying unique kinetoplastid dynein-driven flagellar motility will ultimately help to establish flagellar proteins as potential candidates for pan-kinetoplastid drug development.

P7-04. Identifying Proteins Required for Export of Effectors to the *Plasmodium falciparum* Infected Erythrocyte

David Anaguano-Pillajo, Vasant Muralidharan
University of Georgia

Malaria is a global and deadly human disease caused by the apicomplexan parasites of the genus *Plasmodium*. Infection with one species, *Plasmodium falciparum*, is the deadliest and widest distributed worldwide. Parasite proliferation within human red blood cells is associated with the clinical manifestations of the disease, including severe cardiac complications. During this intraerythrocytic cycle, *P. falciparum* completely remodels the red blood cell to establish a favorable habitat for its proper development. This essential process requires the export of many proteins from the parasite to the RBC cytoplasm and membrane, and the formation of intricate protein interaction networks via poorly understood mechanisms. One crucial but still unidentified mechanism involves the transport of proteins lacking an export-related conserved motif (PNEPs) from the endoplasmic reticulum into the parasitophorous vacuole. To understand the trafficking process of PNEPs within infected red blood cells, I will use a quantitative proteomics approach applying cutting-edge proximity labeling, to identify potential participants on their export pathway in a much more efficient and precise way compared to previous methods. First, I will use TurboID, a recently engineered promiscuous biotin ligase, to label binding partners of the PNEP, PfSBP1, during its export from the parasite plasma membrane to the PV. Then, using immunoprecipitation and mass spectrometry, I will identify the PfSBP1 interactors for further characterization of their role during the export of PNEPs. Achieving these goals will help to extend the current knowledge on effectors export and trafficking, an essential process for *P. falciparum* virulence within the human host.

P7-05. The Role of Oxygen-dependent Glycosylation on SCF (Skp1-Cullin-1-Fbox) Regulation in *Toxoplasma gondii*

Elisabet Gas-Pascual[1,2], Msano Mandalasi[1,2], Bowen Deng[1], and Christopher M. West[1,2,3]
[1]Dept. of Biochemistry & Molecular Biology, [2]Center for Tropical and Emerging Global Diseases, [3]Complex Carbohydrate Research Center, University of Georgia, Athens

The SCF (Skp1/cullin-1/F-box protein) class of E3 ubiquitin ligases plays a critical role in proteostasis in eukaryotes. In this complex, the Skp1 adaptor binds different F-box proteins (FBPs), but how Skp1 selects FBPs for incorporation into the SCF is unclear. In Dictyostelium, *Toxoplasma gondii* and other protists, Skp1 is modified with a pentasaccharide on a hydroxyproline added by the PhyA prolyl hydroxylase in an oxygen dependent manner. Here we report on the TgSkp1 interactome in tachyzoites and how Skp1 glycosylation alters it. We used RH and phyAΔ *Toxoplasma* strains in which Skp1 was endogenously SF-TAP tagged, and performed co-immunoprecipitations followed by nLC/MS analysis. This identified 36 interactors, including Skp1 modifying enzymes (PhyA and Gat1), 15 predicted FBPs (based on sequence features) and SCF subunits (Cul1, Rbx1, Skp1). Interestingly, the representation of seven interaction partners was decreased >3-fold in phyAΔ cells. The interaction with three proteins was further examined after genomic HA-tagging. This allowed confirmation of the Skp1 interaction and revealed that the 3-6-fold decreased representation of FbxO13 and FbxO14 in phyAΔ was explained by a corresponding decrease in their total levels, whereas FbxO1 was essentially unaffected by either measure. A similar effect on FbxO13 in gnt1Δ cells indicated that glycosylation per se is important for the effect. Partial restoration of FbxO13 and FbxO14 levels following proteasomal inhibition of phyAΔ cells is consistent with their selective degradation by autoubiquitination when Skp1 is unmodified. Sequence analysis predicts that FbxO13 is a jumonji C domain containing lysyl hydroxylase that modulates transcriptional processes, and fitness studies suggest that it is essential. In contrast, FbxO14 is present only in apicomplexans that harbor Skp1 modification genes, suggesting a role associated with oxygen sensing. Our studies reveal a novel mechanism of cellular regulation coupled to oxygen sensing, and FbxO13 and FbxO14 offer leads to cellular response pathways.

P7-06. Simultaneous Ingestion of Carbohydrates and Proteins Induces Continuous Oogenesis in Mosquitoes

Ruby E. Harrison[1†], Kangkang Chen[1,2†], Lilith South[1], Mark R. Brown[1], Michael R. Strand[1]
[†]These authors contributed equally to this work. [1]Department of Entomology, The University of Georgia, Athens, GA, 30602 USA. [2]Department of Plant Protection, College of Horticulture and Plant Protection, Yangzhou University, Yangzhou, 225009 China

Female mosquitoes require a blood meal to produce eggs, and while females typically obtain carbohydrate resources from nectar, they remain in a state of reproductive arrest until blood-feeding occurs. Ingestion of proteins sourced from vertebrate blood triggers an endocrine signaling cascade driving maturation of a clutch of ~100 eggs. Females can drink blood and lay eggs in repeated consecutive cycles, greatly facilitating potential transmission of viral, protozoan, and filarial pathogens to their vertebrate hosts. Here, we report that artificial meals containing high levels of both carbohydrates and proteins induced dysregulation of mosquito reproductive endocrine function. Ad libitum access to protein-sugar meals consisting of bovine serum albumin, rabbit hemoglobin, or tryptone solubilized in 10% sucrose promoted continuous, i.e. non-cyclic, egg formation in mosquitoes. Females offered protein-sugar meals imbibed the solution frequently and exhibited perpetual activation of midgut digestive enzymes as well as inhibition of host-seeking behavior. We determined that several genetic markers of reproductive endocrine signaling were constitutively expressed at low levels, in contrast to the strong peak in upregulation followed by rapid decline of these genes in blood-fed controls. Finally, we observed that protein-sugar meals caused maturing oocytes to develop heterogeneously rather than in uniform cohorts. Taken together, our results indicate that simultaneous ingestion of carbohydrates and protein drives continuous reproductive endocrine signaling in mosquitoes, leading to a breakdown of the regimented cyclicity of oogenesis in these animals.

P7-07. Analysis of *P. falciparum* Field Isolates for Mutations in the Chloroquine Resistance Transporter and Kelch13 Propeller Genes

Lui P. Suzuki-Williams[1,2], Alona Botnar[1,3], Anne Elliot[1], Dennis E. Kyle[1,3,4]
[1]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA. [2]Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA. [3]Department of Infectious Diseases, University of Georgia, Athens, GA. [4]Department of Cellular Biology, University of Georgia, Athens, GA

As reported by the World Health Organization, malaria accounts for nearly half a million deaths every year. Among the five human-infecting species of *Plasmodium*, *P. falciparum* is the most lethal. Billions of dollars are invested in malaria mitigation and elimination efforts, but effective antimalarial treatment is dwindling as drug resistance grows. Historically, artemisinin-based combination therapy with dihydroartemisinin-piperaquine was a commonly recommended treatment option by the World Health Organization. In parts of Southeast Asia, endemic regions recorded recrudescence of *P. falciparum* parasites to the antimalarial combination. In various studies, artemisinin resistance was linked to mutations in the propeller domain of the *P. falciparum* Kelch13 gene. Studies also demonstrated an association between piperaquine resistance and mutations in the Chloroquine Resistance Transporter gene. These resistance phenotypes can quickly dominate the population pool of *P. falciparum* strains and requires careful monitoring. Through decades of fieldwork, our laboratory has acquired numerous *P. falciparum* field isolates across several endemic regions of the world. In these studies, we have extracted DNA from these parasites and conducted polymerase chain reactions to amplify the Kelch13 Propeller and *Plasmodium falciparum* Chloroquine Resistance Transporter genes. We then conducted Sanger Sequencing and examined the isolates for amino acid changes or single nucleotide polymorphisms at the loci that might confer drug resistance. Identification of strains containing these mutations can offer a historical context to the development of artemisinin and piperaquine resistance in *P. falciparum*. As a result, this research can help inform us about patterns in drug resistance, specifically how current and past application of treatment plans may be selecting for drug resistant parasites. By surveying for resistance emergence in endemic regions, this knowledge can help healthcare systems quickly adapt and switch to different drug regimens, and hopefully circumvent this problem of drug resistance.

