SPC-1 – **Opening Conference – Samuel Pessoa Award**

Feast or Poison? How Blood-Feeding Arthropods Tame the Toxicity of Massive Meals

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Vertebrate blood is protein-rich, with hemoglobin as its most abundant component. Hematophagous insects consume blood meals that can exceed several times their own body mass, a life trait that provides immense nutritional value but also creates unique physiological stresses. The massive scale of protein digestion generates toxic quantities of otherwise benign nutrients: free amino acids and heme reach harmful concentrations, while the sudden influx of nutrients triggers explosive microbial proliferation. Remarkably, blood-feeding arthropods have evolved mechanisms to detoxify these volume-driven excesses and maintain microbiome homeostasis. These adaptations not only enable their extreme diet but also reveal novel paradigms in cellular signaling, immunity, and metabolism. Over the years, we have explored these mechanisms, adding new knowledge to the biology of insect vectors and uncovering novel targets for vector control, while also providing broader insights into how cells cope with nutrient overload in heme and amino acid metabolism, as well as host-microbiota interactions.

Keywords: hematophagy;heme;aminoacids.

CO – 001 - **A novel type of Plasmodium oocyst division**

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Oocysts grow extracellularly at the abluminal side of the mosquito midgut. After successive rounds of DNA and organelle replication followed by a final budding step, one oocyst can generate thousands of sporozoites, the malaria parasite stage responsible for infecting the mosquito salivary glands and the liver of the vertebrate host. Here, using ultrastructural, dynamic and in vivo imaging, we report a phenomenon with characteristics of a novel type of asexual division of oocysts. This division process is characterized by the budding of a second satellite oocyst leading to the production of multinucleated daughter cells, and by the transitory asymmetric distribution of the capsule, a protein structure surrounding and protecting oocysts. Budding is found in 0 to 20% of the oocyst population in fixed midguts, occurs mostly in the early days of infection, and its percentage inversely correlates with the oocyst density. Longitudinal imaging of infected mosquitoes confirmed the occurrence of this division in vivo. This division may enhance parasite survival, which is particularly important in a low-density infection, leading to an asynchronous and increased generation of sporozoites. This would result in a longer replenishment of salivary glands with newly formed parasites and thus, in a potential increase of sporozoite transmission.

Supported by: PPU, CEPIA, Imaging Platform, ANR LABEX IBEID, Bioimaging.
Keywords: Cell Division;Plasmodium;Oocysts.

CO – 002 - **Why Eat? Elucidating the Purpose of Endocytosis in *Trypanosoma cruzi***

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The protozoan parasite *Trypanosoma cruzi* possesses a unique ancestral feeding apparatus known as the cytostome-cytopharynx complex (SPC) through which it internalizes extracellular nutrients. This endocytic structure initiates as a plasma membrane pore that invaginates into the cytosol, delivering material for digestion. While the SPC has been lost in related dixenous parasites such as T. brucei and Leishmania spp., it is retained in nearly all monoxenous trypanosomatids and in free-living bacterivorous kinetoplastids such as Bodo saltans. To investigate the function of this enigmatic organelle, we generated a series of *T. cruzi* mutants that either lack endocytic activity or fail to assemble a functional SPC. Surprisingly, these endocytic-null parasites grew at wild-type rates in vitro, suggesting the existence of a redundant pathway capable of acquiring putative essential nutrients. The identity of the mystery nutrient, however, remained elusive until our recent work uncovered that the SPC organelle is specialized for heme uptake. As a heme auxotroph, *T. cruzi* must acquire this essential cofactor from its host, and the SPC appears to be fine-tuned to fulfill this critical role. We also recently identified a plasma membrane-localized ATP-Binding Cassette (ABC) transporter operating as a parallel system to the SPC that imports this critical co-factor into the parasite. Disruption of either pathway alone permits parasite survival, but simultaneous inhibition of both the SPC and the ABC transporter abolishes heme uptake and halts parasite growth. Our findings resolve a longstanding mystery in T. cruzi biology, demonstrating that endocytosis is not required for general nutrient uptake but is instead specialized to secure the parasite's most indispensable resource, heme. This discovery highlights a novel vulnerability in the parasite's physiology and suggests new opportunities for therapeutic intervention.