P7-08. Are Plant Derived Adjuvants Providing a Path in Thwarting Emerging Drug Resistant Malaria

Pooja Rani Mina
CIMAP

The investigation of chemotherapeutic agents for the treatment of malaria is essential for malaria elimination. However, most of antimalarial drugs resulted in drug resistance and severe side effects causing failure of malaria elimination programmes. Drug resistance is still a problem to malaria endemic areas. To combat drug resistance there is a requirement of new strategies with low cost and minimal toxicity that may be incorporated in elimination of drug resistant malaria burden itself or in combination. Combination therapy is more emerging concern to control drug resistant malaria. The discovery of combinations of antimalarial drugs that act synergistically with one another is hence of great importance. Plant derived products are highly accessible and available which can be used as partner drug with recommended ACTs and other clinically used drugs. Considering the above problems, a systematic presentation will give how natural product interact synergistically with the existing clinically used drugs and further studies that are required to be taken up for establishing their therapeutic potential.

P7-09. Does a Novel *Toxoplasma gondii* O-fucosyltransferase Modulate the Localization of Target Proteins?

Megna Tiwari, Msano Mandalasi, and Christopher M. West
Center for Tropical and Emerging Global Diseases, Department of Biochemistry and Molecular Biology, University of Georgia, Athens

Apicomplexans are a diverse group of intracellular protozoan pathogens, responsible for the diseases cryptosporidiosis, malaria, and toxoplasmosis that collectively contribute to global morbidity, mortality and substantial economic loss. *Toxoplasma gondii*, the causative agent of toxoplasmosis, infects approximately 30% of the human population worldwide. The parasite propagates between an asexual and sexual life cycle in which the parasite must adapt to its various environments. These adaptations require tight regulation of gene expression and protein activity. While gene regulation has been extensively studied, our understanding of post-translational modifications (PTMs) is lagging. Of interest is the recent discovery of an O-fucosyltransferase (OFT) encoded by the spy locus that modifies Ser/Thr residues of at least 33 different nucleocytoplasmic proteins with a single fucose residue during the proliferative phase of the parasite in fibroblasts. This process is related to the O-GlcNAcylation of cytoplasmic, nuclear, and mitochondrial proteins of animal and plant cells via O-GlcNAc transferases (OGTs), in which N-acetylglucosamine is transferred to Ser/Thr residues. Knock out and complementation studies of the OFT demonstrated that the transferase is involved in the modification of proteins in assemblies associated with the nuclear pore complex. O-fucosylation may be important for sequestering critical proteins that can be rapidly utilized during stress response. Although TgSPY is non-essential, it is required for efficient plaque growth in fibroblasts. Studies based of artificial, overexpressed OFT substrate constructs suggest that O-fucosylation is required for protein stability. We have selected 7 essential Spy target proteins for further study by generating endogenously epitope tagged O-fucosylated proteins in WT and in TgSPY KO cell lines. We are examining the localization and function of the proteins to determine the role of the O-fucosylation PTM in *T. gondii*.

P7-10. Generation of *Trypanosoma brucei* CRISPR/Cas9 Knockouts to Understand the Role of Tubulin Post Translational Modifications

Katherine Wentworth[1,3], Subash Godar[2,3], Lucy Fisher[1,3], Joshua Alper[1,2,3]
[1]Department of Biological Sciences, Clemson University, Clemson, SC [2]Department of Physics and Astronomy, Clemson University, Clemson, SC [3]Eukaryotic Pathogens Innovation Center, Clemson University, Clemson, SC

Trypanosoma brucei relies on a unique motility mechanism for its virulence: the flagellum beats with a bending waveform that alternates between base-to-tip and tip-to-base propagation. The Intraflagellar structure within the flagellum in the axoneme, composed of microtubules and axonemal dynein. Dynein has been observed to be directly regulated by small proteins which bind to the complex; however, the impact of PTMs on the biophysical properties of dynein, such as binding affinity and velocity, remain largely unknown. One mechanism that cells can use to regulate dynein is controlling the biochemical natre of axonemal microtubules through post-translational modifications (PTMs), including detyrosination of the C-terminal tail of tubulin and acetylation of microtubular luminal K40 on alpha-tubulin, and the axonemal microtubules in *T. brucei* are largely acetylated and detyrosinated. To quantify the regulation of axonemal dynein in trypanosomes, we are knocking out the enzymes that remove and add tyrosine and acetyl groups using CRISPR/Cas9. We are confirming knockout generation through genomic sequencing, RT-qPCR, and western blots. We then analyze the phenotypic effects in vivo with high-speed video microscopy and in vitro using dynein ATPase and motility assays performed with tubulin extracted from the knockout strains. We expect to find altered flagellar motility and improper cellular division of *T. brucei* knockouts, and we will quantify the molecular mechanisms behind these phenotypes with in vitro ATPase experiments. Ultimately, this work will further our understanding of dynein regulation mechanisms and how these effect waveform patterns in *T. brucei*.

P7-11. Molecular and Metabolic Resistance Mechanisms in Multiple Insecticides Resistant *Culex quinquefasciatus* Population from Lagos, Nigeria

Ifeoluwa Kayode Fagbohun[1*], Emmanuel Taiwo Idowu[1], Tolulope Amos Oyeniyi[2], Oluwakemi Adesalu[2], Romoke Tawakalitu Jimoh[2], Ahmed Omotayo Idowu[2], Adepado Adeogun[2], Taiwo Samson Awolola[2], Olubunmi Adetoro Otubanjo[1]

[1]University of Lagos, Akoka, Yaba, Lagos State. [2]Vector research unit, Nigeria Institute of Medical Research, Lagos. Nigeria

The control and management of *Culex quinquefasciatus* borne diseases heavily rely on the use of insecticides-based measures but, the emergence and spread of insecticides resistance has been a challenge and led to major setback in recent gains against the control of mosquito borne diseases. Here, we assessed the susceptibility of *Cx. quinquefasciatus* to different classes of neurotoxic insecticides and also determined the possible resistance mechanisms. *Culex* mosquitoes were collected from Kosofe, Alimosho, Ibeju-Lekki and Badagry Local Government Areas (LGAs) of Lagos State. Adult females were exposed to DDT, permethrin, Bendiocarb and piperonyl butoxide (PBO) synergized assays using WHO protocols. Resistance mechanisms were assessed using molecular and biochemical techniques. Resistance to permethrin and DDT in *Cx. quinquefasciatus* was recorded in all the study locations with 24hours mortality ranging from 5% to 86%. Resistance to bendiocarb was also recorded in Alimosho (7%) and Kosofe (19%) LGAs while possible resistance (96%) in Ibeju-Lekki LGA. PBO synergists was able to reduce the KDT50 and KDT95 of the synergized bioassays significantly in contrast to the non-synergized bioassay. Heterozygote resistant Ace1R gene was detected in *Cx. quinquefasciatus* population from Kosofe and Alimosho, kdr L1014S and L1014F were not detected in this study. The activities of Cytochrome P450 monooxygenase and glutathione S-transferase detoxifying enzymes negatively correlates with 24hours percentage mortality of *Cx. quinquefasciatus*. Resistance reported in *Cx. quinquefasciatus* to multiple classes of insecticides may result in difficulty in control of lymphatic filariasis in these areas.