CO – 004 - **Transcriptomic and proteomic analysis of quiescent *Trypanosoma Cruzi* epimastigotes: a resource for investigating persistence.**

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Despite triggering a strong immune response, *Trypanosoma cruzi* infections are life-long and can result in severe cardiac and/or digestive tract pathology. Current drugs have limited efficacy, and treatment failure is a common outcome. The ability to eliminate a non-replicating *T. cruzi* sub-population that can persist after therapy has been a key challenge to the drug-development community. Here, we describe the transcriptome and proteome profiles of quiescent epimastigote forms of the parasite isolated from exponentially growing cultures on the basis of reduced turnover of induced red fluorescent protein. This quiescent sub-population displayed diminished replicative capacity and was characterised by down-regulation of genes/proteins involved in transcription, translation, metabolism, mitochondrial function and DNA replication, and by up-regulation of proteins that promote exit from the cell-cycle in other organisms. Quiescence was a transient phenomenon, and parasites retained a capacity to re-enter the cell-cycle. These data represent a resource that can be exploited to explore parasite biology, to dissect the mechanisms of quiescence in intracellular amastigotes, and to help refine the drug-development screening cascade.
Keywords: Trypanosoma cruzi;Trypanosoma cruzi;quiescence.

CO – 005 - **The conundrum of persistent Leishmania infection and exacerbated inflammation, how do these co-exist?**

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Ulcerated manifestations of cutaneous leishmaniasis (CL) result from exacerbated inflammatory responses in the skin triggered by the infecting parasite, and sustained by deregulated immune pathways. Interestingly, these same inflammatory functions are responsible for parasite clearance, and ultimately healing of lesions. Counterintuitively, chronic CL (lesions with more than 6 months of evolution) caused by *L. Viannia* species, is characterized by a persistent infection with low, and often undetectable, parasite burden, despite strong local pro-inflammatory responses. In this talk, Dr. Gómez will share the most recent findings from studies conducted in Colombia, which investigate the role of innate immune responses in the outcome of CL. Harnessing the power of longitudinal clinical studies and implementation of unbiased transcriptomic profiling, her team´s work has unraveled the local and systemic immune dynamics of healing and non-healing responses to antileishmanial therapy. These studies have allowed identification of immune pathways and mediators as candidates of host directed therapies, and biomarkers of therapeutic responsiveness. Our next step is to work together, as a CL research community, towards validation of these findings and implementation of clinically viable interventions for CL patients.
Keywords: Cutaneous leishmaniasis;therapeutics;persistence.

CO – 006 - **Defining the role of cAMP compartments in the specificity of cellular responses in *Trypanosoma cruzi***

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Trypanosomatids are protozoan parasites that cause deadly infectious diseases. Despite the unique characteristics of their cAMP signaling pathways, little is known about the mechanisms driving signal specificity in these early divergent eukaryotes. From activation of adenylate cyclases in response to environmental cues to the downstream regulation of gene expression, the signaling mechanisms triggering developmental transformations in trypanosomes are poorly understood. Our recent work with the human pathogen *Trypanosoma cruzi* revealed the presence of two putative cAMP microdomains in this parasite: the flagellar distal domain (flagellar tip) and the contractile vacuole complex (CVC). Two adenylate cyclases (AC1 and AC2) and a cAMP response protein (CARP3) located at these compartments were found to modulate cAMP levels, flagellar adhesion, differentiation, response to osmotic stress, host cell invasion, intracellular replication, and colonization of the triatome vector's intestinal tract. In addition, three phosphodiesterases have been previously reported in these compartments: PDEB1 and PDEB2 in the flagellum, and PDEC at the CVC. PDE activity is critical for the compartmentalization of cAMP because it limits its diffusion to the immediate vicinity of synthesis. In this study, we used PDEB1, PDEB2, and PDEC as molecular markers to characterize the flagellum and the CVC as cAMP signaling microdomains in T. cruzi. We confirmed the localization of these proteins by CRISPR/Cas9-mediated endogenous gene tagging. Subsequently, we generated PDEB1, PDEB2, and PDEC2 overexpression, knockout, and rescued cell lines to modulate cAMP signals and investigate the individual role of these compartments. Our data indicate that cAMP generated at the CVC specifically mediates response to osmotic stress while the flagellar cAMP microdomain is mainly involved in metacyclogenesis, invasion of mammalian cells, and intracellular replication. Both cAMP compartments are involved in the parasite's ability to colonize the triatomine vector. Taken together, our results shed light on the signal transduction mechanisms driving response to environmental stress in *Trypanosoma cruzi*, agent of Chagas disease.
Keywords: Contractile vacuole complex;flagellar distal domain;signaling microdomains.