P7-12. Mechanisms of *Borrelia* Surface Lipoprotein Translocation Through the Outer Membrane

Huan He[1], Ankita Pramanik[1], Jacob J. Wiepen[1], Selene K. Swanson[2], Laurence Florens[2], Wolfram R. Zückert[1]

[1]University of Kansas School of Medicine, Department of Microbiology, Molecular Genetics and Immunology, Kansas City, Kansas [2]Stowers Institute for Medical Research, Proteomics Center, Kansas City, Missouri

Borrelia burgdorferi, the spirochetal agent of Lyme disease, is unique among diderm bacteria in its lack of lipopolysaccharide (LPS) in the outer membrane (OM) and its abundance of surface lipoproteins, which play major roles in bacterial transmission and virulence. Despite the importance of surface lipoproteins, little is known about how they are translocated to different cellular compartments. In this study, we characterized *B. burgdorferi* BB0838, an OM LPS assembly protein LptD homologue. Using a newly developed CRISPRi approach, we showed that BB0838 is essential for cell growth. Upon BB0838 knockdown, representative surface lipoproteins such as OspA and OspB were retained in the inner leaflet of OM, as determined by their inaccessibility to in situ proteolysis but presence in OM vesicles. The secretion, insertion and topology of the *B. burgdorferi* OM porin P66 remained unaffected. MudPIT mass spectrometry analysis of the *B. burgdorferi* membrane-associated proteome further confirmed the selective periplasmic retention of surface lipoproteins under BB0838 knockdown conditions. This indicates that BB0838 facilitates the essential terminal step in a distinctive spirochetal lipoprotein secretion pathway that evolved in parallel to the LPS secretion pathway in gram-negative bacteria. Hence, BB0838 and other essential lipoprotein secretion pathway components represent attractive novel targets for antimicrobials. Ongoing experiments are identifying and characterizing the periplasmic lipoprotein secretion pathway components that are feeding into the OM lipoprotein flippase and resolving lipoprotein secretion in time and space using fluorescent lipoprotein fusions.

P8-01. Adenylosuccinate Lyase and Adenylosuccinate Synthetase, Key Enzymes for Purine Salvage Pathway in *Leishmania donovani*

Jigneshkumar Mochi[1], Anju Pappachan[1]
Central University of Gujarat, Gandhinagar-382 030, Gujarat, India

Leishmania donovani is the causal agent of visceral leishmaniasis, also known as dumdum fever or kala-azar, which is the most severe form of leishmaniasis. Leishmaniasis affects more than 2 million people globally and existing treatment strategies of leishmaniasis has limitations due to expensive and toxic drugs, prolonged treatment, and emergence of drug resistant parasites. Hence, there is a need to identify and characterize the novel drug targets and to develop parasite-specific inhibitors. *Leishmania* is auxotrophic for purines and lack the enzymes to synthesize purine nucleotides de novo, therefore, they must depend upon the purine salvage system to utilize purine bases from their mammalian hosts. In the present study, we selected two enzymes Adenylosuccinate lyase (ADSL) and Adenylosuccinate synthetase (ADSS) from purine salvage pathways of *L. donovani* to characterize their structural and functional aspects to exploit them for selective inhibition of *Leishmania*. These two enzymes are crucial for the survival of the parasite at the same time not significant for the human host. The LdADSL and LdADSS genes were cloned in pET vectors. LdADSL protein was expressed, and purified successfully using Nickel affinity chromatography. LdADSS protein was expressed and purification standardization is going on. The tertiary structure of LdADSL was predicted by homology modeling using I-TASSER server. The LdADSL structure is a tetramer, and three monomers come together to form the active site of the enzyme. Based on structural analysis, we hypothesized that R40 and Y41 may be residues important for both the quaternary association as well as activity of the enzymes. To further study this, we have designed three mutations - R40A, R40E and Y41A. Structural and functional characterization of these mutants are underway. Our study may help to design more potential inhibitors for these important enzymes.

P8-02. An Essential Fringe-Like Protein in the *Plasmodium falciparum* Asexual Life Cycle

Alejandra Villegas, Vasant Muralidharan

Malaria is a deadly disease caused by the apicomplexan parasite *Plasmodium*. *Plasmodium* asexual replication occurs in the red blood cell (RBC) and is what causes clinical symptoms of disease. In the RBC, *Plasmodium* moves through 3 developmental stages ending with schizogony and creating 16-32 daughter merozoites. At the end of schizogony, merozoites egress the host cell by breaking out of two membranes, the parasitophorous vacuole membrane and the RBC membrane. Schizogony and successful egress allows for exponential *Plasmodium* replication and RBC infection. Our lab recently identified a B3GLCT-like protein in the *Plasmodium* ER (PfB3ER) that interacts with an essential ER chaperone and whose expression profile is similar to that of proteins required for egress. B3GLCT-like proteins are known to act as glucosyltransferases and work in concert with protein O-fucosyltransferases (POFUT2) to modify thrombospondin-like repeats in mammalian cells. Surprisingly, the *P. falciparum* POFUT2 has been shown to be non-essential while PfB3ER is predicted to be essential in the asexual stages. Based on PfB3ER homology to the mammalian B3GLCT and its predicted expression profile, we hypothesize that the glycosylation function of PfB3ER is required for efficient egress of *P. falciparum* from RBCs. To test this, we have employed CRISPR/Cas9 gene editing to HA-tag PfB3ER and create conditional mutants utilizing the TetR-DOZI aptamer system. We show that PfB3ER localizes to the ER, unlike previous published results that suggest that PfB3ER is exported to the host RBC, and that PfB3ER is primarily expressed during schizogony. Further, we show that PfB3ER is essential for the asexual replication of *P. falciparum*. Future studies will focus on the specific stage of the intraerythrocytic lifecycle that are affected by PfB3ER knockdown and test the requirement of its glycosyltransferase activity. Together, these data have uncovered an essential function for a putative glycosyltransferase in the intraerythrocytic lifecycle of *P. falciparum*.

P8-03.In vitro Hepatocyte Culture of Field-derived *Plasmodium falciparum*

Justine C. Shiau, Anne Elliot, Dennis E. Kyle
University of Georgia. CTEGD and Department of Infectious Diseases

Recent advances in establishing *Plasmodium falciparum* liver stages in human hepatocytes have provided new insights into human hepatocyte host-parasite interactions. Unlike erythrocytic stage cultures, where hundreds of isolates and clones are available to dissect genotypes and phenotypes, the vast majority of sporozoite-induced infections are limited to the Nf54 clone. In our studies, we found that Nf54 produces gametocytes and sporozoites, but the invasion and development rates in hepatocytes in vitro are very low. Therefore, we aimed to establish new transmission competent lines from field isolates that had been recently adapted to in vitro erythrocytic culture. We hypothesized that field-derived parasites that have not been in continuous culture for years would have a higher infection efficiency. In this study, we characterized three *P. falciparum* isolates, from Bangladesh (BD007), Cambodia (CB132), and Thailand (NHP4781), for infectivity and development on our in vitro primary hepatocyte culture system. We found at four-days post-infection, field isolates from Bangladesh and Cambodia had a higher efficiency in infecting hepatocytes than the lab strain, PfNF54, with a mean exoerythrocytic form of 27 ± 3.2, 49 ± 8.0, and 4 ± 0.6 (SEM), respectively. Additionally, we assessed the effect of hypoxia and normoxia on liver-stage parasite development with the Cambodian isolate. With higher hepatocyte infection efficiency, the in vitro *P. falciparum* and primary human hepatocyte model has the potential to further our understanding of host-parasite interactions during the quiescent stage of malaria infection. Lastly, the utilization of field isolates may also provide a glimpse of the ever-changing transmission dynamic out in the field.

P8-04. Structure of the Trypanosome Paraflagellar Rod and Insights into Non-Planar Motility of Eukaryotic Cells

Jiayan Zhang[1, 2, 3, *], Hui Wang[1, 3, 4, *], Simon Imhof[1], Xueting Zhou[1], Shiqing Liao[1], Ivo Atanasov[3], Wong H Hui[3], Kent L. Hill[1, 2, 3, @] & Z. Hong Zhou[1, 2, 3, @]

[1]Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles (UCLA), Los Angeles, California 90095, USA [2]Molecular Biology Institute, UCLA, Los Angeles, California 90095, USA [3]California NanoSystems Institute, UCLA, Los Angeles, California 90095, USA [4]Department of Bioengineering, UCLA, Los Angeles, California 90095, USA

Eukaryotic flagella (synonymous with cilia) rely on a microtubule-based axoneme, together with accessory filaments to carryout motility and signaling functions. While axoneme structures are well characterized, 3D ultrastructure of accessory filaments and their axoneme interface are mostly unknown, presenting a critical gap in understanding structural foundations of eukaryotic flagella. In the flagellum of the protozoan parasite *Trypanosoma brucei*, the axoneme is accompanied by a paraflagellar rod (PFR) that supports non-planar motility and signaling necessary for disease transmission and pathogenesis. Here, we employed cryogenic electron tomography (cryoET) with sub-tomographic averaging, to obtain structures of the PFR, PFR-axoneme connectors (PACs), and the axonemal central pair complex (CPC). The structures resolve how the 8nm repeat of the axonemal tubulin dimer interfaces with the 54nm repeat of the PFR, which consist of proximal, intermediate, and distal zones. In the distal zone, stacked “density scissors” connect with one another to form a “scissors stack network (SSN)” plane oriented 45 degree to the axoneme axis; and ~370 parallel SSN planes are connected by helix-rich wires into a paracrystalline array with ~90% empty space. Connections from these wires to the intermediate zone, then to overlapping layers of the proximal zone and to the PACs, and ultimately to the CPC point out a contiguous pathway for signal transmission. Together, our findings provide insights into flagellum-driven, non-planar helical motility of *T. brucei* and have broad implications ranging from cell motility and tensegrity in biology to engineering principles in bionics.

P8-05. The Malarial Stearoyl-coa Desaturase is Essential Only for Parasite Late Liver Stage Development

Sunil Kumar Narwal, Hadi Hasan Choudhary, Satish Mishra
Division of Molecular Parasitology and Imunology, CSIR-Central Drug Research Institute, Lucknow 226031, India

Apicomplexan parasite, *Plasmodium* replicate exclusively within host cells thus requires nutrients and fatty acids which are essential for membrane formation. One of the principal components of membrane is oleic acid and that is required for maintaining the membrane biophysical properties and fluidity. Malaria parasite *Plasmodium* is capable of modifying fatty acids and Stearoyl-CoA Δ9-desaturase (SCD) is an enzyme, catalysing the synthesis of oleic acid by desaturation of stearic acid. Expression analysis revealed the presence of Scd transcripts across all *Plasmodium* life cycle stages, with maximal expression in liver schizonts. This likely point to its role in membrane biogenesis required during merozoites formation. Disruption of Scd in rodent malaria parasite *P. berghei* did not affect parasite blood stage propagation, mosquito stage development and early liver stage development. However, when Scd knockout (KO) sporozoites were inoculated intravenously or by mosquito bite in mice, they failed to initiate the blood stage infection. Immunofluorescence analysis with MSP1 antibody of late liver stage exo-erythrocytic forms (EEFs) revealed that merozoites formations were impaired that normally initiate blood stage infections. RNA sequencing analysis of 55 h Scd KO EEFs revealed that attenuation at late developmental stage was associated with down regulation of genes central to general transcription, cell cycle, energy metabolism, fatty acid and merozoite formation. Further, C57BL/6 mice immunized with Scd KO parasites were protected against infectious sporozoites challenge. On the basis of these observations, we propose the development of a genetically attenuated *P. falciparum* parasites as a pre-erythrocytic stage vaccine candidate and SCD as a candidate drug target.

P8-06. Study of the Effects of Telomerase RNA (TER) Knockout in *Leishmania major*

Beatriz Cristina Dias de Oliveira, Maria Isabel Nogueira Cano
Chemical and Biologycal Sciences Dept., Biosciences Institute, São Paulo State University (UNESP), Botucatu, SP, Brazil

Parasites from the *Leishmania* genus causes leishmaniasis, a neglected tropical disease that affect people worldwide. The disease presents different clinical forms, high incidence rate and goes from self-healing lesions to the visceral lethal form. Despite of many efforts, until now there are no vaccines and efficient treatment protocols available and therefore, finding new antiparasitic therapeutic tools is crucial. Telomeres, the physical ends of eukaryotes chromosomes are key to maintain genome stability and have been considered potential targets against parasites. *Leishmania* spp. telomeres are composed by the conserved TTAGGG repeated sequence, maintained by telomerase. The enzyme is minimally composed by TERT (Reverse Telomerase Transcriptase), and TER (Telomerase RNA). In all eukaryotes TER is a unique molecule and contains the template sequence copied by TERT during telomere elongation. Our work has the aim to study the effects of LeishTER knockout (KO) in *Leishmania major* life span and development, using the CRISPR-Cas9 system. TER-double KO parasites clones were isolated using Petri dish selection in M199 supplemented with FBS in the presence of G418. LeishTER KO clones were confirmed by PCR and RT-PCR using wild type (wt) parasites as the controls. Our preliminary results show that in *L. major* the absence of LeishTER induces telomere shortening although does not affect cell proliferation and promastigote's cell cycle progression. We also observed that KO clones can stay longer in stationary phase and transform into metacyclics, which compared to wt are arrested in an unknown G1/S checkpoint. We intend to test the infectivity capacity of the KO clones in vivo and to analyze the impact of telomere shortening over long-term parasite cultivation, to verify the importance of telomeres in parasite survival and development.

P8-07. Analytical Sensitivity of Loopamp and Quantitative Real-time PCR on Dried Blood Spots and Their Potential Role in Monitoring Human African Trypanosomiasis Elimination

Charlie Franck Alfred Compaoré[1, 2], Hamidou Ilboudo[3], Jacques Kaboré[1,2], Justin Windingoudi Kaboré[1,4], Oumou Camara[4], Mohamed Bamba[1], Hassane Sakande[1], Minayégninrin Koné[5], Mamadou Camara[4], Dramane Kaba[5], Adrien Marie Gaston Belem[2], Stijn Deborggraeve[6], Philippe Büscher[6], Bruno Bucheton[4,7], Veerle Lejon[6], Vincent Jamonneau[7]

[1]Centre International of Recherche-Développement sur l'Elevage en Zone Subhumide, Unité de Recherche sur les Maladies à Vecteurs et Biodiversité, 01 BP 454, BoboDioulasso 01, Burkina Faso, [2]Université Nazi Boni, Unité de Formation et de Recherche Sciences et Techniques, 01 BP 1091, Bobo-Dioulasso, Burkina Faso, [3]Institut de Recherche en Sciences de la Santé, Unité de Recherche Clinique de Nanoro, 11 BP 218, Ouagadougou CMS 11, Burkina Faso, [4]Programme National de Lutte Contre la Trypanosomiase Humaine Africaine, BP 581, Conakry, Guinea, [5]Institut Pierre Richet, Unité de Recherche « Trypanosomoses », 01 BP 1500, Bouake, West Africa, [6]Institute of Tropical Medicine, Department of Biomedical Sciences, Nationalestraat 155, B-2000 Antwerp, Belgium, [7]Institut de Recherche pour le Développement, UMR INTERTRYP IRD-CIRAD, Université de Montpellier, TA A-17/G, Campus International de Baillarguet, F-34398, Montpellier, France

The objective set by WHO to reach elimination of human African trypanosomiasis (HAT) as a public health problem by 2020 is being achieved. The next target is the interruption of gambiense-HAT transmission in humans by 2030. To monitor progress towards this target, in areas where specialized local HAT control capacities will disappear, is a major challenge. Test specimens should be easily collectable and safely transportable such as dried blood spots (DBS). Monitoring tests performed in regional reference centres should be reliable, cheap and allow analysis of large numbers of specimens in a high-throughput format. The aim of this study was to assess the analytical sensitivity of Loopamp, M18S quantitative real-time PCR (M18S qPCR) and TgsGP qPCR as molecular diagnostic tests for the presence of *Trypanosoma brucei gambiense* in DBS. The sensitivity of the Loopamp test, with a detection limit of 100 trypanosomes/mL, was in the range of parasitaemias commonly observed in HAT patients, while detection limits for M18S and TgsGP qPCR were respectively 1000 and 10,000 trypanosomes/mL. None of the tests was entirely suitable for high-throughput use and further development and implementation of sensitive high-throughput molecular tools for monitoring HAT elimination are needed.