CO – 007 - **ALTERED MONOCYTE RECRUITMENT FOLLOWING CHALLENGE INFECTION WITH NON-HEALING STRAINS OF Leishmania ABROGATES PROTECTIVE Th1 IMMUNITY**

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Leishmaniasis is a vector transmitted parasitic disease causing significant global morbidity and mortality. “Leishmanization”, the inoculation of viable *Leishmania major* (L.m.) parasites into the skin, remains the only effective ‘vaccination’ strategy known to generate protection against subsequent natural vector transmission in mice and humans. In this setting, rapid recruitment of pre-existing CD4 + T helper 1 (Th1) effector cells, not memory-derived cells, and activation of skin-infiltrating phagocytic monocytes mediates parasite elimination. Here, we aim to determine if concomitant immunity provided by Leishmanization can mediate protective immunity against *L. amazonensis* (L.a) challenge, a strain of the parasite that causes complicated, non-healing disease, and two clinical isolates of the L. major strain. Leishmanized C57BL/6 mice were challenged with *L.m.* or *L.a.* in the skin and lesion development and parasite load was followed up to 20 weeks post-secondary challenge. After an early period of robust Th1-mediated control of *L.a.* parasite load in the skin, protection against L.a. challenge began to wane at approximately 9 weeks post-challenge and was characterized by a slow but progressive increase in parasite load and lesion size. Employing leishmanized STAT6 -/- , IL-10 -/- , and STAT6 -/- IL-10 -/- (dKO) mice we observed that neither pro-parasitic STAT6 nor IL-10 played a role in the late loss of protection after *L.a.* challenge. In contrast, and different from L.m. challenge, *L.a.* challenge induced continuous monocyte recruitment to the site of infection that acquired an arginase I + phenotype. These data suggest that effective vaccination strategies against *L.a.* parasites likely requires the induction of Th1 immunity but will also need to account for the impact of moncocyte recruitment and maturation towards a permissive host cell phenotype at sites of challenge infection.
Keywords: Monocytes;Leishmaniasis;Vaccination.

CO – 008 - **Plasmodium simium: birth and evolution of a zoonotic malaria parasite species**

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Plasmodium simium, a parasite of platyrrhine monkeys, is known to cause human malaria outbreaks in Southeast Brazil. It has been hypothesized that, upon the introduction of *Plasmodium vivax* into the Americas at the time of the European colonization, the human parasite adapted to Neotropical anophelines of the Kerteszia subgenus and to local monkeys, along the Atlantic coast of Brazil, to give rise to a sister species, *P. simium*. Here, to obtain new insights into the origins and adaptation of *P. simium* to new hosts, we analyzed whole-genome sequence data from 31 *P. simium* isolates together with a global sequence dataset of 1,086 P. vivax isolates. Population genomic analyses revealed a minimal genome-level differentiation between *P. simium* and present-day New World *P. vivax* isolates, consistent with their common geographic origin and subsequent divergence on this continent. However, *P. simium* clearly comprises a discrete parasite lineage with greatest genetic similarity to *P. vivax* populations from Latin America – especially those from the Amazon Basin of Brazil – and to ancient European P. vivax isolates, consistent with Brazil as the most likely birthplace of the species. We show that *P. simium* displays half the nucleotide diversity of P. vivax from Latin America, as expected from its recent origin. Likely genomic signatures of *P. simium* adaptation to new hosts include the deletion of >40% of a key erythrocyte invasion ligand, PvRBP2a, which may have favored more efficient simian host cell infection. We identified pairs of sympatric *P. simium* isolates from monkeys and from humans as closely related as meiotic half-siblings, revealing ongoing zoonotic transmission of *P. simium*. We conclude that New World P. vivax lineages that switched from humans to platyrrhine monkeys founded the *P. simium* population that infects nonhuman primates and feeds sustained human malaria transmission in the outskirts of major cities in Brazil, such as São Paulo and Rio de Janeiro. Most critically, we show that *P. simium* currently causes most, and possibly all, malarial infections usually attributed to *P. vivax* along the Serra do Mar Mountain range of Southeast Brazil. **Supported by:** National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States of America (U19 AI089681), Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil **Keywords:** malaria; Plasmodium simium;population genomics;Brazil.