P8-08. Developing a *Crithidia* Parasite CURE (Course Undergraduate Research Experience) for Biochemistry Lab Using Metabolically Labelled 1-13C-glucose and 13C-NMR

Ronald Andanje[1] and Dr. Amy Greene[1]
Albright College, 1621 N 13th St, Reading, PA 19604

Course Undergraduate Research Experiences (CUREs) have been shown to increase student engagement and retention in STEM. We developed a CURE for an upper-level biochemistry laboratory using non-pathogenic *Crithidia fasciculata* parasites. *Crithidia* are insect trypanosomes related to the causative agents of Leishmaniasis, African Trypanosomiasis, and Chagas' diseases. These parasites are ideal for undergraduate CUREs because they grow to high density in serum-free inexpensive media and have not been well studied in the literature, providing opportunities for novel discoveries. To facilitate determinations of cell density, we constructed a growth curve correlating OD600 with cell numbers as counted on a hemocytometer. Because biochemistry involves the study of central metabolism, we focused on parasite glycolysis for the first CURE. Isotopically labelled 1-13C-glucose was added to the parasites, and changes in Nuclear Magnetic Resonance (NMR) peak position was monitored over time. The fermentation product ethanol was the primary metabolite observed. Student groups then designed a novel project investigating metabolism in *Crithidia*. Although COVID-19 shutdowns limited our experiments, the results showed students involved in novel thinking and experimentation on metabolic pathways in a little-studied parasite.

P8-09. Chemical Ecology of an Apex Predator Life Cycle

Nicholas C. Mucci[1], Katarina A. Jones[2], Mengyi Cao[3], Michael R. Wyatt II[4], Shane Foye[5], Sarah Kauffman[1], Michela Taufer[4], Yoshito Chikaraishi[6], Shawn Steffan[5, 7], Shawn Campagna[2,8], Heidi Goodrich-Blair[1,3]
[1]Department of Microbiology, University of Tennessee-Knoxville, Knoxville, TN 37996, USA. [2]Department of Chemistry, University of Tennessee-Knoxville, Knoxville, TN 37996, USA. [3]Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, USA. [4]Department of Electrical Engineering and Computer Science, University of Tennessee- Knoxville, Knoxville, TN 37996, USA. [5]Department of Entomology, University of Wisconsin-Madison, Madison, WI 53706, USA. [6]Department of Biogeochemistry, Japan Agency for Marine-Earth Science and Technology, Yokosuka 237-0061, Japan. [7]US Department of Agriculture, Agricultural Research Service, Madison, WI 53706, USA. [8]Biological and Small Molecule Mass Spectrometry Core, University of Tennessee-Knoxville, Knoxville, TN 37996, USA.

Microbial symbiotic interactions, mediated in part by small molecule signaling, drive physiological processes of higher order systems, including the acquisition and consumption of nutrients that support symbiotic partner reproduction. Advances in metabolic analytic technologies provide new avenues to examine how chemical ecology, or the conversion of existing biomass to new forms, changes over a symbiotic lifecycle. Here we examine such processes using the tripartite relationship involving the nematode host *Steinernema carpocapsa*e, its obligate mutualist bacterium, *Xenorhabdus nematophila*, and the insects they infect together. The nematode infective juveniles infect insects into which they release bacteria that help suppress insect immunity and kill the insect. The nematode-bacterium pair consume the insect cadaver and reproduce until nutrients are depleted, causing a new generation of infective juvenile nematodes, colonized by the bacterial symbiont, to leave the cadaver in search of insect prey. To begin to understand the processes by which insect biomass is converted over time to either nematode or bacterium biomass, we took a three-pronged approach integrating information from trophic, metabolomics, and gene regulation analyses. Trophic analysis established bacteria as the primary insect consumers, with nematodes at a trophic position of 4.37, indicating consumption of bacteria and likely also other nematodes. Metabolic changes associated with bioconversion of *Galleria mellonella* insects were assessed using multivariate statistical analyses of metabolomics datasets derived from sampling over an infection time course. Statistically significant, discrete phases were distinguishable from each other, indicating the insect chemical environment changes reproducibly during bioconversion. Tricarboxylic acid (TCA) cycle components and amino acids such as proline and leucine were significantly affected throughout the infection. Hierarchical clustering revealed a similar molecular abundance fluctuation pattern for nucleic acid, amino acid, and lipid biosynthesis metabolites. Together, these findings contribute to an ongoing understanding of how symbiont associations shape chemical environments.

P8-10. Intracellular Calcium Transport in *Crithidia fasciculat*a is Regulated by the Mitochondrial Calcium Uniporter

Camila Gomez and Carlos Ramirez-Morales
Georgia State University, Department of Biology

Mitochondrial calcium contributes to vital cellular processes in protozoan parasites such as host cell invasion, bioenergetics, and cellular signaling. The mitochondrial calcium uniporter (MCU) is a gated channel that controls Ca^{2+} transport across the inner membrane of the mitochondria. In this study, I will characterize properties of a MCU in *Crithidia fasciculata*, a relative of the pathogens *Leishmania* and *Trypanosoma*. We are seeking to characterize function and localization of a gene that I predict encodes subunit D of the MCU in *C. fasciculata*. Because MCU subunit D in *T. brucei* reduces Ca^{2+} uptake by mitochondria, a similar pattern is expected in *C. fasciculata*. To assess this, the Ca^{2+} uptake by permeabilized *C. fasciculata* cells was measured using the fluorescent dye calcium green-5N. Ruthenium red, an inhibitor of the MCU, was used to determine the contribution of the MCU to calcium uptake. By studying the mechanism of mitochondrial Ca^{2+} transport in *C. fasciculata*, we can identify differences in Ca^{2+} regulation across biological models and begin to characterize all the subunits and modulators of the MCU complex in these organisms.

P8-11. Identification and Characterization of *Leishmania* PI3K Class 2 (LdPI3KC2) that Localizes to the *Leishmania* Parasitophorous Vacuole at the Host Parasite Interface

Naixin Zhang[1], Kha Van[1], Phillip Yates[2], Peter E. Kima[1]
[1]University of Florida, Gainesville, FL 32611. [2]Oregon Health and Science University, Portland, OR 97239

Leishmaniasis is a disease with a world-wide prevalence rate estimated at 12 – 15 million. *Leishmania* primarily infects macrophages wherein they reside in membrane enclosed compartments called *Leishmania* parasitophorous vacuoles (LPVs). We have previously shown that in *Leishmania*-infected macrophages, there is sustained activation of Akt, a critical downstream kinase in the PI3Kinase signaling pathway. Experimental approaches that reduce Akt levels or block its activation resulted in clearance of established *Leishmania* infections. This implied that the molecular machinery that mediates sustained Akt activation could contain therapeutic targets for *Leishmania* control. To understand the underlying mechanism by which sustained Akt activation is achieved in parasitized cells, we first assessed the distribution of PI(3,4)P2 and PI(3,4.5) P3 in infected cells, which are the critical and rate limiting lipids to which Akt anchors for activation. Using genetically constructed probes for phosphoinositides as well as performing immunofluorescence assays (IFA) with anti-phosphoinositide antibodies, we found that PI(3,4)P2 and not PI(3,4,5)P3 is displayed on LPVs. In contrast, phagosomes that harbor Zymosan particles displayed PI(4)P, the primary precursor of PI(3,4)P2 but not PI(3,4)P2. We therefore hypothesized that *Leishmania* express a PI3Kinase that functions at the parasite-host interface. We proceeded to develop recombinant parasites that express an mNeonGreen (mNG) tagged PI3KC2 enzyme, which is the member of the PI3kinase family whose primary function is the conversion of PI(4)P into PI(3,4)P2. The LdPI3KC2/mNG localizes on LPV membranes. In addition, we have raised an antiserum to LdPI3KC2, which provided confirmatory evidence of its localization within the LPV. Finally, a recombinant LdPI3KC2 was found to be functionally able to transform PI(4)P to PI(3,4)P2. To our knowledge, this is the first report and characterization of a *Leishmania* derived kinase that localizes to and functions at the host-parasite interface.

P8-12. Contribution of Purinergic Signaling During *Toxoplasma gondii* Infection

Aline C. A. Moreira-Souza, Ph.D.[1] ; Thuany P. Rangel[1] ; Sthefani R.B. Silva[1] ; Robson Coutinho-Silva, Ph.D.[1]
[1]Institute of Biophysics Carlos Chagas Filho Federal University of Rio de Janeiro Rio de Janeiro, RJ. Brazil

Purinergic signaling was first characterized as the one mechanisms responsible for neurotransmission in non-noradrenergic, non-cholinergic nerves. Since then, two families of purinergic receptors were cloned in most tissue and organs: the P1 family (receptors for adenosine) and the P2 family (receptors for purine or pyrimidine nucleotides). Purinergic receptor activation is involved in intracellular signaling cascades that triggers cellular events such as calcium, sodium, and potassium currents, transcription factor activation, induction of motility, cell growth, cell death, production of inflammatory mediators, and microbicide activity. The contribution of purinergic signaling to the parasite-host interaction was investigated during infection by pathogens such as bacteria, viruses, and protozoa. In the last 10 years, the activation of purinergic signaling has been proposed as an essential component of the immune response to infection by *Toxoplasma gondii* in both cellular and systemic contexts of the infection.The finds of our study showed the participation of P2X7 receptor in the control of *Toxoplasma gondii* infection in vitro requires NLRP3 inflammasome activation, reactive oxygen species and lysosomal fusion. Analyzing the murine models of experimental toxoplasmosis (in vivo), it was observed that P2X7 receptor mediates the host resistance inducing local and systemic pro-inflammatory mediators such as IL-1beta, IL-12 and IFN-gamma cytokines and reactive oxygen species. In conclusion, the purinergic signaling is involved in the acute and in the chronic toxoplasmosis contributing to the parasite killing and host protection via innate immune mechanisms.

P9-01. Regulation of Telomere Length and Telomerase Activity During the L*eishmania amazonensis* Developmental Cycle and Population Replication

Mark E. Shiburah[1£]; Beatriz C. D. de Oliveira[1£]; Stepany C. Paiva[1£]; Marina R. Vieira[1]; Edna Gicela O. Morea[1]; Marcelo Santos da Silva[1]; Cristiane de Santis Alves[1]; Marcela Segatto[2]; Fernanda Gutierrez[5]; Julio C. Borges[4]; Rodrigo T. Calado[5]; Maria Isabel N. Cano[1*]

[1]Biosciences Institute of Botucatu, São Paulo State University, UNESP, Botucatu, São Paulo; [2]Faculadade Brasileira Multivix, Vitoria, Espirito Santo; [4]São Carlos Institute of Chemistry, University of São Paulo, São Carlos, SP, Brazil; [5]Hemocentro da Faculdade de Medicina de Ribeirão Preto, Universidade of São Paulo, Brazil

The *Leishmania* sp. developmental cycle comprises three main life stages (amastigotes, promastigotes, and metacyclics) in two different hosts, indicating the parasite is challenged to survive and adapt to drastic environmental changes. Interrupting this cycle is key to discovering new anti-parasite therapies. Telomeres, the physical ends of chromosomes, maintain genome stability and cell proliferation and are considered potential antiparasitic drug targets. Therefore, understanding how telomere length is regulated during parasite development is vital. Here it is demonstrated that telomeric DNA foci are spread in the nucleoplasm of all parasite life stages. It was possible to show that amastigote telomeres are shorter than metacyclics and promastigotes, and telomere length increases in parasites with continuous in vitro passages. The observed differences in telomere length seemed not to be due to lack/inhibition of telomerase activity along the developmental cycle since enzyme activity was detected in all parasite life stages, although the catalysis was temperature-dependent. Therefore, it was important to test if parasite telomere length would be regulated by components of the telomerase ribonucleoprotein complex such as HSP83, the homolog of HSP90 in trypanosomatids. The results showed that similarly to other eukaryotes, an HSP90 inhibitor (17AAG) disturbed parasite growth along the developmental cycle, induced cell cycle arrest and telomere shortening, and inhibited promastigotes telomerase activity. The results strongly suggest a new role for HSP83 as a parasite telomerase component probably involved in control telomere length maintenance and parasite life span.

P9-02. EXP2 is Important for Intrahepatic Parasite Development During the *Plasmodiu*m Liver-stage

Tahir Hussain, Jose Linera-Gonzalez, John M Beck, Manuel A Fierro, Gunnar R Mair, Ryan C Smith and Josh R Beck

Iowa State University, Ames, IA 50010

During vertebrate infection, malaria parasites develop within a parasitophorous vacuole in both hepatocytes and erythrocytes. To transcend this barrier, *Plasmodium* spp. utilize a dual-function pore formed by EXP2 for nutrient transport and, in the context of the PTEX translocon, effector protein export across the vacuole membrane. While critical to blood-stage survival, less is known about EXP2/PTEX function in the liver-stage, although major differences in the export mechanism are indicated by absence of the PTEX motor HSP101. Here, we employed the glucosamine-activated glmS ribozyme to study the role of EXP2 during Plasmodium berghei liver-stage development in hepatoma cells. Insertion of the glmS sequence into the exp2 3'UTR enabled glucosamine-dependent depletion of EXP2 after hepatocyte invasion, allowing separation of EXP2 function during intrahepatic development from a recently reported role in hepatocyte invasion. Post-invasion EXP2 knockdown reduced intrahepatic parasite vacuolar size and significantly decreased expression of LISP2, a marker of mid to late liver-stage development. As an orthogonal approach to monitor development, EXP2-glmS parasites and controls were engineered to express nanoluciferase. Again, activation of glmS after invasion substantially decreased nanoluciferase signal at 48 hours post-infection in hepatoma monolayers and in culture supernatants at later time points corresponding with merosome detachment that marks the culmination of liver-stage development. Collectively, our findings extend the utility of the glmS ribozyme to study liver-stage protein function and indicate EXP2 is important for intrahepatic parasite development, paving the way to understanding liver-stage vacuolar transport mechanisms.

P9-03. cAMP-dependent Phosphorylation of Flagellum Matrix Proteins in *Trypanosoma brucei*

Daniel Velez-Ramirez[1]; Michelle Shimogawa[1]; Alexis Elliott1; Tim Britton[1]; Rebecca Pasquarelli[1]; Kent Hill[1,2,3]
[1]University of California Los Angeles. Department of Microbiology, Immunology & Molecular Genetics. Los Angeles, California, USA. [2]University of California Los Angeles. Molecular Biology Institute. Los Angeles, California, USA. 3University of California Los Angeles. California NanoSystems Institute. Los Angeles, California, USA

Trypanosoma brucei is a protozoan parasite and causative agent of sleeping sickness. *T. brucei* flagellum plays critical roles in parasite transmission, pathogenesis and biology. The flagellum is surrounded by membrane and is laterally connected to the cell body along almost its entire length. We used APEX2-proximity proteomics to examine protein composition of *T. brucei* flagellum, using Dynein Regulatory Complex subunit 1 (DRC1) as bait. The resulting DRC1-proximity proteome gives good overlap with known flagellum proteomes, validating the APEX2 system in trypanosomes. In addition to its canonical role in cell motility, the flagellum is also a signaling platform. Adenylate cyclases located at specific flagellum domains, produce cAMP that controls motility, cell signaling and parasite transmission. cAMP is removed by the flagellar-restricted phosphodiesterase B1 (PDEB1). To investigate the flagellar cAMP cell signaling pathway, we sought to identify cAMP-dependent protein phosphorylation in the flagellum and assess its role in cell motility. By performing APEX2-proximity labeling in tandem with TiO2-mediated phosphoenrichment, we have been able to define the flagellum phosphoproteome. We next obtained the flagellum phosphoproteome of a PDEB1 knockout, to identify cAMP-dependent phosphorylation. Principal component analysis of control and PDEB1-knockout samples revealed that the flagellum phosphoproteomes are indeed different, with several proteins differentially phosphorylated in the PDEB1-knockout. Gene ontology (GO) analysis of the biological process of this group of proteins retrieved microtubule-based movement as the top GO term. In terms of cellular component, the GO analysis retrieve intraciliary transport particle as the top term, followed by ciliary tip. Finally, the top molecular function was phosphoric ester hydrolase activity, with would refer to phosphatases catalytic activity. We are now working to assess the role of these cAMP-dependent flagellum protein phosphorylations in cell motility and signaling.