CO – 009 - **Beyond the Genetic Code: Insights into Trypanosome Epigenome on Gene Regulation**

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In *Trypanosoma cruzi*, gene expression of protein-coding genes is regulated mainly post-transcriptionally due to polycistronic transcription and the absence of canonical promoters. However, our group and others have accumulated evidence suggesting that gene expression is also reflected in chromatin marks, genome accessibility, and three-dimensional genome structure. Here, I will show that these features are dynamically modulated across the parasite's cell and life cycle, correlating with its gene expression patterns and phenotypic differences. I will focus on our recent findings on the dynamics of histone variants and post-translational modifications, nucleosome occupancy, 3D genome interactions, and nascent transcription profiles, using different omics strategies such as high-resolution proteomics, transcriptomics, epigenomics, and Hi-C. I will also present the characterization of H4.V, a novel histone variant in *T. cruzi*, enriched at telomeres and convergent strand-switch regions (cSSRs), and strongly associated with chromatin in metacyclic forms. Together with H2B.V—previously characterized by our group—these variants show stage-specific expression, distinct chromatin affinities, and an association with regions enriched in virulence genes. Finally, integrative Hi-C and tRNA-seq analyses revealed that 3D chromatin folding, codon usage, and anticodon availability further shape gene expression and translational efficiency in these organisms.
Supported by: FAPESP, CAPES and CNPq. **Keywords:** chromatin;histone, epigenetics,;T;cruzi.

CO – 010 - **Synchrotron light: can it help my research?**

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The investigation of different biological samples depends on the physical techniques employed for their analysis, the correct interpretation of the resulting data, as well as on the knowledge of their limitations and potential artifacts. A powerful set of techniques involves the acquisition of images of biological samples. Incredible advances in light and electron microscopy have revealed increasingly greater details of cellular and tissue architecture. In parallel, modern synchrotron light sources have established a diverse range of beamlines with different optics and capabilities that can be used to analyze biological material. By using synchrotron radiation at different intensities and wavelengths, it is possible to scrutinize the cellular structure without causing physical damage to the material necessary for tissue preparation. Focusing on the beamlines currently in operation at the SIRIUS synchrotron in Campinas (Brazil), we intend to present the potential capabilities of beamlines such as CATERETÊ, CARNAÚBA, MOGNO and IMBUIA, and present preliminary results that illustrate the applications that can be considered for cellular investigation as well as its limitations.
Keywords: Synchrotron;3D image;Cellular structure, Tissue structure.

CC – 001 - **What DNA Replication Tells Us, So Far, About the Biology of *Trypanosoma cruzi***

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Trypanosoma cruzi is a generalist parasite that completes its life cycle across multiple host species. In both mammalian and insect hosts, it alternates between a proliferative form and a specialized, non-replicative one—likely as a strategy to preserve its genome, similar to what occurs in differentiated cells. The balance between genome maintenance and the generation of genetic variability is essential for adaptability. For *T. cruzi*, this is particularly critical, as the parasite must overcome diverse host-imposed challenges while remaining capable of performing its key function: host cell infection. Our group has been investigating proteins involved in DNA replication and its dynamics in this parasite, aiming to understand the extent to which DNA duplication contributes to genetic diversity during replicative stages, and how this process is silenced in non-replicative forms, where genome integrity must be maintained. In this talk, I will present proteins involved in the pre-replication complex that license replication origins, highlighting differences in their expression and DNA-binding capacity between replicative and non-replicative stages. Additionally, I will show that distinct *T. cruzi* genome compartments exhibit different replication dynamics, which impact the mutation rates of each genomic region, evidencing the DNA replication contribution to the diversification of fast-evolving genomic regions while preserving the conserved core compartment. Therefore, we suggest that infection success relies on a regulated transition from a proliferative, diversity-generating stage to a non-replicative, genome-preserving one, highlighting how parasites can harness core biological processes not only for survival, but as active strategies for adaptation. **Supported by:** FAPESP
Keywords: DNA replication;Trypanosoma cruzi;genetic variability.