P9-04. Description of a New Species, *Microcotyle visa* (Monogenea: Microcotylidae), a Gill Parasite of *Pagrus caeruleostictus* (Teleostei: Sparidae) Using Integrative Taxonomy

Chahinez Bouguerche[1,2], Jean-Lou Justine[2], Delphine Gey[3,4], Fadila Tazerouti[1]
[1]Laboratoire de Biodiversité et Environnement : Interactions – Génomes, Faculté des Sciences Biologiques, Université des Sciences et de la Technologie Houari Boumediene, Alger, Algérie. [2]Institut Systématique Évolution Biodiversité, Muséum National d'Histoire Naturelle, Paris, France [3]Service de Systématique Moléculaire, Muséum National d'Histoire Naturelle, Paris, France [4]UMR7245 MCAM, Muséum National d'Histoire Naturelle, Paris, France

Parasite biodiversity of fish of the southern part of the Mediterranean Sea is still incompletely explored. We described a new species *Microcotyle visa* from the gill filaments of the bluespotted seabream *Pagrus caeruleostictus* (Valenciennes) (Sparidae) collected off the Algerian coast. The identity of fish hosts was confirmed by barcoding. Analysis of the cox1 gene of the monogeneans revealed minor intraspecific variation (1.4%), an order of magnitude lower than the distance between this species and other *Microcoty*le species (10–15 %). *Microcotyle vis*a was distinguished from *Microcotyle erythrin*i van Beneden & Hesse, 1863, a congener infesting sparids, on the basis of morphological (size of clamps, number of testes) and molecular (cox1) differences. This is the fourth member of the genus known to parasitise a sparid host. A species of *Paramicrocotyle* sp. Included in the molecular analysis was nested within a robust *Microcotyle* + *Paramicrocotyle* clade; in the absence of demonstrated molecular and morphological differences, we considered that *Paramicrocotyle* Caballero & Bravo-Hollis, 1972 is a junior synonym of *Microcotyle* van Beneden & Hesse, 1863 and transferred two species of *Paramicrocotyle* as *Microcotyle danielcarrioni* (Martinez & Barrantes, 1977) and *Microcotyle moyanoi* (Villalba & Fernandes, 1986).

P9-05. Lymphatic Filariasis Elimination Status: *Wuchereria bancrofti* Infections in Human Populations and Factors Affecting Continued Transmission after Seven Rounds of Mass Drug Administration in Masasi District, Tanzania

Eliza Lupenza[1*], Dinah B. Gasarasi[2] and Omary M Minzi[3]

[1,2]Department of Parasitology and Medical Entomology, School of Public health and Social Sciences, Muhimbili University of Health and Allied Sciences, Box 65011, Dar es Salaam, Tanzania [3]Department of Clinical Pharmacy, School of Pharmacy, Muhimbili university of Health and Allied Sciences Box 65013, Dar es Salaam, Tanzania

Background: Lymphatic filariasis (LF) affects more than 120 million people globally. In Tanzania, nearly six million people live with clinical manifestations of the disease. The National LF control program was established in 2000 using Mass drug administration (MDA) of Ivermectin and Albendazole to individuals aged 5years and above. This study assessed the infection status in individuals aged 15 years and above who are eligible for participation in MDA; compliance to MDA and factors affecting continued transmission after seven rounds of MDA in Masasi District. **Methods:** A community based cross-sectional study was conducted in two villages of Masasi District. A total of 590 participants aged 15 years and above were screened for the circulating filarial antigen (CFA) using the rapid diagnostic test. Night blood samples from CFA positive individuals were further analyzed for detection and quantification of *Wuchereria bancrofti* microfilaria (Mf) using the counting chamber technique. A pre-tested questionnaire was administered to collect information on compliance to MDA and the factors affecting continued transmission. Data were analyzed using SPSS Version 20. Chi-square test was used to compare the prevalence of CFA by gender and village where a P-value ≤0.05 was considered statistically significant. **Results:** Out of 590 participants, 30 (5.1%) were positive for CFA and one (0.2%) was found positive for microfilaria of *Wuchereria bancrofti*. The differences in CFA prevalence between the two villages were not statistically significant, (P=0.72). Drug uptake during the last round of MDA, year 2019 was 56% which is below the minimum coverage recommended by WHO. Being absent from home during MDA, perceptions of being free from hydrocele or elephantiasis were the major reasons for noncompliance. **Conclusion:** There is a significant decline in LF transmission in Masasi District after seven rounds of MDA. However, the presence of systematic non-compliants may delay elimination of LF in the District.

P9-06. Tell Me What You Eat, I Will Tell You What You Are! A Study of a Hyperparasite *Cyclocotyla bellones* (Monogenea, Platyhelminthes) Using Integrative Taxonomy

Chahinez Bouguerche[1,2], Fadila Tazerouti[1], Delphine Gey[3,4], Jean-Lou Justine[2]

[1]Laboratoire de Biodiversité et Environnement : Interactions – Génomes, Faculté des Sciences Biologiques, Université des Sciences et de la Technologie Houari Boumediene, Alger, Algérie. [2]Institut Systématique Évolution Biodiversité, Muséum National d'Histoire Naturelle, Paris, France [3]Service de Systématique Moléculaire, Muséum National d'Histoire Naturelle, Paris, France [4]UMR7245 MCAM, Muséum National d'Histoire Naturelle, Paris, France

P9-07. Extracellular Vesicles Released by *Leishmania donovan*i Infected Macrophages Contain Parasite Molecules and May Contribute to Lesion Development and Immune Modulation

Anna Gioseffi[1], Keily Ortega1, Kha Van[1], Austin Sheppe[1], Lisa Emerson[1], Phillip A Yates[2], Mariola J Edelmann[1], Peter E Kima[1]

[1]Microbiology and Cell Science, University of Florida, Gainesville, FL 32611. [2]Department of Biochemistry and Molecular Biology, Oregon Health and Science University, Portland, OR, 97239

Visceral leishmaniasis is a neglected tropical disease that causes significant morbidity and mortality across the globe. How *Leishmania* parasites communicate with their host to enhance their pathogenesis remains uncertain. To address this knowledge gap, extracellular vesicles (EVs) have emerged as an intriguing prospect because of their ability to transport active molecules to other cells and tissues. We performed proteomic analysis of *Leishmania*-infected macrophage EVs (LieEVs) and determined that *Leishmania donovani* infection of macrophages results in quantitative and qualitative changes in the protein profile of EVs released by infected cells. We validated these results by performing Western blots analyses for selected molecules that were identified in LieEVs. Using ingenuity pathway analysis (IPA), we learned that several of the identified host-derived proteins upregulated in LieEVs had been previously implicated in promoting vascular changes in other systems. We also identified 59 parasite-derived proteins in LieEVs, including a putative *L. donovani* homolog of mammalian vasohibins (LdVash), which in mammals contribute to angiogenesis. To study LdVash more closely, transgenic parasites were developed that expressed an endogenously tagged LdVash/mNeonGreen (mNG) and confirmed that LdVash/mNG is indeed expressed in infected macrophages and traffics to EVs. Moreover, using antibodies to LdVash, we know that it is expressed in infected tissues. We proceeded to evaluate LieEVs in surrogate assays of angiogenesis. We observed that LieEVs induce endothelial cells to release angiogenesis promoting mediators, including IL-8, G-CSF/CSF-3, and VEGF-A. In addition, LieEVs induce epithelial cell migration and tube formation by endothelial cells. Finally, treatment of primary peritoneal macrophages with LieEVs revealed that they promote an M2-like polarization of macrophages, suggesting the EVs from infected macrophages may also play a role in immunological processes that influence disease progression. These studies provide evidence that LieEVs may play an important role in *Leishmania* pathogenesis, including in the promotion of vascularization of *Leishmania* lesions.

P9-08. Targeting the Isoprenoid Pathway of the Apicomplexan Parasite *Toxoplasma gondii*

Melissa A. Sleda, Zhu-Hong Li, Ranjan Behera, Catherine Li, Silvia NJ Moreno

Department of Cellular Biology, University of Georgia, Athens, GA 30602

Toxoplasma gondii is an obligate intracellular parasite that infects humans and animals and it is the most prevalent zoonotic infection. It is estimated that one-third of the world's human population is infected. The isoprenoid pathway is a metabolic pathway present in both humans and *T. gondii* that synthesizes essential metabolites like cholesterol, dolichol, heme, and ubiquinone. The host relies on the Mevalonate pathway while the parasite utilizes the DOXP/MEP (non-mevalonate) pathway for synthesizing short-chain isoprenoids followed by a peculiar enzyme that synthesizes the longer units. Due to these differences the synthesis of isoprenoids appears to be a good target for chemotherapy. In addition, there is evidence that several enzymes of the isoprenoid pathway involved in the synthesis of some metabolites like farnesyl diphosphate (FPP) and sterols, and in protein prenylation have been reported to be excellent drug targets against parasites. Previous studies have shown that the *Toxoplasma* enzyme, farnesyl diphosphate synthase (FPPS), can be inhibited by bisphosphonates. However, *T. gondii* knock-out mutants for this enzyme are able to survive because they can salvage intermediates from the host synthesized through the Mevalonate pathway. We wondered if other enzymes downstream to the salvage point could be targeted for improved chemotherapy. We characterized an enzyme termed polyprenyl diphosphate synthase (PPS) and found it to be essential for parasite growth. PPS localizes to the mitochondrion and is essential for normal mitochondrial function. The defects in growth and respiration can be rescued by complementation of the mutants with the TgPPS or with a solanesyl diphosphate synthase from *Trypanosoma cruzi* (TcSPPS). The enzyme can be inhibited by a bisphosphonate derivative, BPH-1218, which also inhibits in vitro parasite growth and protects against *T. gondii* infection in vivo.

P9-09. *Plasmodium* and the Bone Marrow: Uncovering Novel Host-parasite Interactions

Tamar Feldman[1], Yana Ryan[1], Elizabeth Egan[1]
[1]Stanford University, Stanford, CA 94305.

Severe anemia of malaria is a large and understudied contributor to maternal and infant mortality in areas of endemic malaria. Severe anemia results from both red blood cell (RBC) destruction and aberrant production (dyserythropoiesis). While the pathogenesis of dyserythropoiesis in malaria is poorly understood, both asexual and sexual stage *Plasmodium falciparum* parasites have been observed in the bone marrow in clinical samples, suggesting they may influence erythroid development. In vitro studies have demonstrated that *P. falciparum* is capable of infecting late-stage erythroblasts and that incubation of parasites or parasite metabolites with erythroid precursors can perturb host cell proliferation and induce transcriptional changes in bulk culture. However, the host cell response to infection remains unknown as do the specific changes that drive dyserythropoiesis. To characterize the erythroid precursor responses to *P. falciparum* infection, we implemented an approach to infect primary human erythroid progenitor cells at different stages of differentiation and measure the stage-specific transcriptional responses by RNA-seq. We induced primary CD34+ hematopoietic/stem progenitor cells to proliferate and differentiate down the erythroid lineage and inoculated them with late-stage, GFP-expressing *P. falciparum* on day 7 and 13. We observed robust infection of erythroid precursors at all stages of terminal differentiation and demonstrated that parasites were viable at 20 hours post-invasion. In an study to profile the host transcriptional response to *P. falciparum* at specific stages of erythroid maturation, we sequenced mRNA from unexposed, exposed, and infected erythroblast populations at 20 hours post-addition of parasites to the cells. We found extensive transcriptional changes in infected late-stage erythroblasts. Enrichment analysis revealed alterations in expression of cell cycle pathways in infected cells. We also detect parasite transcripts in infected erythroblasts that are hallmarks of ring-stage development. This information will inform functional studies aimed at dissecting the molecular interactions between host and parasite that contribute to dyserythropoiesis.

P9-10. Investigating the Role of Gibberellic Acid on Dihydroartemisinin-induced Dormant *Plasmodium falciparum*

Alona Botnar[1,2], Lili Huang[3], Chungsik Kim[3], Roman Manetsch[3], Dennis E. Kyle[1,2,4]
[1]Department of Infectious Diseases, University of Georgia, Athens, GA [2]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA [3]College of Science and the Bouvé College of Health Sciences, Northeastern University, Boston, MA. [4]Department of Cellular Biology, University of Georgia, Athens, GA

As the COVID-19 pandemic has spread rapidly across the globe, it has become even more crucial that other infectious diseases, such as malaria, are not neglected. Currently, malaria is treated with Artemisinin (ART) based combination therapies (ACTs), which are our last line of defense in effectively treating all strains of *Plasmodium*. ART is known to induce a dormancy phenotype in the early ring stage parasite, during which parasite growth is arrested for several days before recovery and normal growth resume. ART induced dormancy is hypothesized to be a stress response that allows parasites to withstand the cytotoxic effects of the drug. Currently, the exact mechanism by which the parasite enters this state of dormancy and later recrudesces to continue development is unknown. Furthermore, reports of Artemisinin resistance are rising, highlighting the urgency to develop better cures and increase our understanding of the parasite biology. Recently, Duvalsaint and Kyle discovered that Gibberellic Acid (GA) treated parasites recovered from dormancy (expressed as resumption of normal morphology) 48 hours earlier than non-GA-treated parasites. In this study, we prepared and conducted limited structure-activity relationship studies of GA derivatives on ART-induced dormant *Plasmodium falciparum*. We synthesized GA derivatives with alkyne substituents and incubated them in growing and dormant parasites and localization was then assessed by click chemistry with an azide linked fluorescent tag. We then synthesized biotinylated GA derivatives and employed immunoprecipitation methods and successfully pulled down potential interacting proteins of GA. We next plan to conduct mass spectrometry experiments to identify these proteins and further characterize the early recrudescence phenotype caused by exogenous addition of GA. These studies will broaden our scope of understanding of the dormancy mechanism of *Plasmodium* and will be important for the development of novel drugs.

P9-11. Adenine DNA Methylation, 3D Genome Organization, and Gene Expression in the Parasite *Trichomonas vaginalis*

Ayelen Lizarraga[1], Zach Klapholz O'Brownb[2], Konstantinos Bouliasb[2], Lara Roach[2], Eric Lieberman Greer[2], Patricia J. Johnson[3], Pablo H. Strobl-Mazzulla[4], Natalia de Miguel[1]

[1]Laboratorio de Parásitos Anaerobios, Instituto Tecnológico Chascomús, National Scientific and Technical Research Council-National University of San Martin (CONICET-UNSAM), B7130IWA Chascomús, Argentina. [2]Department of Pediatrics, Harvard Medical School, Boston, MA 02115. [3]Department of Microbiology, Immunology & Molecular Genetics, University of California, Los Angeles, CA 90095. [4]Laboratorio de Biología del Desarrollo, Instituto Tecnológico Chascomús, CONICET-UNSAM, B7130IWA Chascomús, Argentina.

Trichomonas vaginalis is a common sexually transmitted parasite that colonizes the human urogenital tract causing infections that range from asymptomatic to highly inflammatory. Chronic infections have been associated with high risk pregnancies, increased risk of acquiring HIV and higher susceptibility to developing cervical or prostate cancer. Despite their importance in other organisms, the epigenetic mechanisms involved in gene regulation in the parasite remain poorly understood. Recent works have highlighted the importance of histone modifications in the regulation of transcription and parasite pathogenesis. However, the nature of DNA methylation in the parasite remained unexplored. Using a combination of immunological techniques and UHPLC, we analyzed the abundance of DNA methylation in strains with differential pathogenicity demonstrating that N6-methyladenine (6mA), and not 5-methylcytosine (5mC), is the main DNA methylation mark in *T. vaginalis*. We performed an adapted methylated immunoprecipitation assay followed by high-throughput sequencing (MeDIP-seq) on a patient-derived strain to obtain genome-wide distribution of 6mA mark. Our results revealed that this mark is enriched at intergenic regions, with a preference for certain superfamilies of DNA transposable elements. We show that 6mA in *T. vaginalis* is associated with silencing when present on genes. Interestingly, bioinformatics analysis revealed the presence of transcriptionally active or repressive intervals flanked by 6mA-enriched regions and results from chromatin conformation capture (3C) experiments suggest these 6mA flanked regions are in close spatial proximity. This finding revealed a new role for 6mA in modulating 3D chromatin structure and gene expression in this parasite.